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Microbial Biodiversity, Biotechnology and Ecosystem Sustainability

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Contents

Dryland Microorganisms: Ecology and Biotechnological Potential

Cristian Torres-León, Jorge A. Aguirre-Joya, and David R. Aguillón-Gutierrez

Abstract Drylands are ecosystems whose biodiversity has developed the ability to adapt to extreme conditions, such as scarcity of water and high temperatures, and has evolved to survive in an environment where biomass and energy flow are limited. Microorganisms are a fundamental part of biodiversity in drylands since they have ecological and functional interactions with plants and animals, maintain the adequate soil conditions where other organisms live and grow and participate in the biogeochemical cycles of some elements. Also, dryland microorganisms can be used as bioindicators of environmental health, especially in arid zones that are particularly sensitive to anthropogenic impact. Furthermore, from the point of view of biotechnology, the biochemical and physiological processes of dryland microorganisms can be exploited with industrial and research purposes in the production of enzymes, antioxidants, antimicrobials, biosurfactants, growth promoters, antifungals, polysaccharides, and lipids. Therefore, in this chapter, we provide a general overview of the microbial ecology in drylands and its biotechnological potential. The use of microorganisms and/or their metabolites in biotechnological industries represents a sustainable and efficient process with a huge number of plausible applications.

1 Biological Characteristics, Isolation, and Identification of Dryland Microorganisms

Some microorganisms are adapted to extremely dry environments, like deserts and semideserts, where one of the most common survival strategies is to colonize the interiors of rocks. This habitat provides thermal buffering, physical stability, and protection against UV radiation, among other factors. Through water retention in the rocks and soils, moisture is made available. Microbial cells need to withstand the biochemical stresses created by the lack of water, along with temperature

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fluctuations, pH, atmospheric pressure, and/or high salinity. The diversity and abundance of microorganisms in arid and semiarid zones also depend on the soil depth (the maximum density of microorganisms is found at a depth of 15 to 60 cm), and as the soils became drier and warmer the total number of microorganisms decreased. Also depends on the presence of water, the temperature, and the chemical composition of the soil (Vollmer et al. 1977; Wierzchos et al. 2012).

Research in Sonoran Desert soils concluded that culture-independent surveys of bacterial and archaeal communities in soil have illuminated the importance of both edaphic and biotic influences on microbial diversity. The results indicate that culture-independent approaches can reveal large portions of previously undetected microbial communities and highlight the potential importance of these previously cryptic taxa to extreme environments (Bernard et al. 2012).

Powell et al. (2015) reported that Cyanobacteria are the dominant organisms in desert crusts that are metabolically active during brief periods of moisture. These microorganisms have developed an array of adaptations to hot, arid climates with intense UV radiation, extreme diurnal temperature fluctuations, and high soil salinity. Crust microorganisms positively contribute to their harsh ecosystems, by preventing evapotranspiration, fixing nitrogen, and blocking solar radiation, then Cyanobacteria positively contribute to desert biomes. Thus, biological soil crusts provide important ecosystem services in dryland regions, including erosion control and contribution to nitrogen and CO_2 fixation (Maier et al. 2014).

Microbiological properties of arid soils also varied depending on the type of land use pattern: grasslands, in general, supported higher numbers of microorganisms than three plantations, cultivated fields, or barren land. Stabilization of shifting and dunes introduction of vegetation has markedly increased the soil microflora. In general, the low organic matter content and poor moisture availability of desert soils were the major factors limiting optimum microbial activity. Research in the Western United States showed that an inorganic nutrient or other factors may limit microbial activities in some vadose zone environments. It means that the hypothesis that water limits the abundance and activities of microorganisms in vadose zones was supported in the case of some, but not all, bacterial samples. For instance, lack of inorganic nutrients (e.g., N, P) may be the limiting factor (Kieft et al. 1993).

Dryland microorganisms are also in the air. Research in the twentieth and twenty-first centuries has shown that microorganisms mobilized into the atmosphere along with desert soils are capable of surviving long-range transport on a global scale. As with the better-known pathogens that use aerosolization to move from host to host, the microbiological research conducted to date has identified a wide range of dust-borne pathogenic microorganisms that move great distances through the atmosphere (Griffin 2007).

The isolation, culture, and microscopic examination of microorganisms require the use of suitable selective media and special microscopic slide techniques. Microorganisms collected in samples (soil, aquatic, air, and clinical, etc.) are characterized by culture-based assays (identification via substrate utilization) and/or non-culture-based assays (morphology-based identification; cell, spore, physiological, and nucleic acid stains; identification via nucleic acid amplification and/or hybridization;

immunological identification; direct counts; and fatty-acid analysis). Microscopy is one of the oldest tools used for the study of bacteria and fungi. Identifications or descriptions are based on staining characteristics, cellular morphology, spore shape, the presence or absence of spores, and pigmentation (Griffin 2007), depending on the macro and micro-environment in which the microorganism lives. Fluorescence microscopy or scanning electron microscopy can be used to observe a specific microorganism in a soil particle (Madigan and Martinko 2004).

Currently, in addition to classical microbiological techniques, molecular techniques such as metagenomics are widely used to achieve the identification of microorganisms, broadening the spectrum of microorganisms found in a sample. For example, in the Cuatro Cienegas basin (Coahuila, Mexico) in the Chihuahuan desert, were found 250 different phylotypes of microorganisms among the 350 cultivated strains. Ninety-eight partial 16S rRNA gene sequences were obtained and classified. The clones represented 38 unique phylotypes from ten major lineages of Bacteria and one of Archaea (Souza et al. 2006).

Kuske et al. (1997) performed a phylogenetic survey of microbial species present in two soils from northern Arizona (Southwestern United States); microbial DNA was purified directly from soil samples and subjected to PCR amplification with primers specific for bacterial 16S rRNA gene sequences (rDNAs). Their results indicate that some microorganisms comprise a phylogenetically diverse, geographically widespread, and perhaps numerically important component of the soil microbiota.

It is known that microorganisms play an important role in promoting the germination process of some cacti like *Echinocactus* seeds (Castillo-Reyes et al. 2014). This may have ecological implications using native species. For example, restoration of highly eroded desert land was attempted in the southern Sonoran Desert that had lost its natural capacity for self-revegetation using native leguminous trees (mesquite Amargo *Prosopis articulata*, and yellow and blue palo verde *Parkinsonia microphylla* and *Parkinsonia florida*), demonstrating that restoration of severely eroded desert lands was possible (Bashan et al. 2012).

Other types of microorganisms such as fungi can be found and isolated in deserts. For example, in order to facilitate the discovery of novel actinomycetes from the Egyptian deserts, which can be useful as new sources for bioactive metabolites, were used the media glucose–yeast extract agar, soil extract agar, and a new minimal medium (MM) containing glucose, yeast extract and mineral salts (Hozzein et al. 2008).

Microbiota of desert ecosystems is not only responsible for the productivity, biogeochemical cycling of elements and ecosystem balance, but also soil neogenesis and improvement of soil structure (Bhatnagar and Bhatnagar 2005).

2 Desert Microorganisms as Bioindicators

Bioindicators are living organisms that can be used to determine the state of health of their environments. These organisms are sensitive to environmental changes and therefore, their absence or scarcity indicates that there is some factor modifying the normal conditions of the environment. Changes in the abundance and diversity of these organisms can be used as a measuring tool to determine habitat health. Bioindicator organisms are subject to changes in pollutant load. They are often more effective than laboratory-based measuring tools as they are unable to escape the effects of the pollutants and are cheaper to use. Besides the physicochemical parameters, the use of bioindicators significantly complements the landscape of environmental quality and health (De la Lanza-Espino et al. 2000). In general, a bioindicator must comply with all or some of the following characteristics: (1) the alterations suffered by environmental changes must be measurable at a morphological, ethological, or physiological level, (2) the organism must be sufficiently dispersed in the area to be studied, it must be relatively abundant and easily detectable, (3) it must be as sedentary as possible to reflect local conditions, (4) it must be of a size that permits the study of tissues, (5) it must tolerate contaminants in concentrations similar to those observed in the environment without lethal effects, (6) must survive outside the natural environment, (7) make it easy to capture, (8) that sufficient information is available on the life cycle of the species and (9) preferably not be in taxonomic conflict (De la Lanza-Espino et al. 2000).

Organisms usually react differently to environmental disturbances. Accordingly, the bioindicators are classified as follows (Zuarth et al. 2014): Detectors: organisms that in the presence of environmental stressors suffer an increase in mortality, alteration in reproductive activity, and a decrease in their abundance; Exploiters: organisms that, faced with the disappearance of competition or the enrichment of nutrients caused by environmental disturbances, suffer an explosive population growth, so their presence evidence this disturbance; Accumulators: organisms that, due to their resistance to certain contaminants, can accumulate them in their tissues in concentrations that can be measured without suffering any apparent damage. Species that should be considered as bioindicator organisms (Zuarth et al. 2014): Flagship species, that by their charisma or beauty, easily attract the attention of the general public and, to a large extent, to the decisión makers; Sentinel species, which by having physiology or diet sufficiently similar to humans, or because they are very sensitive to chemical contaminants, pathogens or toxins, can provide an early indication of possible adverse health effects and provide information on the toxic mechanisms of a given hazardous agent; Keystone species, which because of their effects on the ecosystem, greater than those expected due to their abundance, play a vital role in maintaining the structure of an ecological community; Umbrella species, which by requiring an extensive habitat to maintain viable populations, facilitate with their protection, the conservation of other species.

It is difficult to consider a microorganism as a Flagship, Sentinel, Keystone or Umbrella species, but that does not mean that we cannot use them as bioindicators of

environmental quality. There are some places in the world where microorganisms are relevant to indicate the health of the environment, as in the following example. Cuatro Cienegas, an oasis of the Chihuahuan Mexican desert, is probably the only place in the world where communities of microorganisms form the base of a complex trophic chain. There is evidence of the molecular clock that Cuatro Cienegas is a "time machine" and the marine origin of its microbial mats, which descent from those that transformed an inhospitable planet in a blue and full of life planet. When this ecosystem loses its deep sulfur-rich water, or nutrients reach it by anthropogenic pollution, these biosignals of our past disappear. For this reason, microbial mats become bioindicators of these ecosystems´ health (Souza et al. 2014).

Bacterial communities change when the environment faces any disturbance such as the entry of pollutants, organic, or inorganic. In some cases, it is possible to register very rapid changes in the structure of bacterial communities due to the sudden discharge of contaminants. On the other hand, chronic contamination of sites tends to decrease bacterial diversity and the environment is enriched with the most resistant bacteria with a greater capacity for degradation of a particular contaminant. The effects that pollutants discharge has on the structure of bacterial communities are presented to stress the importance of considering bacterial communities in the monitoring and conservation of ecosystems (Le Borgne and Avitia 2014).

Microorganisms are the predominant portion of the soil's biological phase and they are indicators of soil health and quality. Soil microorganisms (a) take part in the degradation of organic and inorganic compounds, (b) their activity, number, and diversity may serve as bioindicators of toxic effects on soil biological activity, (c) some microbial species may be used for soil bioremediation, and (d) some sensitive microbes are used in eco-toxicity tests (Milolevic et al. 2010). Liu et al. (2000) concluded that substrate availability was the most important factor affecting the diversity and activity of soil microorganisms within a season in the northern Chihuahuan desert; in this case, soil moisture was not the factor causing differences in microbial diversity and activity, but it was a predictor for some microbial responses under particular stress. Then there are certain factors under which microorganisms in deserts can be used as bioindicators. Su et al. (2004) pointed out that the count of culturable microbes from an arid zone in the northwest of China, after enrichment with different media were statistically significantly correlated with soil organic matters, total nitrogen content, soil water content, and surface vegetation.

There are for example, isolated arsenic resistant bacteria from sediments of an arsenic-contaminated river (Escalante et al. 2009), that can be used as bioindicators in heavy metals environmental pollution. In some deserts, there are wetlands, where also is possible to use the microorganisms as bioindicators. Water quality routinely is determined using chemical analysis; however, the analysis of microbial populations is an additional tool that has been developed to determine the condition of aquatic environments (Guillén et al. 2012).

3 Biotechnological Potential for the Production of Value-Added Bioproducts

The biotechnological production of bioproducts is very important for obtaining ingredients with multiple industrial applications. Natural sources such as microorganisms, plants, or animals have caught the attention of consumers. In this sense, microorganisms are of particular interest because of their great metabolic diversity for modifying and upgrading a variety of complex molecules (Palmerín-Carreño et al. 2015), such as enzymes, antioxidants, antimicrobials, biosurfactants, growth promoters, antifungals, polysaccharides, and lipids (Fig. 1). In this way, the selection of new isolates is a wide field of commercial exploration (Pessôa et al. 2019).

4 Production of Value-Added Compounds

4.1 Enzymes

Enzymes are organic molecules that can catalyze chemical reactions, these molecules have important applications in different industries and can be obtained biotechnologically by desert microorganisms. Cruz-Hernadez et al. (2005) isolated 4 *Penicillium commune*, 2 *Aspergillus niger*, 2 *Aspergillus rugulosa*, one *Aspergillus terricola*, one *Aspergillus ornatus,* and one *Aspergillus fumigatus* en the Mexican

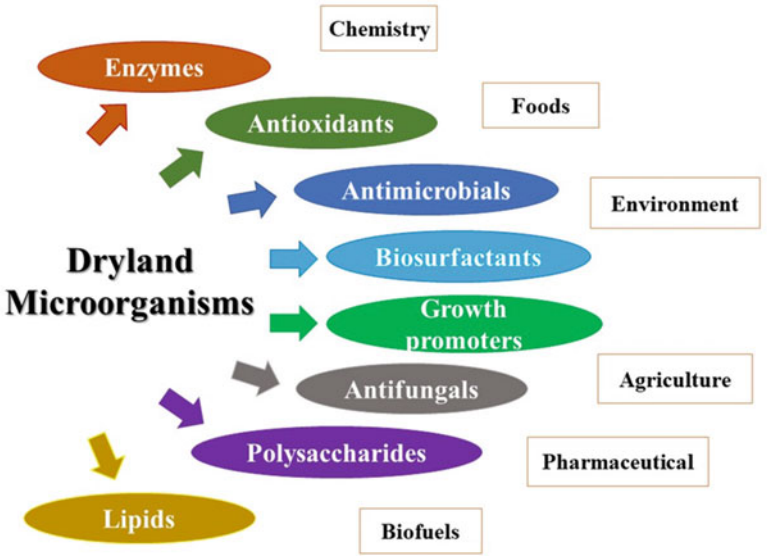

Fig. 1 Value-added bioproducts obtained through microbial biotechnology with dryland microorganisms and its industrial applications

Desert by their capacity to degrade tannins. *Aspergillus niger* GH1 and PSH showed the highest tannin-degrading capacity 67 and 70%, respectively. Additionally, *Aspergillus niger* PSH, and *Penicillium commune* EH2 degraded 79.33% and 76.35% of catechin (condensed tannin). Tannin degradation is caused by the production of enzymes. Ascacio et al. (2014) reported that *A. niger* GH1 has a high cellulase, xylanase, b-glucosidase, polyphenol oxidase, and tannase activity.

De la Cruz et al. (2015) evaluated the production of the enzyme ellagitannase from 4 strains of *A. niger* (PSH, GH1, HT4, and HC2) by fermentation. The fermentation was carried out into a column bioreactor packed with polyurethane foam impregnated with an ellagitannin's solution as a carbon source. The production of the enzyme was 938,8 U/g. According to the researchers, this was 10 times higher than that reported by other authors. De la Cruz-Quiroz et al. (2018) also investigated the production of enzymes from *Trichoderma* strains isolated in the Mexican desert by Osorio et al. (2011). The *Trichoderma* strains presented a high production of endoglucanase (40.8 U/g), exoglucanase (2.9 U/g), and chitinase (20.5 U/g). The enzymes produced by microorganisms can be used in two ways. The first consists of the use of pure enzymes (extracted and purified). In the second option, the enzymes can be used in fermentation processes for the extraction of antioxidant compounds of commercial interest.

4.2 Antioxidants

Antioxidants are used in dietary supplements to maintain good health and prevention from deadly diseases. As synthetic antioxidants exhibit adverse health effects (carcinogenic), researchers are encouraged to look for natural antioxidants. Microorganisms are a rich source of bioactive compounds with antioxidant properties (Chandra et al. 2020)**.** During the fermentation process, antioxidants are either produced by microorganisms through a secondary metabolic pathway or released from the matrix of the substrate by extracellular enzymatic action (Dey et al. 2016).

Aguilar et al. (2008) studied the production of antioxidant phenolic compounds by Solid-State Fermentation (SSF) with the fungus *A. niger* isolated from the Mexican semi-desert (Cruz-Hernandez et al. 2005). The substrates used in the fermentation were Pomegranate (*Punica granatum*) Peel and Creosote Bush (*Larrea tridentata*) Leaves. The results showed an increase in the concentration of ellagic acid due to the action of the microorganism. Recently, Torres et al. (2019) reported the use of *A. niger* GH1 in the production of antioxidant phenolic compounds in the SSF process using mango seed as support. SSF mobilized the polyphenolic compounds and improved the antioxidant properties. The total phenol content in ethanol extract increased from 984 mg GAE/100 g to 3288 mg GAE/100 g. In the SSF process, agro-industrial by-products can be used as a support, this can contribute to generating added value (Torres-León et al. 2018).

4.3 Antimicrobials

In recent decades, new microorganisms capable of producing compounds as a potential source of new antibiotics have been discovered. Alotaibi et al. (2020) isolated fungal and bacterial species from Sabkha and desert areas in Saudi Arabia, these microorganisms may have important biological properties such as antimicrobial activity. In Saudi Arabia also investigated the biological activity of soil microorganisms. Antimicrobial activity of the extracellular extract of *Alternaria alternata* isolated from soil against *Proteus vulgaris* and *Salmonella typhi* has been reported (El Hamd et al. 2014). Also, ten fungal filamentous fungi strains were isolated from Saudi Arabia soil. Most of the fungal extracts showed activity against different *Candida* species (Al-Enazi et al. 2018). Also, isolated actinobacteria from 10 different locations in the desert of Riyadh, Saudi Arabia showed broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, as well as against fungi (Nithya et al. 2018).

4.4 Biosurfactants

Chemical surfactants constitute a major component used in a large number of industrial applications as surface-active substances that lower the surface tension of fluids. However, chemical surfactants are often toxic (Gutnick and Bach 2019). Biosurfactants have generated wide interest as potential non-toxic alternatives. Biosurfactants can be obtained by fermentation using desert microorganisms. Korayem et al. (2015) isolated species of *Streptomyces* from arid soil in Egypt (19 different arid locations) and evaluated its biosurfactant activity. The authors reported that 17 isolates (out of 37 primary isolates) exhibited biosurfactant activity. The authors emphasize that the 5S isolate presented the highest biosurfactant production, having an emulsification index equal to 42.68%, in an optimized culture medium.

4.5 Plant Growth-Promoting Bacteria

Many studies have focused on the use of microbes to change the soil rhizosphere and enhance the availability of macro and micronutrients (Patel et al. 2017). Plant Growth Promoting Bacteria (PGPB) improves the synthesis of plant hormones and the availability of mineral nutrients in the soil (Patel et al. 2017). 67 bacteria were isolated from *Commiphora wightii rhizosphere* soil collected in the desert region of the Kutch, India. The drought-resistant bacterized seeds of chili (*Capsicum annuum)* were evaluated in the potting soil supplemented with 50 mM NaCl, the isolates showed higher root and shoot lengths, respectively, compared with control. The

authors mentioned that drought-resistant bacteria isolated from the rhizosphere of *C. wightii* grown on desert lands could be used for alleviating salinity stress in crop plants (Patel et al. 2017).

4.6 Antifungals (Biological Control)

Diseases caused by fungi have caused severe losses in industrial crops and food like fruits. Control of pathogenic fungi is both difficult and costly, and currently relies on chemical fungicide application. However, the extensive use of these chemicals is of concern in many countries as it leads to resistant fungal strains and accumulation that can pose human health risks (Sunpapao et al. 2018). Therefore, biological control with natural antagonistic microorganisms is actively pursued as an alternative approach that could reduce fungicide usage in agriculture (Sunpapao et al. 2018). Desert microorganisms have the potential to inhibit pathogenic fungi of agricultural interest. Osorio et al. (2011) isolated strains of *Trichoderma* from the soil, seeds, and plant in the desert of Mexico (Northeast region). The authors report that one *Trichoderma* strain which inhibited *Phytophthora capsici* (phytopathogen) in a range from 4.3 to 48.8%. Recently De la Cruz-Quiroz et al. (2018) investigated the inhibitory potential of these *Trichoderma* strains against the phytopathogens *Phytophthora capsici* and *Colletotrichum gloeosporioides*. The *Trichoderma* strains showed a percentage of inhibition on *P. capsici* and *C. gloeosporioides* up to 22.5%. *Trichoderma* strains evaluated showed to be an excellent potential agent to be used as a controlled pest against *C. gloeosporioides* and *P. capsici*.

4.7 Carbohydrates

There is a growing interest in the development of functional foods with health benefits. Among the functional ingredients, fructooligosaccharides (FOS) fully comply with all the described requirements. These are healthy sweeteners with important biological properties (de la Rosa et al. 2019). FOS are carbohydrates that promote the growth of beneficial bacteria in the host gut leading to improved health. FOS can be obtained biotechnologically using micro-organisms from the desert via sucrose biotransformation by partially purified Fructosyltransferase (Ojwach et al. 2020). De la Rosa et al. (2020) studied the production of FOS via SSF using *Aspergillus oryzae* (a strain isolated in the Mexican desert). The authors reported that *Aspergillus oryzae* has a great capacity for the synthesis of FOS (7.64 g/L).

4.8 Lipids

The deserts represent particularly harsh habitats (nutrient limitation, water-stress, and desiccation). To withstand and survive, the majority of prokaryotes have developed the ability to deposit lipophilic storage compounds (Röttig et al. 2016). Lipids have important advantages for microorganisms. These are very energy-rich, water-insoluble, osmotically inert, and readily accumulated in the cell. Most bacteria synthesize polymeric lipids, like polyhydroxyalkanoate (PHA), however extremophilic organisms (which grow in hydric external environments) can accumulate triacylglycerols (TAG) or wax esters (WE). Bequer et al. (2013) isolated 12 bacterial strains from High-altitude Andean Lakes in Argentina (an ecosystem characterized by periodical desiccation) producing TAG and WE (between 2 and 17% of cellular dry weight) Among these strains, the extremophile *Rhodococcus* sp. A5 accumulated17% and 32% of TAG during growth on glucose and hexadecane, respectively.

Röttig et al. (2016) isolated 10 strains affiliated to the genera *Bacillus*, *Cupriavidus*, *Nocardia*, *Rhodococcus,* and *Streptomyces* (from arid desert soil). The results showed that the *Streptomyces* sp. strains and *Rhodococcus* sp. accumulate significant amounts of TAG or WE. The stored TAG was composed of 30–40% branched fatty acids, such as anteiso-pentadecanoic or iso-hexadecanoic acid. These strains train could be used in biotechnology for the production of microbial lipids for sustainable production of biodiesel, or other industrially relevant compounds.

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An Overview of the Biodiversity, Ecosystem Services and Conservation Actions in the Western Ghats, India

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Abstract The Western Ghats, a global biodiversity hotspot, is particularly rich in floral and faunal wealth and endemism, covering an area of 164,280 km^2 in a stretch of 1600 kilometres. This majestic mountain chain rises from the coastal region at its western borders, reaching up to a height of 2695 m and then merging with the Deccan plateau to the east at an altitude of 500–600 m. This mountain range is home to ca. 7402 species of flowering plants, of which more than 1270 are endemic. Another ca. 1814 species of non-flowering plants, ca. 139 mammals, ca. 179 amphibia, ca. 508 bird, ca. 6000 insects and ca. 290 freshwater fish species are known; with even more to be discovered. Even though the area covers less than 5% of India's total land area, 30% of all flora and fauna are found here. As the case with a high degree of endemism in flora, 50% of amphibians and 67% of fish species in India are endemic to this region. The Western Ghats region is the source of about 13 major river systems. It encompasses a diversity of ecosystems and accomplishes essential hydrological and watershed functions. However, due to the increasing anthropogenic pressure, as in any other region, regional and global drivers of biodiversity change and ecosystem loss are prevalent in this region and increasing. Assorted efforts are in place to conserve the biodiversity here; however, only an integrated and ecosystem-based approach, at the landscape level, can achieve conservation of the rich bioresources and sustainable development.

With its unique assemblies of flora and fauna and rich endemism, the Western Ghats and Sri Lanka biodiversity hotspot are universally critical for conserving representative regions of the Earth's biodiversity. Running almost parallel to the west coast of

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India for 1600 km, the Western Ghats [WGs] falls across six south Indian states—Tamil Nadu, Kerala, Karnataka, Goa, Maharashtra, and Gujarat. Formed of a string of low hills to high mountains rising to over 2695 m above sea level, it is almost continuous except for the Palghat Gap, 30 km wide. The WGs being part of the Gondwana landmass has experienced complex geological history and confronted massive volcanic activity by the end of the Cretaceous epoch. The landscape is heterogenous for its topography and climate. Thus, the WGs provide a unique environment to study the effect of geological and ecological processes on its biota's existing diversity and distribution (Joshi and Karanth 2013). The Western Ghats forests are bestowed with a montage of landscapes—thorn scrub to dense evergreen forests—that holds an interesting collection of flora and fauna, represented by a large subset of endemics (MoEF 2009). The WGs has the highest protected area coverage in India, with over 15%. It also includes two of the eighteen biosphere reserves in India—the Nilgiri Biosphere Reserve (11,040 km^2) and the Agasthyamala Biosphere Reserve (3500 km^2). Geographically, the WGs is divided into three major regions (Pascal 1988): Surat to Goa, Goa to Nilgiri mountains and South of the Palghat Gap.

1 The Evolution

The breakup of the Gondwana supercontinent and consequent modification because of the subsequent tectono-thermal events is considered the triggers leading to the formation of the Western Ghats (Tiwari et al. 2018). The origin traced back to the separation of Madagascar from the Indian continent and the associated rifting event about 88 Ma ago (Storey et al. 1995). The peninsular India southern part encircled by passive continental margins where continents rifted away from each other to form the Arabian Sea and the Bay of Bengal (Corfield et al. 2010); which includes several tectonic events like Deccan trap eruption, separation of Seychelles from India (Hofmann et al. 2000; Hooper 1990). The onshore part of the west coast abodes topographic escarpments of varying heights to form the Western Ghats.

The development of a long topographic feature extending from the north to south (Satpura Range to Kanyakumari) with dissimilar rock types is a less understood but important geodynamic problem (Dubey and Tiwari 2018). The formation of the Western Ghats is not well understood, and the trigger for such an elevated topography in passive margins is being discussed a lot (Campanile et al. 2008; Gunnell 2001; Gunnell and Fleitout 2000; Gunnell and Radhakrishna 2001; Moore et al. 2009; Nielsen et al. 2010; Nielsen et al. 2009; Widdowson and Cox 1996). The isostasy, crustal buoyancy or the lithospheric flexure are the major theoretical cause (s) for the topography rise of the Western Ghats (Gupta et al. 2018). Some studies even suggest that the Himalayan collision from the late Miocene caused the Western Ghats formation (Gowd et al. 1992).

Patro et al. (2018) divided the entire Western Ghat belt and the adjacent region towards the east in the Indian Peninsular shield geologically, into three zones viz., the southern, northern and the middle or transitional. The southern zone corresponds Malabar coast (8–12° N), which exposes Precambrian high-grade rocks and

gneisses. The northern zone, corresponding to the Konkan coast (16–20° N), exposes mainly the Deccan traps and the middle one, i.e., the transition zone (12–16° N) occupied by Precambrian volcano-sedimentary sequences. Recent modelling (Sinha-Roy 2018) suggests that the persistence of the WGs is because of the combined effect of possible crustal mass addition by mid-crustal flow along an intra-crustal detachment and denudational isostasy. They also confirmed that it links the evolution of the WGs with the Deccan plateau uplift and to offshore crustal attenuation, both processes creating an upper crustal taper floored by a mid-crustal detachment.

2 The Rich Biodiversity of the Western Ghats Region

Mountain regions occupy 23% of the world's forests, covering over nine million square kilometres. The forests in these regions provide ecosystem goods and services to millions of people in mountain communities and contiguous lowland areas. The mountain biodiversity hotspots and the associated forests deliver many benefits, such as erosion control, improved water quality and quantity, carbon fixation, recreation and aesthetic appeal, timber, fuelwood, and non-timber forest products (Mountain-Partnership 2014) (Fig. 1). The Western Ghats is no exception to this.

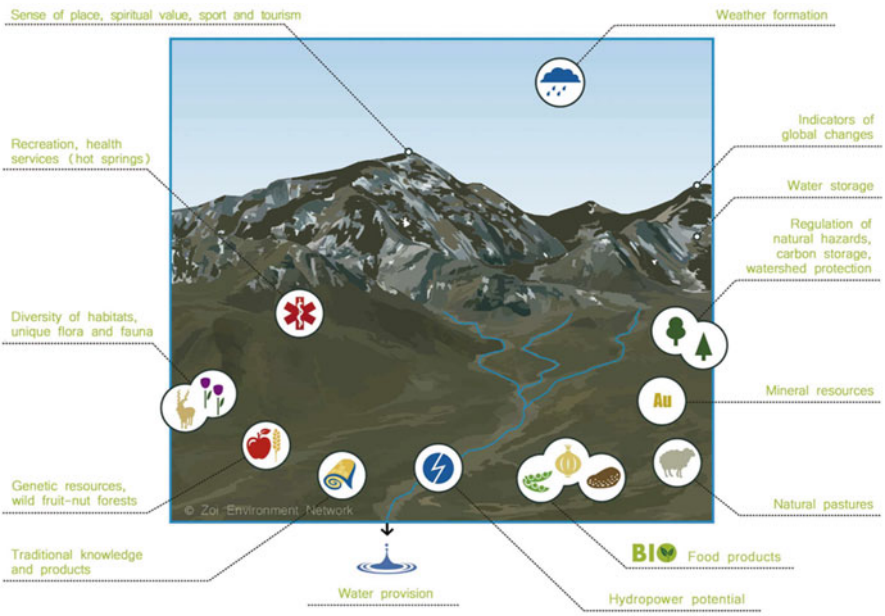

Fig. 1 Graphical representation of the Mountain ecosystems and the services provided [reproduced from (Mountain-Partnership 2014)]

The Western Ghats climate illustrates two rainfall gradients—West-East gradient and South-North gradient, and a temperature gradient related to the increase in elevation. Its proximity to the equator and the sea, good rainfall from both monsoons (1800–2000 mm), warm, humid tropical climate, heterogeneous soil strata, wide altitudinal range and the perennial hill streams traversing the area favoured the development of very rich tropical forest types in the WGs, sheltering unique biodiversity of great ecological and economic importance. The positioning makes the WGs biologically rich and biogeographically distinctive—an absolute treasure house of biodiversity.

It is a mega-biodiverse region with a species count of *ca*. 7400 flowering plants, *ca*. 508 birds, *ca*. 139 mammals and *ca*. 179 amphibians (WWF n.d.), with high endemism and species under different threat categories. The areas supporting intact natural ecosystems and where native species and communities associated with these ecosystems are well represented are considered biodiversity hotspots. Locally endemic species, which are not found or rarely found outside the hotspot, have a high diversity within these areas (Mittermeier et al. 2011). The WGs is considered a biodiversity hotspot and one of the "hottest" of its category for its incredible biological diversity (Myers 1990; Nag 2017). Besides the many endemic and endangered species, the WGs is home to many wild relatives of economically important and cultivated plants. We could attribute the rich biodiversity coupled with higher endemism to the geographical isolation with the semiarid Deccan Plateau to the east and the Arabian Sea to the west, geological and topographical characteristics, and humid tropical climate.

The forests of the WGs represent one of the best non-equatorial tropical forests in the country (Pascal 1988). The mountains in WGs can be considered as the water tank of south India as several rivers originate here other than the three major rivers flowing eastward, along with its tributaries, and joining the Bay of Bengal, all other rivers run westward, joining the Arabian Sea (Dikshit 2001; Radhakrishna 2001; Ramachandra et al. 2016a). These rivers are very important reserves for irrigation, electricity and drinking water in peninsular India (Chandran et al. 2010).

2.1 Ecosystem Diversity

The WGs encompasses different vegetation types: forest types such as tropical evergreen, semi-evergreen, moist deciduous and dry deciduous, scrub jungles, and grasslands at low altitudes, as well as montane grasslands, and unique sholas embracing evergreen forest patches interspersed among montane grasslands. The evergreen forest montane grassland matrix form a distinctive vegetation mosaic (Thomas and Palmer 2007). The region keeps its rich diversity due to its complex topography and varying abiotic conditions, particularly high rainfall. A recently conducted study (Reddy et al. 2015), following a hybrid classification approach that followed the ecological rule bases of Champion and Seth (1968), quantified six main forest types in the WGs (Table 1). The WGs are also home to Myristica swamps,

Table 1 Forest types in the Western Ghats [source: (Reddy et al. 2015)]

Sl No.	Forest type	Area (km^2)
1	Tropical wet evergreen forest	18,821
2	Tropical semi-evergreen forest	21,678
3	Tropical moist deciduous Forest	15,235
4	Tropical dry deciduous forest	5002
5	Subtropical broadleaved hill forest	139
6	Montane wet temperate forest	636

highly endangered fragmented ecosystems restricted to small patches with a high watershed value (Senthilkumar et al. 2014).

2.2 Species Diversity

The Western Ghats is home to over 7402 flowering plant species, 1814 non-flowering plants, 139 mammalian species, 179 amphibian species, 508 avian fauna, 320 freshwater fish species and 6000 insect species; the probability of many undiscovered species occurring in the WGs is high, considering the reports of new species from this region every year. More than 325 globally threatened species occur in the WGs (Dahanukar et al. 2004; Dahanukar and Raghavan 2013; Myers et al. 2000; Nayar et al. 2014). 2253 flowering plant species occurring here are endemic to India, of which 1273 species are only confined to the WGs. Apart from these, some taxa of doubtful occurrence are also recorded, raising the total to 8080 taxa of flowering plants (Nayar et al. 2014).

Even though the list is still incomplete, of the 139 mammal species recorded, 16 are endemic, and 32 are threatened. The Malabar large spotted civet, which is critically endangered; lion-tailed macaque, Nilgiri Tahr, Bengal tiger and Indian elephants, which are considered endangered and the vulnerable Nilgiri langur, Indian leopard, and gaur are some of the threatened species found in this region (Malviya et al. 2011; Singh and Kaumanns 2005). The region has a good gathering of large mammals—roughly 30% of the global population of the Asian elephant (*Elephas maximus*) and 17% of the population of tigers (*Panthera tigris*) occur here (WWF-India n.d.). Several endemic reptile genera occur here. The major population of the Uropeltidae (snake family) is restricted to the region (Greene and Mcdiarmid 2005). The amphibians of the WGs are distinct, with over 80% endemic to the rainforests (Daniels 1992; Greene and Mcdiarmid 2005; Radhakrishnan et al. 2007; Vasudevan et al. 2001). Ninety-seven freshwater fish species from the WGs were threatened, including 12 critically endangered, 54 endangered and 31 vulnerable (Molur et al. 2011). Sixteen species of avian fauna are endemic to the WGs (Pande n.d.). There are roughly 6000 insect species: 334 butterfly species, 174 odonate species (67 damselflies and 107 dragonflies), of which 69 are endemic (Mathew and Binoy 2015; Mathew and Mahesh Kumar 2015; Molur et al. 2011). About 258 gastropods species 77 freshwater mollusc species (25 bivalves and 52 gastropods) have been

documented from the WGs, but the actual number is likely higher (Madhyastha et al. 2015; Molur et al. 2011). The WGs is also well known for their macro and micro-fungal, algal, bryophyte and pteridophyte diversity.

3 Ecosystem Services

The concept of ecosystem services, which first appeared in the 1980s, is becoming ever more influential (Chaudhary et al. 2015b; Go'mez-Baggethun et al. 2010). Millennium Ecosystem Assessment (MEA 2005) defines Ecosystem services as 'the benefits ecosystems provide to human well-being'. The allied terms for this are' environmental services' or 'ecological services'; however, 'ecosystem services' remains the most frequent term in scientific publications (Abson et al. 2014). The concept's implications are swiftly growing as researchers, policymakers, and managers delve into the benefits ecosystems provide for people (Haines-Young and Potschi 2009).

While the importance of protecting mountain ecosystems is widely accepted (Xu et al. 2019), traditional conservation methods have become a subject of debate, and the idea of ecosystem services has risen to prominence (Chaudhary et al. 2015a, b; Naidoo et al. 2008; Singh 2002). Studies on ecosystem services have grown exponentially over the past two decades, and the idea has been debated and integrated into many decision-making processes (Chaudhary et al. 2015a, b; Díaz et al. 2015).

An analysis of the effect of human actions on ecosystems and human well-being by the United Nations have identified major groups of ecosystem services: provisioning, regulating, cultural and supporting services (see also Fig. 2) (MEA 2005).

(a) **Provisioning Services:** This includes any benefit to people that can be drawn from nature. This includes food, drinking water, wood fuel, timber, natural gas, oils, clothes and other materials from plants and sources of medicines.
(b) **Regulating Services:** The benefits from ecosystem processes that regulate natural phenomena, viz., water purification, carbon storage and climate regulation, pollination, decomposition, erosion and flood control. Other indirect benefits provided by forest ecosystems are gas regulation, soil retention, groundwater recharge, waste treatment, refugium function, nursery function etc. (de Groot et al. 2002). Pollination is an important ecological service, and the pollinators living in the forest (such as bees) contribute substantially to the production of a broad range of crops (Costanza et al. 1997).
(c) **Cultural Services:** Benefits that are non-material and contributes to the cultural advancement and development of people, comprising the ecosystem's role in the cultures at local, national, and global scales. The edifice of knowledge and dissemination of ideas; creativity developed from exchanges with nature (architecture, art, music); and recreation are also grouped as cultural services.

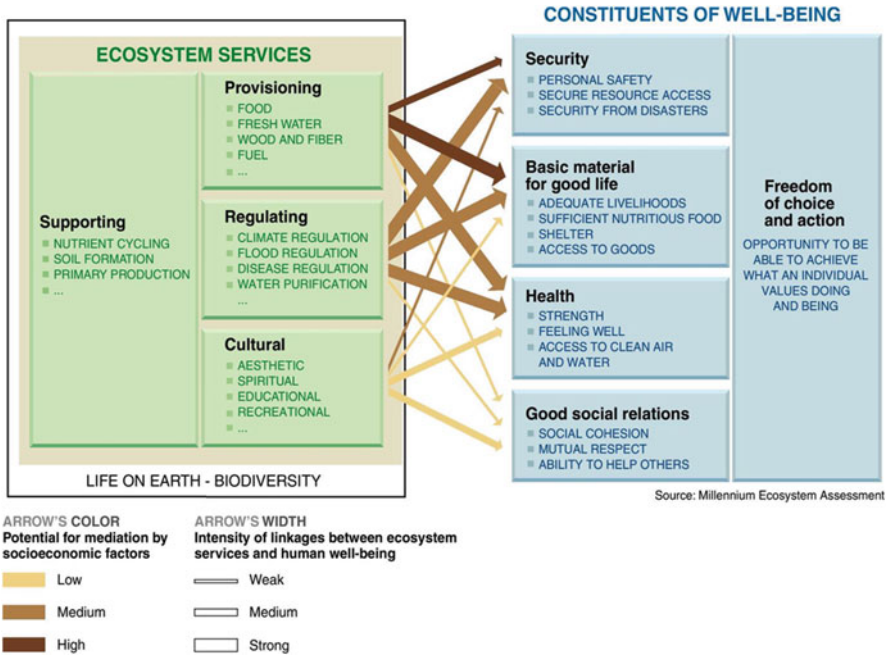

Fig. 2 Links between Human Well-being and Ecosystem Services. The figure shows the linkages and their strength between ecosystem services and human well-being. (Source: MEA 2005)

(d) **Supporting Services:** There are some most fundamental services that nature provides and overlooked. Without these underlying natural processes, ecosystems themselves couldn't prolong; this includes nutrient cycling, soil formation, water cycle and photosynthesis. Such processes sustain basic life forms, and without these, other ecosystem services cannot exist.

The natural ecosystems along with the species in them will support and fulfil the requirements of the human through the ecosystem goods and services. Ecosystems can be thus considered the natural capital assets maintaining and providing services precious to the sustenance of humanity (Daily and Matson 2008; Gunderson et al. 2016; MEA 2003; Newcome et al. 2005; Ramachandra et al. 2017). More than any other terrestrial biomes, forests, especially tropical forests, impact climate and biodiversity-related processes more (Nasi et al. 2002). From the perspective of the biological diversity and ecological functions, services provided by forest ecosystems are of great economic value making these critically valuable habitats (OECD 2002; Ramachandra et al. 2011; Ramachandra et al. 2016b). While being a foundation of ecosystem services vital for the well-being of humans, these ecosystems also affect Earth's biogeochemical systems (Gonzalez et al. 2005; Villegas-Palacio et al. 2016).

The WGs perform important watershed and hydrological functions. Most of the water supply for approx. 245 million people living in peninsular India are through the rivers originating from the WGs. Except for the Indo-Malayan region, none of

the other biodiversity hotspots affects the lives of such a large population. As per the Millennium Ecosystem Assessment (MEA 2005), an analysis of the effects of the different drivers on biodiversity in the various ecosystem in the past 50–100 years showed that Habitat change has a very high impact, followed by over-exploitation with an increasingly high impact. Climate change, invasive species, and pollution also show an increasing impact on change in Biodiversity and Ecosystems (CWG). The case is the same with the WG too. However, these are providing the stakeholders of this region prospect of closer cooperation to build sound schemes for handling the continual flow of ecosystem services. A shift from a species-centric approach to a landscape and ecosystem approach, with action beyond conventional methodologies, is needed for managing these life-sustaining systems.

There have been some earlier studies to assess the ecosystem values of the forest in the Western Ghats (Murthy et al. 2005; Ramana and Patil 2008), carbon sequestration potential (Chandran et al. 2010; Lal and Singh 2000), ecotourism benefit (Anitha and Muraleedharan 2006; Gera et al. 2008; Manoharan 1996; Mohandas and Remadevi 2011; Panchamukhi et al. 2008). The table below (Table 2) shows the trends in the dependency on ecosystem services along the Western Ghats.

4 Conservation and Management Practices

Much of the original forest extent of Biodiversity Hotspots is lost, when retaining forest cover in these areas is essential as they support many endemic and threatened species (Krishnadas et al. 2018), regardless of a slowdown of trends of deforestation in tropical areas over the past (Butler and Laurance 2008; Wright and Muller-Landau 2006). Nearly half of Earth's biodiversity is held in tropical forests, and millions of people are benefitted from the ecosystem services they provide. The global agreements (CBD; Aichi Biodiversity Targets, Strategic Goals C, Target 11) to curb biodiversity losses and climate change decree that 17% of the forest area should be sustained as biodiversity habitats. There is a need for strong public policies for protection of forest and understanding and curbing the drivers for deforestation to meet these global biodiversity goals (Abood et al. 2015; Kremen et al. 2000; Margono et al. 2014).

Protected areas (PAs) are regarded as cornerstones of conservation efforts; however, changes in land use accelerate habitat loss and fragmentation around these areas affecting the entire ecosystem process within and beyond PAs (Athira et al. 2017; Hansen and DeFries 2007). Anthropogenic pressures in the form of industrialisation, infrastructure development, demographic activities etc., affect the effectiveness of forests and management of protected areas (Dudley et al. 2010). Even though the PAs are not frequently subjected to deforestation, biological invasion and fires may affect them (Hiremath and Sundaram 2005). This being the case, there is no adequate spatial data on the PAs available to assess their conservation effectiveness (Athira et al. 2017).

Table 2 Human Use of Ecosystem Services and its trends along the Western Ghats [Adopted and modified from MEA 2005]

Service	Sub- category	Human Uses	Enhanced or Degraded
Provisioning services			
Food	Crops	↔	↔
	Livestock	↑	↔
	Capture fisheries	↑	↓
	Aquaculture	↑	↑
	Wild plants and animal products	↑	↑
Fibre	Timber	↑	↑
	Cotton hemp, silk	NA	NA
	Wood fuel	↔	↔
Genetic resources		↔	↔
Biochemicals, natural medicines and pharmaceuticals		↑	↑
Ornamental resources		↑	↔
Fresh water		↑	↑
Regulating services			
Air quality regulation		↑	↔
Climate regulation	Global	↑	↑
	Regional and local	↑	↓
Water regulation		↑	↓
Erosion regulation		↑	↓
Water purification and waste treatment		↑	↓
Disease regulation		↑	↔
Pest regulation		↑	↔
Pollination		↑	↔
Natural hazard regulation		↑	↓
Cultural services			
Cultural diversity		↑	↑
Spiritual and religious values		↑	↑
Knowledge systems		↑	↑
Education values		↑	↔
Inspiration		↑	↑
Aesthetic values		↑	↓
Social relations		↑	↑
Sense of place		↔	↔
Cultural heritage values		↑	↑
Recreation and ecotourism		↑	↔
Supporting services			
Soil formation		◦	◦
Photosynthesis		◦	◦
Primary production		◦	◦

(continued)

Table 2 (continued)

Service	Sub- category	Human Uses	Enhanced or Degraded
Nutrient cycling		◦	◦
Water cycling		◦	◦

↑ = Increased or enhanced
↓ = Decreased or degraded
↔ = Mixed (trend increases and decreases over past 50 years or some components/regions increase while others decrease)
NA = Not assessed
◦ = These services are not directly used by people

The Western Ghats region includes two Biosphere Reserves. The Nilgiri Biosphere Reserve was established in India as the first Biosphere Reserve in 1986. The region has high species richness and endemism, with species still waiting to be described by researchers (Pandurangan et al. 2012). This Biosphere Reserve is in the confluence of three south Indian states—Kerala, Karnataka and Tamil Nadu. Agasthyamala Biosphere Reserve is in the south end of the WGs and includes a towering peak, 'Agasthyarkoodam' at 1868 m. The reserve hosts one of the most diverse ecosystems in Peninsular India and an important 'Hot Spots' in the WGs, because of its high endemism (Pandurangan and Rai 2012).

4.1 Protected Areas Management

In the Indian mainland, the highest protected area coverage (15%) is for the Western Ghats, with 21 national parks (Table 3) and over 68 sanctuaries. 40% of the area lies beyond any formally protected areas graded as Reserve Forest, and Bandipur National Park is the largest PA in the WGs. The Silent Valley National Park and Kudremukh National Park abode the important virgin tropical forest tracks.

For identifying the zones of conservation significance in the WGs, a considerable amount of work has already been done (Daniels et al. 1991; Gadgil and Meher-Homji 1986; Karanth 1986, 1992; Nair 1991; Prasad et al. 1998; Ramesh et al. 1997; Rodgers and Panwar 1988; Rodgers et al. 2002; Venkatraman et al. 2002). Das et al. (2006), based on patterns of land use, the density of population and the highest conservation value area in grid level, identified six key areas: Periyar–Agasthyamalai, Anaimalai and Palni hills, Nilgiri–Wayanad area, Kodagu area, Malnad area, Sahyadri–Konkan region. Among these high conservation areas, the study also identified 21 irreplaceable grids for their conservation value (Table 4).

Table 3 List of National Parks in the Western Ghats

Sl. No.	Protected area	Species	State	Year	Area (km^2)
1	Periyar national park	Tiger	Kerala	1982	305
2	Eravikulam national park	Nilgiri Tahr	Kerala	1978	97
3	Silent Valley National Park		Kerala	1984	89.52
4	Mukurthi national park	Nilgiri Tahr	Tamil Nadu	1990	78.46
5	Kudremukh national park	Lion-tailed macaque	Karnataka	1987	600.32
6	Pambadum shola national park		Kerala	2003	1.32
7	Indira Gandhi national park		Tamil Nadu	1989	117.1
8	Chandoli national park	Tiger	Maharashtra	2004	317.7
9	Anamudi shola national park		Kerala	2003	7.5
10	Mathikettan shola national park		Kerala	2003	12.82
11	Bandipur national park	Tiger	Karnataka	1974	874.20
12	Nagarahole national park		Karnataka	1974	642.4
18	Anshi national park		Karnataka	1987	417.34
19	Mudumalai national park		Tamil Nadu	1990	103.23
20	Mollem national park		Goa	1992	107
21	Bansda national park		Gujarat	1990	23.99

4.2 Flagship and Keystone Species Conservation

Conservationists increasingly use flagship species to encourage community engagement in conservation actions (Bowen-Jones and Entwistle 2002; Dietz et al. 1994; Entwistle and Stephenson 2000; Leader-Williams and Dublin 2000; Smith and Sutton 2008; Walpole and Leader-Williams 2002; White et al. 1997; Zhi et al. 2000). Flagship species is a popular, "cute," enigmatic animal/plant used as a sign to rouse public attention for the species and its habitat and foster clearer ecological and economic values of conservation. A species is considered flagship: (1) when it is endemic to a particular area and not known beyond that region; (2) has economic relevance as part of a culture; (3) can serve as umbrella species; and (4) has a population decline (Bowen-Jones and Entwistle 2002; Caro and O'Doherty 1999; Entwistle and Stephenson 2000; Simberloff 1998; Walpole and Leader-Williams 2002). The success of conservation actions in China for the Giant Panda (Zhi et al. 2000) and in Brazil for Tamarins (Dietz et al. 1994) demonstrate that international and local public support for conservation of species and habitat rise if a flagship species is present (Caro and O'Doherty 1999; Dietz et al. 1994; Ginsberg 2001; Myers Jr. and Saunders 2002; Zhi et al. 2000). It necessarily not be the case that a flagship species will be an indicator or keystone species.

The species which helps to define an entire ecosystem is generally known as a keystone species. In the absence of which, there would be a dramatic difference in

Table 4 Irreplaceable grids in the Western Ghats with high conservation value. [adopted from Das et al. (2006)]

Sl No.	Survey of India (1: 25,000 grid number)	Corresponding site (s)	Irreplaceable species in this region
1	58 H/7/ NE	Virapuli RF	*Homalium jainii* (EN)
2	58 H/6/ NW	Kalakkad Mundanthurai TR	*Cinnamomum walaiwarense* (CR), *Eugenia singampattiana* (CR)
3	58 H/6/ SE	Kalakkad Mundanthurai TR	*Symplocos pulchra* (EN)
4	58 H/5/ NW	Kuttalam RF	*Litsea nigrescens* (EN), *Nothopegia aureo-fulva* (CR)
5	58 H/1/ NE	Puliyarai RF and Kuttalam RF	*Drypetes travancorica* (EN)
6	58 H/1/ NW	Kulathupuzha RF–Thenmala range	*Canthium pergracilis* (EN)
7	58 G/6/ SW	Periyar TR	*Syzygium chavaran* (EN)
8	58 F/8/ NE	Amburuvi RFand Shingalvariyar RF–Periyakulam range	*Elaeocarpus blascoi* (EN)
9	58 F/4/ SW	Munnar area	*Philautus chalazodes* (CR), *Philautus griet* (CR)
10	58 B/9/ NE	Palghat division	*Syzygium palghatense* (CR)
11	58 A/8/ NE	Souhern old Amarambalam FR, northern Silent Valley NP, southwestern Mukuruthi WLS, northern Attappadi RF	*Actinodaphne lanata* (CR), *Ilex gardneriana*, *Glochidion sisparense* (EN), *Microtropis densiflora* (EN)
12	58 A/7/ NE	Low elevation evergreen forest in Gudalur taluk –west of Naduvattam RF	*Atuna indica* (EN), *Pittosporum virudulum* (CR)
13	58 A/11/ NW	Naduvattam RF	*Fejevarya murthii* (CR)
14	58 A/2/ SW	Chedaleth FR, Kozhikode FD	*Cynometra beddomei*, *Eugenia argentea*
15	48 P/10/ SW	Kilarmale RF and Sampaji RF	*Cinnamomum heyneanum*, *Hopea Jacobi* (CR)
16	48 O/3/ SW	Kudremukh NP	*Hopea canarensis*
17	48 O/14/ SW	Bababudan hills	*Croton lawianus* (CR)
18	48 J/14/ SW	Siddapur FR–RF no. 63	*Syzygium utilis*
19	48 I/6/ SW	Barpede cave	*Otomops wroughtonii* (CR)

(continued)

Table 4 (continued)

Sl No.	Survey of India (1: 25,000 grid number)	Corresponding site (s)	Irreplaceable species in this region
20	48 I/1/SE	Degraded semi-evergreen forests in Kalasgade RF and Parle RF, Changadh range and NE of Dodamarg	*Cinnamomum goaense*, *Nothopegia castanaefolia* (CR)
21	47 F/8/ NE	Sinhagad RF	*Millardia kondana* (EN)

RF Reserve Forest; *FR* Forest Range; *FD* Forest Division; *WLS* Wildlife Sanctuary; *TR* Tiger Reserve; *NP* National Park

the ecosystem, or it may cease to exist altogether. The functional redundancy of keystone species is quite low, implying that no other species could fill its ecological niche if they disappear from the ecosystem. Example: *Cullenia exarillata*, *Ficus* spp., top predators like Bengal Tiger (*Panthera tigris*), etc. An indicator species, sometimes called sentinel species, describes an organism that is extremely sensitive to environmental changes in its ecosystem, and can provide early warning on the health of a habitat.

Some flagship species observed in the Western Ghats are: the Bengal tiger (*Panthera tigris*), Indian elephant (*Elephas maximus indicus*), Asiatic Wild Dog (*Cuon alpinus*), Indian leopard (*Panthera pardus fusca*), Lion-tailed macaque (*Macaca silenus*), Nilgiri Langur (*Semnopithecus johnii*), Nilgiri tahr (*Nilgiritragus hylocrius*), Malabar large-spotted civet (*Viverra civettina*), Hump-backed Mahseer (*Tor remadevii*), cane turtle (*Vijayachelys sylvatica*), Malabar pit viper (*Trimeresurus malabaricus*), purple frog (*Nasikabatrachus sahyadrensis*), Nilgiri laughing thrush (*Montecincla cachinnans*), Banasura laughing thrush (*Montecincla jerdoni*), Nilgiri wood pigeon (*Columba elphinstonii*), white-bellied blue robin (*Sholicola albiventris*), Nilgiri pipit (*Anthus nilghiriensis*), Nilgiri flycatcher (*Eumyias albicaudatus*), Malabar grey hornbill (*Ocyceros griseus*), Malabar parakeet (*Psittacula columboides*), white-bellied blue flycatcher (*Cyornis pallidipes*), grey-headed bulbul (*Brachypodius priocephalus*), rufous babbler (*Argya subrufa*), small sunbird (*Leptocoma minima*) etc.

4.3 Conservation through Traditional Knowledge

The social systems, as well as the ecosystems, are intricate and adaptive (Marten 2001). In traditional societies, the management of natural resources sustainably is inspired by their intricate connections with nature and the beliefs and culture of the communities. The overall goal of species conservation, along with conservation of genetic diversity and ecosystems, has been well achieved by the unification of

traditional, religious, and cultural knowledge systems (Anthwal et al. 2010; Gadgil et al. 1993; Negi et al. 2018; Rist et al. 2003; Salick et al. 2007). Worshipping nature and managing it has always been a major influence in formulating human mindsets about sacred conservation and utilisation of natural resources sustainably (Negi et al. 2018). Traditional knowledge and 'folk tradition' inherited by generations of the local communities associated with the use of natural resources played a major part in the health systems (Bhatia et al. 2015; Maikhuri et al. 1998; Nautiyal et al. 2000; Simbo 2010; Uprety et al. 2012; Kala 2005b; Malik et al. 2015; Samant et al. 1998).

Small patches of forests known as Sacred groves are classic examples of community-based, culturally sensitive management of natural resources (Ormsby and Bhagwat 2010; Rath and Ormsby 2020). The Western Ghats' biodiversity-rich regions are recognised for the presence of many sacred forests (Bhagwat et al. 2005b; Chandrakanth et al. 2004; Chandrakanth and Nagaraja 1997; Chandran and Hughes 1997; Chandrashekhara and Sankar 1998; Kalam 1996; Kushalappa and Bhagwat 2001; Ormsby and Ismail 2015; Ormsby 2013).

The sacred areas considered divine for various gods and goddesses representing not only species but also land, water, and air act as *de facto* protected areas apart from their materialistic uses (Brandt et al. 2015). All religions teach to live in harmony with nature respecting every element (Dafni et al. 2005; Gowda 2006; Jain and Kapoor 2007; Kala 2005a, b, 2006, 2011; Kamla et al. 2006). However, these religious rules and modes of nature conservation have often been overlooked, undervalued, or deliberately falsified with time (Avise et al. 2008; Boyd 1984). The exponential increase of anthropogenic stresses and the growing disconnection from nature owing to urbanisation result in severe loss of biodiversity and environmental degradation (Barlow et al. 2012; Lawton and May 1996; Miller 2005).

Most of the tribal communities in India are nature worshippers. The hill tribes account for hardly 5% of the area population in the Western Ghats but are key players in the conservation through their traditional knowledge. They are used to associate useful species, forests, rocks, rivers, mountains with local deities, and prohibit the exploitation of certain plants and animals and are used in various indigenous systems of medicine. The significance of sacred species, landscapes, and sacred groves must not be seen simply in the livelihood and economic prospects; these must be recognised as the historical indication of a human bond with nature and its elements. More than a nature reserve, the sacred grove is an important part of the lives of local communities or the janitors (Appfel-Marglin and Mishra 1993; Bhakat 1990; Hasnain 1992; Hembram 1983; Pathak 2009; Rath and John 2018; Ray 2002; Roy Burman 1982).

Traditional Ecological Knowledge (TEK) systems are imbued with practices and concepts passed on through generations that can be associated directly and indirectly with the management of resources and conservation at different scales. The concept of Totemism, an aspect of religious belief cantered upon the veneration of sacred objects called totems, is prevalent among some tribal communities. A totem is an object or symbol representing a plant or animal or any being that acts as a motif of a subset of people, such as a lineage, clan, family, or tribe, as a reminder of their lineage (or past mythology). This plays a significant role in the internal structure of

the tribal communities. Hundreds of sacred landscapes and groves are present in the WGs region as a testament to society's commitment to conservation.

4.4 The Landscape Approach in Conservation

This forms a framework to combine policy and practice for various land uses, in an area, to safeguard equitable and sustainable use of land while improving ways to alleviate and become accustomed to climate change (Reed et al. 2015). This is more of an effort in the best interest of the environment and human well-being by balancing the land use needs. It involves designing solutions that consider livelihoods, sustenance, rights, finance, restoration and progress towards development and climate goals (GLF n.d.; Harvey et al. 2014; Milder et al. 2010; Reed et al. 2015; Sayer et al. 2013; Scherr et al. 2012).

There has been an intentional and unintentional anthropogenic influence on the landscapes by continuously modifying them to fulfil people's purposes who live in and use them (Ellis and Ramankutty 2008). To respond to the predisposition of coping with landscape characteristics in disciplinary silos, the term' landscape approach' has been widely used in the last two decades (Sayer et al. 2017). Central elements of the landscape approach are improving governance, Capacity building, local empowerment, and resource management negotiations transparently (Buck et al. 2006; Milder et al. 2014; Pfund 2010; Smith et al. 2009).

A 'landscape' can either be ecological or spatial characteristics which help define targets of development and conservation, or it can refer to those which minimise development trade-offs and conservation governance and other social interactions (Redford et al. 2003; Reed et al. 2015). An approach centred only on an assessment of ecosystem services does not take into account the dynamics of landscape transformation. Such dynamics are primarily driven by socioeconomic constraints and need to be incorporated into conservation strategies for maximum effectiveness (Ayensu et al. 1999; Nagendra 2001; Warren et al. 1997). Maintaining and restoring the integrity of ecosystems at the landscape level is essential to support viable populations of a species and to maintain critical ecosystem functions.

Very limited efforts/actions are taken in the WGs region for landscape-level conservation activities apart from studies on the sacred groves, coffee plantations and forest areas in Kodagu (Bhagwat et al. 2005a) or Muthikulam in Kerala (Sreekumar et al. 2013). A study in Karnataka has established that management at the landscape-level is crucial for alleviating human-elephant conflicts (Ramanan 2019). In the Periyar–Agasthyamala landscape of the southern Western Ghats, studies on tiger (*Panthera tigris*) and elephant (*Elephas maximus*) (Gangadharan et al. 2011) showed the need for ecosystem connectivity for large mammal conservation and to alleviate the adverse effects of inbreeding and demographic stochasticity.

ATREE and CEPF (2013) identified 80 priority locations and 53 critical links clustered in five landscape-level conservation corridors: Periyar-Agasthyamalai,

Anamalai, Mysore-Nilgiri, Malnad-Kodagu, and Sahyadri-Konkan, to improve ecological connectivity at the landscape level. These are crucial to sustaining critical ecosystem functions and viable wildlife populations. With the support of local stakeholders, strategies were established to secure seven critical Asian elephant habitat corridors in the Mysore-Nilgiri corridor by the Wildlife Trust of India (WTI).

There are initiatives in the Vazhachal Forest Division and Parambikulam Tiger Reserve for the protection of Hornbills and trees in which they nest by participating in the Kadar tribal group. In the Mysore-Nilgiri and Anamalai corridors, Rainforest Alliance and Nature Conservation Foundation (NCF) have facilitated sustainable ways on tea and coffee estates, providing technical advice to estate managers and creating markets for certified products. FERAL and Rainforest Alliance had an analogous approach with rubber plantations in the Periyar-Agasthyamalai Corridor. There were also efforts in the Malnad-Kodagu Corridor in coffee agroforestry landscapes for native tree conservation.

4.5 Practices in Forest Resources Management through Participatory Approach

The support of local people determines the success of natural resources management for the long-term in a sustainable way (Heinen and Shrivastava 2009; Infield and Namara 2001; Macura et al. 2011; Mehta and Heinen 2001; Shibia 2010; Sudha et al. 2006; Triguero-Mas et al. 2010). Participatory Forest Management gave a significant breakthrough in India in relations between the local communities and Forest Department and is currently a key driver in managing forests (Mukerji 2006). The fate of participatory forest management programmes mostly depends on the communities' attitude to the protected area staff and their perceptions towards the management practices (Allendorf 2007; Ormsby and Kaplin 2005). For example, rules prohibiting access to forest resources, tensions with protected area managers because of resource extraction, limited or no job provisions, fear of resettlement (Allendorf 2007; Heinen and Shrivastava 2009; Shibia 2010), the behaviour of staff (Ormsby and Kaplin 2005; Infield and Namara 2001) create a pessimistic approach towards protected areas.

The concept of participatory management was introduced in the National Forest Policy to deescalate the evolving conflicts among several actors over forest use rights (Rishi 2007). Different models are employed in the WGs as part of Participatory Forest Management like: Enrichment of the natural forest and gap planting, small scale industry and bamboo and cane plantations, multipurpose forestry management based on local needs, management of ecologically sensitive, archaeologically important areas, natural regeneration, trench mound cum pit plantation, teak plantation, irrigated fruit orchard, foreshore plantation, and strip plantation (roadside, railway line side and canal slide). As an option for degraded forests conservation and restoration, and sustainable use of tropical forests, participatory forest management

can be considered viable (Balaji 2003; Murthy et al. 2004; Singh 2000). An earlier study (TERI 1999) showed an improvement in regeneration in 40% of the sites as a result of efforts on the part of the community.

4.6 Biodiversity Conservation through Policies and Legislations at the International and National Level

Biodiversity loss at an extensive rate over the past has affected both developed and developing countries. Species extinctions, over-exploitation, exotic species, loss of habitats, climate change and pollution, and associated biodiversity loss most affect the marginalised communities. India has signed various key international conventions concerning the conservation and management of wildlife and is also a party to the treaty of Convention on Biological Diversity (CBD). The constitution of India [e.g., Article 48A and 51A (g)] cherishes the concept of biodiversity conservation, and there are different laws in place to govern the environment. The important global policies, National policies and laws are detailed in Table 5.

5 Conclusion

The Western Ghats is the source of several major river systems. The lush biodiversity and distinct ecosystems perform a key role in supporting the well-being of about 50 million people. The provisioning services provided by the diverse ecosystems include drinking water, timber, wood fuel, plants that provide raw materials for clothes, lifestyle products and a source of medicines. Various indirect benefits in the form of soil retention, groundwater recharge, gas regulation, climate regulation and carbon storage are also provided by the forest ecosystems. We also know the Western Ghats for its cultural and other supporting services. However, these are primed for significant shifts in the present scenario of pressures, largely because of changing climate and land-use changes.

The integrity of ecosystems is threatened by linear intrusions, for example as road networks, power lines, etc., and it is essential to assess the effects of these intrusions on ecological connectivity and evolve mitigation measures. We need productive partnerships between government officials and conservationists in complementary roles in the planning and execution of conservation actions. Awareness-raising is required to conserve the Western Ghats ecology in the current climate change scenario and empower and equip decision-makers with comprehensive information and knowledge. Cooperation between different stakeholders, governments, and citizens is vital for protecting the Western Ghats region. The current policies and their execution still do not signify the assimilation essential across diverse sectors to address various challenges. It is critical to have more financing in the Western Ghats

Table 5 Key Global policies, National policies and National acts associated with biodiversity conservation

Global Policies	
Sustainable Development Goals, 2015	Millennium Development Goals, 2000
UNFCCC: Paris Agreement, 2015	International Geosphere and Biosphere Programme, 1997
CBD: Strategic Plan 2010–2020 with Aichi targets, 2010	Convention on Biological Diversity, 1992
IPBES established, 2010	Convention on the Conservation of Migratory Species of Wild Animals, 1979
Programme of Work on Mountain Biodiversity, 2004	Convention on international trade in endangered species of wild Flora and Fauna (CITES), 1975
Programme of Work on Protected Area, 2004	World Heritage Convention, 1972
MEA report for policy makers launched, 2005	Ramsar Convention, 1971
International Treaty on Plant Genetic Resources for Food and Agriculture, 2004	International plant protection convention (IPPC), 1951
MEA framework launched, 2003	International Convention for Protection of Birds, 1950
Earth Summit, 2002	
Millennium ecosystem assessment launched in 2001	
Key National Policies	
The Wildlife Action Plan, 2002–2016	
National Action Plan on Climate Change, 2008	
National Biodiversity Strategy and Action Plan, 2009	
Forest Policy, 1952	
National Forest Policy, 1988	
National Conservation Strategy and Policy Statement, 1992	
Key National Laws	
Scheduled Tribes and Other Traditional Forest Dwellers (Recognition of Forest Rights) Act, 2006	Wildlife protection act (amendment act), 1991
Biological Diversity Rules, 2004	Environment Protected Act, 1986
Wildlife protection act (amendment act), 2002	Forest (Conservation) Act, 1980
Biological Diversity Act, 2002	Wildlife Protection Act, 1972
The Geographical Indications of Goods Act, 1999	Indian Forest act amendments, 1980
Panchayati Raj Act, 1996	Prevention of Cruelty to Animals Act, 1960
Foreign Trade (Development and Regulation) Act, 1992	Mining and Mineral Development (Regulation) Act,1957
	Indian Forest Act, 1927

region to secure greater economic growth and human well-being goals, ensuring the sustainable use of assets.

A spatially explicit approach is crucial to highlight the ecosystems for biodiversity conservation. Protecting the ecology of the Western Ghats, considering it at a landscape level, is of critical significance because it would save numerous endemic species from becoming extinct and counteract the calamities experienced in this region in recent years.

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Origin and Evolution of Peninsular India, Western Ghats, and its Diverse Life Forms

Pankajakshan P and Richard Scaria

1 Introduction

Western ghats is a chain of precipitous mountain range lying parallel to the western coast of India for a length of 1600 km and is a biodiversity hotspot with high endemicity. The ghats running North to South through the states of Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu, and Kerala cut across the Deccan Basalts and the Precambrian Peninsular shield. It is interrupted only by a gap stretching for a length of the 32 km, known as the Palghat Gap at 10° 45' North latitude. It is the home to the largest population of tigers and Asian elephants and host a variety of endemic species such as Lion-tailed macaque and Nilgiri Tahr. Apart from the significant diversity in fauna and flora, the ghats also harbours human population, often living adjacent to the forest areas.

The Western Ghats, the source of all peninsular rivers of India, play a crucial role in determining the regional climate. They are oriented perpendicular to the Southwest monsoon winds forcing them to precipitate their moisture on its windward slopes. The copious rainfall thus received, together with the variation in climate due to the altitudinal gradation and its nearness to the equator transformed the Western Ghats into a unique biophysical region. Yet the significance of geology as a determinant of biodiversity cannot be overlooked.

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2 Geological Evolution of the Indian Carton

Throughout the history of the Earth, there has been several incidences of amalgamation and dispersal of continents (Chetty 2017) forming a series of super continents. Worsley et al. (1982) identified five supercontinent episodes prior to Pangaea which include Pannotia, Rodinia, Columbia, and Kenorland (Li et al. 2007). Of these five super continent evolutions only the amalgamation and rifting of the latest event (Pangea) is precisely documented (Roberts and Bally 2012). Amalgamation of Pangaea started with the breakup of Rodinia (Meert 2003) during the Neoproterozoic, (Powell and Li 1994) between 830 and 750 Ma. The crust of Peninsular India, which was part of Gondwana, in the last episode of super continent assembly underwent considerable modifications during these events. The high-grade crustal provinces of peninsular India such as the Southern Granulite Terrain (Braun and Kriegsman 2003) and the Eastern Ghats Belt (Dobmeier and Raith 2002) were mainly affected during these events (Radhakrishna and Vasudev 1977).

According to Shiraishi et al. (1994) the granulite facies metamorphism, that occurred during 610–550 Ma and 550–520 Ma, has intensely deformed the Southwestern Complex of Sri Lanka and the Lutzow-Holm Complex of Antarctica respectively. These two terranes which were in two different continents now, were part of continuous orogenic belt at that time. Radhakrishna and Mathew (1996) suggest that the Mozambique orogenic belt, which was one of the many Pan-African orogenic belts was formed by the convergence of Peninsular Indian Shield and the African shield during the amalgamation of Gondwana. This is supported by the correlation between the Mozambique Belt in Africa and the Central Highland/Southwestern Complex in Sri Lanka. According to Fitzsimons (2003), the Gondwanaland position of India along the western margin of Australia was achieved by about 550 Ma.

The disintegration of Gondwana, which was made up of continents like Africa, Antarctica, Australia, India together with Madagascar, and South America occurred between Late Triassic and Early Jurassic period from 225 to 175 Ma. (Rogers and Santosh 2004). By around 165 Ma the combined landmasses of Madagascar, India, and Antarctica started drifting towards the north (Coffin and Rabinowitz 1988). The separation of the combined landmass of India and Madagascar from Antarctica was the first to occur. This rift was initiated by the Kerguelen plume during the early Cretaceous, about 140 Ma (Baksi et al. 1987; Ghatak and Basu 2011). The eastern part of the Indian Subcontinent experienced extensive mantle plume-related volcanism during the drifting of Antarctica and the resulting formation of the Bay of Bengal (Roy and Purohit 2018). The combined landmass of India and Madagascar was separated from Africa during 130 to 120 Ma (Reeves and de Wit 2000). Storey (1995) suggests that the Marion plume outburst, that occurred during 88–99 Ma separated Madagascar from India. Kumar et al. (2001) points out that the evidence of the separation of Madagascar and India can be traced in different parts of Karnataka coast, central and northern Kerala, and even in Madagascar. The formation of the present day western Indian margin took place during the Cretaceous i.e., around

66 Ma, because of Reunion mantle plume. This mantle plume event resulted in the breakup of Seychelles and India (McKenzie and Sclater 1971). This breakup is one of the reasons that increased the height of the Western Ghats. Roy and Purohit (2018) suggest that the Western Ghats attained its present-day prominence after the tilting of the Peninsular India to its southeast.

Another event of notable scale that occurred during the Reunion mantle plume event was the formation of Deccan traps (Norton and Sclater 1979; Hooper 1990), The Deccan flood basalt province is a thick sequence of horizontal basalt flows covering almost 500,000 km^2. According to Duncan and Pyle (1988) and Courtillot et al. (1988), the radiometric ages of Deccan volcanism is roughly between 65 and 69 Ma. Based on magneto stratigraphic and absolute age data, Jaeger et al. (1989), believed that the Deccan basalts were deposited at a rate of 0.5 meter per year at around 66 Ma. The present-day Arabian Sea is formed by the rapid sea floor spreading that followed this Deccan volcanic event (Courtillot et al. 1986; Miles and Roest 1993).

3 Geological Evolution of Western Ghats

Gunnell and Harbor (2008) divide the Western Ghats escarpment, into three structural segments: the Deccan traps of Maharashtra, Dharwar craton of Karnataka and the southern granulite terrane of Kerala. The west facing slope of the ghats is steep and is characterized by a multiple precipitous escarpment. These landing stair like escarpment feature is known as the western ghat. Valdiya (2016) points out that the mountain range is known as Sahyadri, and Western Ghats is its escarpment.

The Western Ghats is made up of different rock types. The rock types include the Deccan Basalts of the northern part, the Precambrian migmatite gneisses and volcano-sedimentary sequence of the Dharwar in the middle and the high-grade granulite gneisses to the south. Thus, as pointed out by Radhakrishna (1965) and Ollier and Powar (1985) that though the lithology and the ages of rocks are noticeably different, their geomorphic expression is similar. Subrahmanya (1998) suggest that the Western Ghats had originated much earlier than the Deccan traps.

The origin and evolution of Western Ghats and other passive margins of the world were one among the most widely studied topic in Geology. Numerous models have been proposed in connection with their origin and evolution. These models have been grouped into five categories. They are;

1. Models that discuss the origin of the great escarpments similar to the Western Ghats,
   elsewhere in the world.
2. Models that discusses the evolution of plateaus elsewhere in the world.
3. Models that discusses the origin of post-rift escarpments.
4. Models that discuss the origin of Western Ghats specifically.
5. Hybrid multistage models on the evolution of the Western Ghats.

The models that discuss the origin of the great escarpments similar to the Western Ghats, elsewhere in the world consists of the following.

Rift-related crustal thinning by McKenzie (1978)—In his simple model on the evolution of sedimentary basins McKenzie proposed the thinning of continental lithosphere due to its rifting and breakup, which results in its stretching. This produces an upwelling of hot asthenosphere and is associated with block faulting and subsidence. Due to the heat conduction to the surface, the lithosphere initially thickens, and subsides slowly when it cools.

Magmatic under-plating by Cox (1980)—This model proposes that the magma that form the continental flood basalt provinces formed during the Mesozoic-Tertiary periods in areas such as Deccan, Parana, Karroo, Antarctica, and Siberian Platform reach the surface in areas of crustal thinning as a series of sills. This model infers that in areas of flood vulcanism a potentially large contribution to the crust is made by under-plating of magma.

Thermal event aided by mantle convection model by Cochran (1983) suggests that the tectonic subsidence of continental margins was the result of thermal contraction following heating of the lithosphere. It is the horizontal temperature gradient due the lithospheric thinning that controls the subsidence rates and the stratigraphy of the crust.

Stretching and flexural unloading due to secondary convection by Buck (1986). According to this model the rift shoulders as well as the areas adjacent to rifts are often uplifted. In areas of passive rifting, the magnitude of the uplift of the rift shoulders is not only the result of heat transfer between the rift and the rift flanks through conduction, but also due to the small-scale convection set off in the mantle beneath a rift.

Unloading of the lithosphere by mechanical process and the resulting Isostatic rebound of the flanks of the rift by Weissel and Karner (1989). This model propose that the uplift of the rift flanks is caused by thermal and dynamic processes. It also considers the magmatic crust thickening as the cause for uplift of the rift flanks. It is hypothesized that lithospheres have finite mechanical strength when stretched due to extension, and that isostatic rebound of the lithosphere occurs as a result of the development of surface topographic depressions caused by the stretching and extension of the crust.

Rifting of crust by the thermal and dynamic effects of mantle plumes by White and McKenzie (1989). This model explains that new basaltic materials are added to the earth's crust whenever the rifting of continents takes place due to the thermal effects of mantle plumes. Plume generated magma rises quickly until it is either extruded as basalt flows or intruded into or beneath the crust. The study considers the formation of deccan traps of India as the result of rifting of the crust by the effects of mantle plumes.

Kusznir and Ziegler (1992) proposes the uplift due to the cantilever effect caused by the upward flexure of the lithosphere. This model investigates the possibilities of the lithosphere's geometric, thermal, and flexural isostatic reaction in response to extension generated by faulting of the upper crust and distributed deformation in the

lower crust and mantle. During faulting, the upper crustal foot wall and hanging wall blocks operate as two mutually sustaining flexural cantilevers. The crustal extension caused because of isostatic forces results in the uplift of the foot wall and collapse of the hanging wall. The model also predicts that decompression causes partial melting of the lower lithosphere and upper asthenosphere, resulting in lithosphere extension along the passive border rift.

Van der Beek et al. (1994) postulated lithospheric necking as a result of dynamic stretching resulted in the rift-flank uplift. This study conducted along the rifted margin of blue mountain region in Australia suggest that the migration of river knick zones towards the drainage divide control the increase in the relief. This model suggests that the present-day morphology of the blue mountain region is shaped by both lithological controls, and the early Cenozoic tectonic uplift related to mantle diapirism and magmatic under-plating.

As per the numerical model proposed by Gunnell and Fleitout (2001), the elastic nature of the lithosphere, or the upward bend caused by asymmetric erosional unloading, along with sediment flow, may regulate the uplift of passive continental margins.

The second set of models that deal with the uplift and persistence of western ghats are those that look at the evolution of plateaus such as the Deccan and others throughout the world. In this regard, a variety of models have been presented, which are described below.

Bird (1984) in his displacement of the lower crust model argued that the transit of the ductile lower crust from the SW to the NE by shear stresses exerted by the Farallon plate on the base of the North American lithosphere is the reason for the crustal thickening of the rocky mountain foreland.

Spencer (1996) in his model attributes the Cenozoic uplift of the Colorado Plateau area to thinning of the North American mantle lithosphere occurred due to low-angle subduction, which occurred during Laramide to mid-Tertiary periods.

McQuarrie and Chase found that crustal thickening occurs as a result of deformation and intra crustal pressure flow (2000). This hypothesis was presented to explain how the Colorado Plateau evolved in the United States. It's possible that the Sevier orogenic belt's thickened and overheated hinterland crust caused an intra-crustal flow of heat and pressure, resulting in a thickened crust and isostatically uplifted Colorado Plateau.

The third set of models considers the origin of post-rift escarpments due to rifting and continental breakup processes.

When examining magmatic underplating processes during continental flood basalt vulcanism, Cox (1992) points out that the Karoo province in Southern Africa underwent at least one kilometer of persistent uplift linked with the emplacement of a 5-kilometer-thick under-plated gabbroic layer.

King and Anderson (1995) while discussing large continental igneous provinces found along the margins of lithosphere, propose that small scale convection mantle plumes are induced by the non-uniform nature of cratonic boundary.

Osmundsen and Redfield (2011) suggest that there is a direct link between the gradient of crustal thinning and topography of the passive margins.

The fourth group of models are those that specifically proposes the origin of Western Ghats.

The Western Ghats, according to Ollier and Powar (1985), are a massive escarpment that extends parallel to India's western coast, cutting through Deccan basalts in the north and the metamorphic rocks of the Precambrian Shield in the south.

Watts and Cox (1989) proposed that the load that formed the Deccan traps was compensated by a sequence of cones that migrated at a steady rate over the Indian plate as it drifted north. The traps' current thickness is due to flexural rebound after the cone stresses were removed. Subsidence and uplift linked with the development of the Seychelles coast and Indian passive continental margins, as well as the Arabian sea's oceanic crust, drastically altered the pattern of rebound.

The Western Ghats escarpment, according to White and McKenzie (1989), evolved during a thermal anomaly caused by a mantle plume that separated peninsular India and the Seychelles around 64 Ma. He also pointed out that throughout the geologic time, mantle plumes have played a significant role in expanding the volume of the continental crust.

Bridges (1990) considered western Ghats as a fault scarp extending for 1500 km from Kanyakumari to the Tapi river. According to him the highest portions of Nilgiris and Cardamom hills form a summit plain which is possibly the Gondwana surface found on other parts of the former Gondwana continent.

Subrahmanya (2001) attributed the genesis and evolution of the western Ghats to the Reunion plume impact response. According to him, doming happened around 93 Ma and rifting began around 88 Ma, marking the beginning of India's west coast and thus the Sahyadris. Around 67 Ma, the northern part of the west coast and the Sahyadris witnessed basaltic magmatism linked to the Reunion hotspot. The lava flow created a monocline facing the Arabian Sea, since the west-facing escarpment was already in place before the volcanic event, which is now known as the Western Ghats.

Valdiya (2001) attributes the formation of central Sahyadri mountain to significant vertical motions associated with northward strike-slip displacement along the NNW-SSE faults. The fault-delimited blocks' northerly thrust triggered breaking up at their margins on the ESE-WNW oriented reverse faults and shear zones, giving rise to the Western Ghat escarpment's high mountain ranges. Through headward erosion, the west-flowing rivers significantly modified the ancient escarpment. While a portion of the Dharwar craton was uplifted to build horst mountain, the coastal belt remain at an elevation between 40 to 120 meters above sea level, resulting in an undulating topography of low ridges and shallow depressions.

According to Tiwari et al. (2006), the crust beneath the ghats varies in thickness and is 3–4 km thinner than the crust beneath the coastal plains. The buoyancy forces of the rift-related shallow upper mantle low-velocity zones under the ghats may be responsible for the elevation of the ghats.

In their modelling of the lithosphere's isostatic response to sediment loading offshore and denudation onshore, Campanile et al. (2008) deduce that flexure responses are an important component in the formation of the Western Indian Margin.

As Gunnell and Fleitout (2001) opined, no one geophysical model appears to be sufficient to explain the relief, and continuity of the Western Ghats. The evolution and durability of rifted margin escarpments over geological time has been attributed to the combined effects of erosion, slope recession, and the lithosphere's flexural isostatic response to stretching along rifted margins (Sacek et al. 2012). This is demonstrated by Subrahmanya (1987) and Sinha-hybrid Roy's multistage models of Western Ghats evolution (2018).

Subrahmanya (1987) attributes the western coast of India's genesis to a combination of six factors. These stages begin with a tectonic episode characterized by crustal doming and subsequent rifting, followed by the drifting of the Indian subcontinent, resulting in a first escarpment along India's western edge around 88 Ma. The Deccan magmatism in the northern sections of the Western Ghats followed the split. Isostatic subsidence was caused by the heaping up of Deccan lava, as well as the thermal contraction of the upper mantle owing to cooling that occurred after the lava eruption. This was followed by the Ghats' eastward retreat due to maritime erosion. The development of westerly drainage was preceded by the withdrawal of the sea during the glacial period. Fluvial erosion of the Western Ghats and coastal plain, as well as a slow post-glacial rise in sea level, have resulted in lagoons, estuaries, and drowned valleys.

Sinha-Roy (2018) provides a multistage model of the Western Ghats' evolution and persistence, incorporating a series of overlapping stages linked to offshore tectonics. The Marion hotspot activity, which separated Greater India from Madagascar around 91–88 Ma, is the starting point for this model (Torsvik et al. 2000; Storey et al. 1995). Greater India drifted at a pace of 12 to 20 cm per year after the partition (Norton and Sclater 1979). Seychelles and the Mascarene plateau were divided in reaction to the Reunion hotspot. Between 66 Ma and 64 Ma, the Chagos-Laccadive and Laxmi ridges broke away from Greater India (White and McKenzie 1989). Between 66 and 65 Ma, the Reunion hotspot activity generated the Deccan traps volcanism in its northwestern part (Ramana. et al. 2015). Later, at around 55 Ma, India collided with Asia in the Himalayan orogenic belt, reducing the Indian plate's NNE drift from 16 cm/year to 6 cm/year (Besse and Courtilott 1989). India's rifted and thinned western continental margin functioned as a magnet for low-viscosity mid-crustal material. The thickened, heated, and under-plated mafic lower crust of Western Darwar Carton, Southern Granulite Terrain, and the thinning western continental margin crust created the pressure gradient that triggered the mid-crustal flow. The Western Ghats' channelized crustal flow and low-viscosity crust caused density differences and strain partitioning within the crust, resulting in crustal thickening, and raised topography (Royden 1996; Bott 1999).

Due to the combined influence of the mid-oceanic ridge's push and collisional boundary resistive forces, intraplate stress throughout the Western Continental Margin of the Indian shelf region rose after 33 Ma, with the maximum horizontal compression directed roughly NNE (Muller et al. 2015; Gowd et al. 1996). Studies of subsidence rates at India's offshore Western Continental Margin have revealed a rather rapid rate of post-rift sinking due to thermal relaxation, beginning around 25 Ma (Whiting et al. 1994) and peaking around 7.5 Ma. This period roughly

corresponded to the commencement of the Southwest monsoon at 20 to 15 Ma, which grew stronger at 5 Ma (Clift et al. 2003). The Southwest monsoon increased denudation boosted the sediment supply to offshore basins and caused the Western Ghats escarpment to recede. Because of the increased sediment load, the rate of basin subsidence increased, creating the ideal circumstances for flexural rebound along the continental margin.

Radhakrishna et al. (2019) after investigating a section of stratigraphy within a drill hole located in the Western Ghats together with existing geological and geophysical data, proposes that igneous underplating may be the cause of initial epeirogenic uplift in the Western Ghats.

4 Western Ghats and its Biodiversity

The known inventory of plant and animal species in the Western Ghats demonstrates the importance of the region's biodiversity (Gunawardene et al. 2007). With roughly 40% of the total number of endemic species, the Western Ghats is considered a hotspot (Myers et al. 2000). The Western Ghats represent a distinct biogeographic zone in India, recognized for its biotic richness, and are characterized by a unique blend of geographic, geologic, edaphic, and climatic gradients. Several authors have addressed the geological age, evolutionary history, and biogeographic patterns of the Western Ghats, with a focus on the endemism of the flora and fauna (Blasco 1970; Krishnan 1974; Subramanyam and Nayar 1974; Nayar 1977, 1980a, b; Ahmedullah and Nayar 1986). The area is diverse, hosting over 145 wild relatives of crop plants from 66 genera (Arora and Nayar 1984).

5 Biogeographic History

Wet evergreen taxa would have faced a physiological barrier to occupying the new habitats after the uplift of the Western Ghats. Thus, historical and evolutionary reasons may have influenced the existing latitudinal and altitudinal endemic species richness gradients in the Western Ghats (Carpentier. 2003). Latham and Ricklefs (1993) proposed a similar idea for the flora of northern temperate trees. On the one hand, Laurasian flora is restricted to high elevation places with an altitude greater than 1800 metres (Raven and Axelrod 1974). These species are thought to have migrated from the Himalaya during glacial eras and sought sanctuary in the ghats' highest peaks (Vishnu-Mittre. 1974). Species occurring in low and intermediate elevations with a brief dry season, on the other hand, show a Gondwanan affinity. These species are thought to be either relicts or recently evolved, with the southern half of the ghats serving as a former sanctuary for wet evergreen forest species. Indeed, the onset of monsoon rains throughout the Quaternary (Van Campo 1986), along with the complicated terrain of the mountains, particularly in the southern

section of the Ghats, may have aided the development and survival of new species (Fjeldsa and Lovett 1997). The Ghats' remarkable species variety can be linked to the complex environmental variability that has formed as a result of climate and mountain interactions (Korner 2004). Other elements such as the mountain chain's direction and terrain are also relevant in this regard (Elsen and Tingley 2015).

6 Floristic Diversity

The Western Ghats in India are one of the most important habitats for tropical evergreen forests, with a huge floristic diversity. Many authors, including Gamble and Fischer (1915-1936) and Nayar, have highlighted the region's floristic variety (1996). According to Yoganarasimhan (2000) and Nair and Henry (1983), the ghats support 2100 indigenous blooming plants, accounting for roughly 27% of the total Indian flora. The flora of the Western Ghats is strikingly similar to that of East Africa, Malaysia, and Sri Lanka. When compared to the Himalayan mountains, which are younger, it hosts a great amount of relict or paleoendemic plants. Another aspect of the Western Ghats is the prevalence of monotypic taxa.

The Western Ghats are unique because of its geology, lengthy geological history, altitudinal and latitudinal range, variety of soil types, and other factors. They're also active speciation hotspots.

This significant speciation is due to the altitudinal variety in terrain and the microclimatic zones. In the Western Ghats, Rao (2012) classified the following forest types: (i) Dry scrub vegetation; (ii) Dry deciduous forests; (iii) Moist deciduous forests; (iv) Semi-evergreen forests; (v) Evergreen forests; (vi) Shoals; and (vii) High altitude grasslands. There are various subtypes, formations, or connections within each of these forest types, each with a different floristic makeup.

The ghats have a total of 490 arborescent taxa, with 308 of these being indigenous. The Western Ghats are home to 56 genera and 1500 species of flowering plants. Orchids are famous among plant lovers because of their long-lasting and gorgeous blossoms. They primarily consist of epiphytic and terrestrial plants. More than 300 orchid species are estimated to be found in the Western Ghats, out of India's total of 1230 orchid species. The region also has wild relatives of crop plants with a wide range of old lineages. The location is also a haven for wild cereal and millets. Due to a variety of physiographic and physio-gnomic variables, medicinal plant diversity is also rather great.

7 Faunal Diversity

The distribution of amphibians in the Western Ghats appears to be patchy. Wetter rainforests in India are home to over 90% of amphibian species, while drier forests have just about 20%. (Daniels 1992). Because of sophisticated study in the ghat

region, fresh amphibian reports have proliferated during the last two decades. One such breakthrough finding is Nasikabatrachus sahyadrensis, a frog species belonging to the Sooglossidae family.

In the Western Ghats, butterflies are found in five families, 166 genera, and 330 species, 37 of which are endemic. In the ghats, there are approximately 218 species of primary and secondary freshwater fish. All fish species in the Western Ghats are indigenous, with 116 species in 51 genera.

The Western Ghats are home to 157 reptile species, including the crocodile Crocodylus palustris. Snakes make up the majority of reptile species. There are 97 species in all, representing 36 genera, with two turtle/tortoise genera, 20 snake genera, and 14 lizard genera being endemic. There are 144 aquatic or coastal birds among the 508 birds recorded. The Western Ghats are home to nineteen species that are classified endemic. Insectivores, with 11 species, bats, with 41 species, and rodents, with 27 species, including the porcupine, dominate the mammalian fauna.

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Microbial Diversity and Conservation of Local Biodiversity Heritage Sites in Western Ghats with Community Participation-a Novel Conservation Effort of Kerala

V. Balakrishnan, N. P. Sudheesh, and Preetha Nilayangode

Abstract Microbial diversity is mostly an unexplored bioresource till date that deserves greater attention and hence, Convention on Biological Diversity (CBD) has given special emphasis towards the conservation of microbial diversity. Similar to other organisms, both *in-situ* and *ex-situ* preservation technologies are widely used for the overall restoration of the microbial diversity in a given ecosite. In India, National Biodiversity Authority (NBA) has declared 5 Microbial-Designated National Repositories (M-DNRs) under 'Section 39' of Biological Diversity Act 2002 as an important aspect of infrastructure for biodiversity conservation. These bio-resource centres follow operational guidelines of World Federation for Culture Collections (WFCC) and best practices of the Organisation for Economic Development and Cooperation (OECD). Most recently, NBA has constituted a 'core expert group' for developing guidelines for identification of repositories for plant, animal and microbe under Section 39 of the BD Act 2002. This is the first ever approach to intensify identification of more repositories for India. In another effort, NBA has developed Guidelines for Selection and Management of the Biodiversity Heritage Sites (BHS) under 'Section 37' of BD Act 2002 in order to strengthen the biodiversity conservation in traditionally managed areas and to stem the rapid loss of biodiversity in intensively managed areas, which need special attention. BHS can be smaller areas, which serve as corridors or offer refuge for threatened and endemic flora or fauna and serve as a positive interface between nature, culture and society so that both conservation and livelihood security can be achieved. In Kerala, studies have reported presence of numerous micro-organisms from the Western Ghats which is one of the world's biodiversity hotspots. It is interesting to note that the tropical climate and extremely acidic soils of Kerala are home to many microorganisms. Several studies for microbial diversity collection recorded over thousand microbial taxa from Kerala, especially from the wetlands, mangrove forests, sacred groves etc., during the last few decades. In Kerala there are numerous small biodiversity rich patches like sacred groves, wetlands, Mangrove forests, *Myristica swamps etc.,*

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*which is the home to numerousunidentified and very important*groups of microbial species. But during last decade, climate change, unsustainable use of bio resources, invasive species, land use change and habitat loss have resulted in huge microbial biodiversity loss. Microbial diversity plays an important role in sustaining ecosystem functioning, hence conservation of such areas as Local Biodiversity Heritage Sites (LBHS) is important. As per the Section 37 of BD act, and as per the Section 19 of Kerala Biological Diversity Rules–2008, Kerala State Biodiversity Board (KSBB) had identified numerous LBHS which are unique in their biodiversity and they are now protected through the Biodiversity Management Committees (BMC's). This paper presents an overview of the status of microbial diversity in India, the vital role played by microorganisms in ecosystem functioning, strategies for conservation of microbial diversity and the International and national legislations relating to access and transfer of microbial resources.

Keywords Microbial diversity · Biodiversity · Convention on biological diversity · Western ghats · Local biodiversity heritage sites

1 Microbial Diversity of India: An Overview

The term 'Biodiversity' is defined by Convention on Biological Diversity (CBD) as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems' (CBD website). Microbial diversity is the most diverse and least explored type of biodiversity constituting bacteria, virus, protozoans, fungi, unicellular algae etc. As per the website of Microbial Diversity resource portal of ICAR-National Bureau of Agriculturally Important Microorganisms (https://mgrportal.org.in/MicrobialConservation.html), 85 to 90% of the plants and vertebrate animals in the world have been described, but it is conservatively estimated that **<1%** of the bacterial species and **<5%** of fungal species are currently known. Due to the difficulties in cultivation of a number of microorganisms, still most of the earth's microbial wealth is unknown to the world.

The World Conservation Monitoring Centre (WCMC) of the United Nations Environment Program has identified a total of 17 mega-diverse countries: Australia, Brazil, China, Colombia, Ecuador, United States, Philippines, India, Indonesia, Madagascar, Malaysia, Mexico, Papua New Guinea, Peru, Democratic Republic of Congo, South Africa and Venezuela. Among these, **India is harbouring nearly 7–8%** of the recorded species of the world, and representing 4 of the 34 globally identified biodiversity hotspots (Himalaya, Indo-Burma, Western Ghats and Sri Lanka, Sundaland). India is also a vast repository of traditional knowledge associated with biological resources. As per the Convention on Biological Diversity (CBD) website, more than 91,200 species of animals and 45,500 species of plants have been documented till date in the ten biogeographic regions of the country. India also harbours a significant proportion of World's microbial

diversity ie., about 10.60% of Virus/Bacteria, 17.90% of Algal, 20.10% of Fungal and 16.40% of Lichen diversity of the world (Khoshoo 1995).

The biodiversity richness of India is mainly because of the presence of Western Ghats which is one of the major biodiversity hospots in India. Even though numerous studies have been conducted on the biodiversity of this area, there are still huge gaps in these data. These studies are mainly focussed on the plants in these areas, however, the microbial diversity studies are very rare in this area. Nampoothiri et al. 2013 had summarised most study reports on the microbial diversity of Western Ghats and as per his report, 21 different genera of Bacterial species such as 58% of Proteobacteria, 26% of Firmicutes, 13% of Actinobacteria and 3% of Bacteroidetes were isolated from the water samples of rivers flowing through the Western Ghats region, confirming the abundance of microbial diversity in this ecosystem. In addition to these, the report also summarized the various microorganisms collected from Western Ghat region (Nampoothiri et al. 2013).

2 Biodiversity of Kerala

Kerala occupies only 1.88% of India's landmass, but it contains approximately 28.41% of flowering plant species and 26.59% of Pteridophytes recorded from India. Kerala is the house to 1709 taxa of flowering plants which are endemic to Peninsular India among which 237 species distributed in 47 families are exclusively endemic to Kerala (Nayar et al. 2008). According to Nameer (2015) Kerala is having 1847 species of vertebrates 500 species of birds, 173 species of reptiles, 151 species of amphibians and 118 species of mammals. 36% of terrestrial vertebrates reported from Kerala are endemic to Western Ghats and Kerala. Endemism is greatest among amphibians with 90% endemic species, of which 66% are endemic to the Western Ghats, while 24% are endemic to Kerala (Das 2015; Palot 2015). The diverse physical features of Kerala have resulted in 13 agroclimatic zones and a variety of ecosystems such as forests, grasslands, wetlands, coastal and marine ecosystems which harbours high biodiversity. Kerala also have the Kuttanad below sea level farming which has been recognized as 'Globally Important Agricultural Heritage System' (GIAHS) by Food and Agricultural Organization of the United Nations (UN). In addition to these identified biodiversity richness, Kerala is also gifted with many unexplored biodiversity rich areas like wetlands, mangrove forests, *myristica* swamps, sacred groves etc. that are found outside the protected areas which needs special conservation interventions for the future.

2.1 Microbial Diversity of Wetlands in Kerala

Studies showed that the wetland ecosystem and its microbial communities are mutually dependent since they actively take part in the major functions of wetland

ecosystems such as nutrient recycle and various biogeochemical cycles (Bodelier and Dedysh 2013). Hence, analysing microbial communities in the wetland ecosystems is fundamental for understanding the functioning of wetlands, such as regulating the cycling, retention, and release of nutrients and soil carbon.

Kerala's unique wetland ecosystems constituting of 217 wetland areas includes marshy and water logged areas, paddy fields, backwaters, lakes and the Myristica Swamps seen in the Western Ghat areas. Kerala also have contains Nationally and Internationally recognized Ramasar sites such as Vembanad—Kole, Ashtamudi and Sasthamcotta lakes. Keratinophilic fungi have been reported from soil samples of Ernakulam and Thrissur districts, (Mini et al. 2012) and 38 species of fungi from the soil samples collected from oil palm agricultural fields in the wetland agroecosystem of Kerala (Thomas 2017).

Kerala State Biodiversity (KSBB), had conducted various studies post August 2018 floods in Kerala which indicated that natural calamities as flooding had a strong effect on the bacterial community structure. The presence of different anaerobic bacteria in the flood affected samples of Manimala River has been reported. A depletion in soil enzyme activity compared to buffer and non-flooded areas indicate that there is a decrease in beneficial organisms in the soil (nitrogen fixing and phosphate solubilizing) due to flooding.

2.2 Microbial Diversity of Sacred Groves of Kerala

Sacred groves are the fine example of in-situ conservation. Sacred groves are forest fragments, size varying between 0.5–500 ha, (some groves are more than 500 ha in size) which are protected by religious communities, and have a significant religious connotation for the protecting community. (Murugesan 2016). Around 100,000 to 150,000 sacred groves have been reported from different parts of India (Malhotra et al. 2007). They are of central importance in the State and National level ecological conservation policies since, they play an important role in ensuring smooth ecosystem services such as clean environment, that is, air, soil, and water conservation, flora and fauna conservation, carbon sequestration, temperature control, and conservation of traditional knowledge. (Ray and Ramachandra 2010).

Biological diversity of sacred groves and their conservation need have been well recognized by various International organizations including UNESCO since they are apt example for biodiversity conservation using traditional wisdom and practices there by helps to implement the article 8(j) of the Convention on Biological Diversity (CBD). owing to their significant role and potential. Sacred groves represent diverse ecosystems with widely varying species composition, and the vegetation within the grove has a definite set of ecological features. Biodiversity inventories have been carried out in many sacred groves, and checklists of flora and fauna are available, but a thorough analysis of biodiversity and functional ecology with conservation focus is limited (Ray et al. 2014). In 1927, studies documented about 15,000 sacred groves in

Kerala (Ward 1891), however the number had been declined to about only 7000 as per a recent study by KSBB.

Studies showed that Sacred Groves provides a unique habitat for the sustainable growth of numerous micro organism especially fungi. (Brown et al. 2006). More than 27,000 species of fungi had been reported from India which is around 1/3rd of the total reported fungal biodiversity of the world (Sarbhoy et al. 1996). Out of the estimated 2040 lichen species in India (Awasthi 2000), Western Ghats are known to harbor around 1096 lichen species which is around 47% of the total lichens of the country including 257 endemic species (Nayaka and Upreti 2011). Though the Western Ghats have been explored by many researchers for documenting the lichen diversity and richness (Balaji and Hariharan 2013a, b), no studies have not yet been done on the lichen diversity of groves from Western Ghats except the study of lichens from the Ugai sacred grove of Maharashtra by Nayaka and Upreti 2004 and the study by S. Swaminathan Research Foundation Centre for Research on Sustainable Agriculture and Rural Development in Kerala on the lichen diversity of Western Ghats. As on the whole, the sacred groves in general of the Western Ghats are on the road to decadence, due to various reasons.

2.3 Below Ground Microbial Biodiversity

The richness of below ground level biodiversity is essential for the productivity and resilience of forest and agricultural ecosystems. Among them, earthworms have the ability to alter the physical chemical and biological properties of the soil there by maintaining the soil structure and fertility. In Indian sub-continent, 590 species and 67 genera of earthworms were reported (Julka 2001). Bourne (1889) reported Indian earthworms for the first time. An overview of biodiversity of Indian earthworms were furnished by Julka (2010) and Julka et al. (2009). The description of the earthworm diversity on the western ghats in India stretching from Kerala in the South to Gujarat State in the North is furnished by Stephenson (1915, 1923, 1925), Gates (1945), Soota and Julka (1972), Jamicson (1977), Julka and Rao (1982), Blanchart and Julka (1997), Kale and Karmegam (2010), Mahesh et al. (2011), Shylesh et al. (2012) and Siddaraju et al. (2013). Not much information is available on the occurrence, distribution and diversity of earthworms in relation to geographic and edaphic factors of different regions of Kerala State in general and southern Kerala in particular. However, recently few important studies have reported from Kerala. A study was conducted in Wayanad district from 2017 to 2018 to collect and identify the earthworm species present in three agro-climatic regions of Wayanad. John et al. 2019 recently reported 15 earthworm species from Waynad district of Kerala including two new species. Similarly, 12 species of earthworms were reported from Kolam district of Kerala (Nair et al. 2017).

3 Importance of Conservation of Microbial Diversity

The environmental "super challenges" of the twenty-first century includes Global warming, Climate change, Pollution, Overexploitation of bioresources and recently the spread of zoonotics. The increased incidence of zoonotics has been attributed to loss of habitat and deforestation. Hence, recently many scientists have concentrated on the importance of conservation of microbial biodiversity. There is a widespread concern worldwide for conservation of microorganisms. Microbial resource centres (MRCs) plays important roles in the conservation as well as for research purpose in the microbial biodiversity. Cockell and Jones 2009 had put forward, a "roadmap" toward microbial conservation. Important elements in this includes habitat conservation, legislation, education, and ethical considerations.

4 International and National Legislations

The Convention on Biological Diversity (CBD), Cartagena Protocol on Biosafety, Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purposes of Patent Procedure, Biological Weapons Convention 1975, World Federation of Culture Collections (WFCC) are some of the major international regulations on the microbial biodiversity utilization. Among them, Convention on Biological Diversity (CBD 1992) ensures conservation, sustainable utilization and sharing of the benefits aroused from the bioresources in the countries who have entered this agreement. Indian Forest Act, 1927; Wildlife Protection Act, 1972; and Environment Protection Act, 1986 are some of the national rules related to the microbial diversity. Based on the India Biodiversity Act National Biodiversity Authority was established in 2003 in India and NBA has declared Microbial-Designated National Repositories (M-DNRs) under 'Section 39' of Biodiversity Act for biodiversity conservation. These M-DNRs aims for the preservation and maintenance of type and reference of microbial strains, genomes of organisms and information relating to heredity and function of microbial systems.

5 Strategies for Conservation of Microbial Diversity in Kerala

Microbial diversity is normally conserved in '*in situ*', '*ex situ*' and 'in factory' forms of conservation. Among them, In Situ Conservation Methods refers to the conservation method where the microbes are being conserved in theie natural habitats through ecosystem preservation such as National Parks, Wildlife Sanctuaries Community Reserves etc.

5.1 *Conservation of on-Farm Practices and Agrobiodiversity Hotspots: This Incvolved the Conservation through Traditional Farmers and Traditional Faring System*

Subramanian et al., (2013). It is important to protect the on-form conserved areas towards conserving agrobiodiversity. In Kerala, the agrobiodiversty activities demonstrated by agrobiodiversity management of the M. S. Swaminathan Research Foundation (MSSRF) is a perfect example of such conservation model. There are numerous traidiotnal farmers who have been conserving the native varieties in theory farms through generations.

5.2 **In Situ** *Methods with Community Participation: Biodiversity Heritage Sites (BHS)*

Biological Diversity Act 2002 provides for conservation of biodiversity rich areas outside protected area network as Biodiversity Heritage Sites (BHS). Under Section 37 of Biological Diversity Act, 2002 (BDA) the State Government in consultation with local bodies may notify in the official gazette, areas of biodiversity importance as Biodiversity Heritage Sites (BHS). As per the NBA, BHS is recognized as are 'a well-defined areas that are unique, ecologically fragile ecosystems having rich biodiversity, high endemism, presence of rare and threatened species, keystone species, species of evolutionary significance, wild ancestors of domestic/cultivated species and fossils with or without long history of human association with them'. So far, 18 BHS have been identified in India Asramam, Kollam, Kerala is the only BHS declared till date in Kerala. Kerala has about 2000 sacred groves, which are distinct and unique in biological diversity varying in size from one cent to 20 or more hectares. The BHS declared in Kerala., ie., Asramam in Kollam district is of 57.53 ha, hosts a unique diversity of Mangrove species with diverse flora and fauna. The area harbours 15 sp. of true mangroves, 22 associated mangrove species, 122 sp. of plants, 34 sp. of edible fish and about 62 sp. of birds. Most importantly, the site has rare and endangered heritage trees of *Syzygium travancoricum* which is listed as critically endangered in IUCN Red List. However, so far there is no area that has been selected which is rich in unique and rare microorganisms that can be utilized for many purposes of human benefit.

The Government of Kerala vide G.O.(MS) No.05/2020/Envt. dated 03.03.2020 has empowered BMCs for declaring locally specific biodiversity rich areas or trees as local biodiversity heritage sites/ trees through a resolution for the purpose of conservation without causing restrictions on legitimate use by the stakeholders and local community. This is a new initiative for the first time in India undertaken by KSBB through the BMCs for promoting conservation and management of locally specific biodiversity rich areas/ecosystems/trees. BMCs declare such kind of local BHS by passing a resolution as per the provisions of the BD Act, Section 41, with the

Table 1 The list of local BHS/trees declared so far by the BMCs in Kerala State

Sl. No.	Name of BMC & District	Name of Local BHS/ Tree	Date of Resolution passed
1	Mallappuzhasseri Grama panchayat, Pathanamthitta (Fig. 1)	'Pannivelichira'—a wetland ecosystem	26.10.2019
2	Pandalam Thekkekara GramaPanchayat, Pathanamthitta	A two hundred year old cashew nut tree in the fifth ward of panchayat	16.10.2019
3	Pandalam municipality, Pathanamthitta	16 number of trees (heronries) seen in the town area of the municipality	12.11.2019
4	Anchuthengu Grama panchayat, Thiruvananthapuram	Two Chempaka trees seen in second ward of panchayat (about 250 year old)	05.12.2019
5	Chirayinkeezhu Grama panchayat, Thiruvananthapuram	'Nattumavu'(150 year old) seen in fifth ward of panchayat	07.12.2019
6	Mudakkal Grama panchayat, Thiruvananthapuram	'Kadalkandam'—a biodiversity rich wetland area & a two hundred year old tree—'Poovanam' as local biodiversity heritage tree.	11.12.2019
7	AyarkunnamGramaPanchayat, Kottayam	A two hundred year old 'Nattumavu' tree, a keystone species seen along the bank of Meenachil River	12.12.2019
8	Alakkode Grama panchayat, Kannur	'Eeyyabharanithuruthu'—a biodiversity rich island area in the Kuppampuzha	27.12.2019
9	Pallikkal Grama panchayat, Pathanamthitta	'Arattuchira'- a wetland ecosytem	03.01.2020
10	Mooliyar BMC, Kasargode	Neyyamkayam, a Pool (Fig. 2)	01.06.2020

prior consent of the Board, after conducting thorough study regarding the biological importance of the proposed site/ tree with the support of district level technical support group. So far, 10 such sites/ trees were declared by BMCs as local BHS/biodiversity heritage trees and the resolution of the same has been submitted to KSBB. The list of local BHS/trees declared so far by the BMCs is given below (Table 1):

In the scenario that microbiomes are inadequately described and microorganisms play a major role in ecosystem functioning, conservation of such pockets of biodiversity rich areas outside protected areas by community participation serve as novel methods of conservation of biodiversity of flora, fauna and microorganisms.

Fig. 1 Pannivelichira impoundment in Mallappuzhasserry Grama Panchayat, Pathanamthitta

5.3 Ex Situ *Conservation Methods*

Ex situ conservation is the technique of conservation of all levels of biological diversity outside their natural habitats through different techniques like zoo, captive breeding, aquarium, botanical garden, and gene bank etc.

6 Access to Microbial Resources and Fair and Equitable Sharing of Benefits

Microbial wealth significantly contribute to the global economy through multibillion-dollar biotechnology industry. As per the Section 21 of the Act and Rule 20 of the Biological Diversity Rules corresponding to Articles 5, and 12 of the Nagoya Protocol ensure that benefits derived from the utilization of biological resources and associated knowledge from India are shared with the benefit claimers in the country (including communities through the local Biodiversity Management Committees). The microbial diversity of Kerala can be an immense source of potent microorganisms that could be potentially exploited for commercial use. A review of around 80 research papers from Kerala perspective conducted by Kerala State Biodiversity Board (KSBB), covering bacterial resources of terrestrial and marine nature shows that more than 45 species of bacteria have been used in *in vitro* and *in vivo* clinical studies, The proportion researches using microbial resources shows that bacterial and fungal species have been utilised most in generating metabolites and bioactive molecules with 43.1% and 41.9% respectively. The researches on the lichen for clinical studies are still in the growing stage and the research on algae is found to be very less compared to others and is still in the beginning stage with more scope of development of drugs and active moieties of therapeutic potential.

The bacterial resources found in Kerala have been a study material in 80% of research for developing therapeutic products out which only 2% went *in vivo* trials (Fig. 3). The endophytic bacteria, actinomycetes (both marine and terrestrial) have

Fig. 2 Neyyamkayam, a Pool at Mooliyar BMC, Kasargode

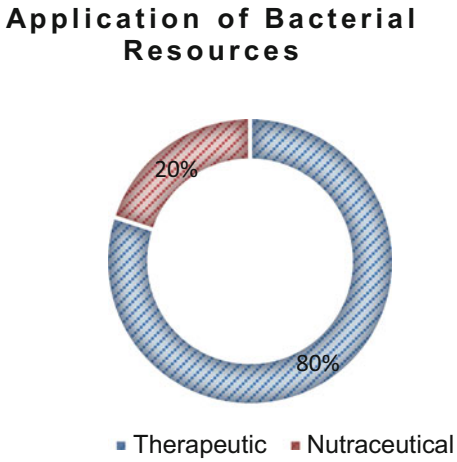

Fig. 3 Application potential of bacterial resources

been a treasure of bioactive molecules and the most studied species fall in the genus Bacillus and Streptomyces. *Spirulina sps*. and LAB (Lactic Acid Bacteria) was found to be the prominent species on which development of nutraceuticals have been worked out. Among the therapeutic applications, 36% of the researches were devoted in developing antimicrobials for human pathogens with highest in antibacterial activity followed by antifungal agents and very few works have been reported for antiviral property. Actinomycin, Tautomycins, Bacteriocins, flavanioids and L-asparaginase were some of the identified active ingredients along with polysachrides and proteins of nutraceutical potential. More number of the studies focussed on the marine algal species, the reservoirs of metabolites than the terrestrial and fresh water species.

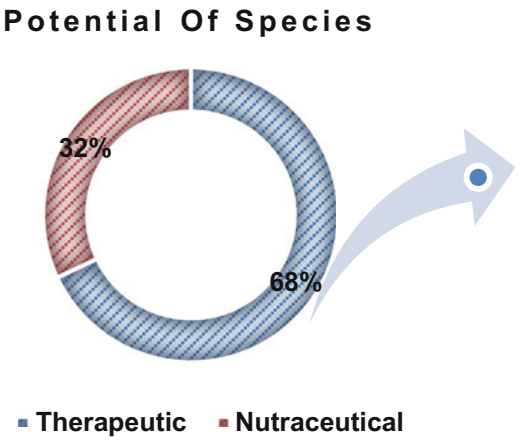

Fig. 4 Proportion of species based on potential

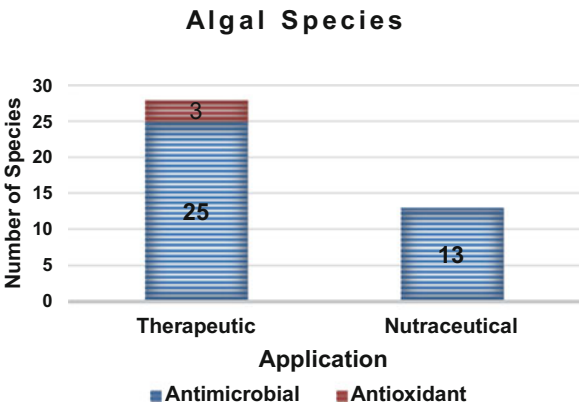

Fig. 5 Number of species according to their application

Figures 4 and 5 shows that most of the species found in Kerala region possess therapeutic potential and some have nutraceutical value with high minerals and polysaccharides, fatty acids and esters. Of the total species recorded, 26 possessed antimicrobial compounds and 3 with antioxidant and anticancerous properties. All the studies conducted with algal metabolites are pre-clinical in nature.

Lichens from Kerala are a rich resource of novel bioactive compounds. The lichen extracts, especially of Parmotrema sp. have shown promising potential in both antidiabetic and antioxidant assays. On scrutinizing the available research materials 16 species of lichens found in the Kerala part were found to have subjected to studies related to its therapeutic applications (Fig. 6).

Figure 6 shows that the bioactive molecules extracted from the lichens possess therapeutic potential and are proved to be antimicrobial (44.4%) in researches followed by antioxidant property (25.9%). 7.4% of the preclinical studies prove the potential of metabolites in treating cancer, hyperglycaemia, as cardioprotectives and as anti-inflammatory agents. No *in vitro* studies were observed and

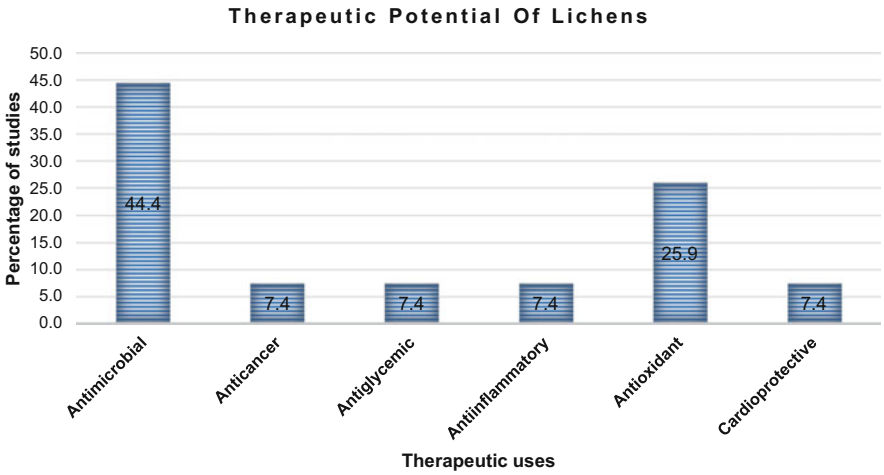

Fig. 6 Proportion of researches on therapeutic potential of lichens

Everniastrum cirrhatum a common lichen species was found to have minerals and nutrients that can be used as an edible species similar to Himalayan lichen species. *Everniastrum cirrhatum* and *Parmotrema sps* are among the species that have been studied extensively.

Of the more than 2000 applications received by NBA for access to genetic resources, 19% seek grant for access to microbial resources. The Indian Institute of Oilseeds Research (IIOR) is one of the first ICAR institute to take up the initiative to follow the National Biological Diversity Act, 2002 in accessing local biological resources by obtaining necessary permissions, for their research patents and commercial exploitation. This is also the first instance of accessing microbial resources with intimation to respective SBBs and NBA IIOR accessed microbial bio-resources. Bt-1, a local isolate of the bacterium *Bacillus thuringiensis* (Bt) var. kurstaki from a dead larva of castor semilooper *Achaea janata* from a castor field in Kothakota, Mahabubnagar district, Telangana, Trichiodermaviride B-16 was collected from Kothagudem BMC, Nalgonda (District), Telangana State and *Trichoderma harzianum* -Th4d was collected from Gurajala Biodiversity Management Committee (BMC) (Grampanchayat), Guntur (District), Andhra Pradesh. The isolates were utilized for R & D to develop eco-friendly insect pest and plant disease management. Research was carried out to standardize the mass production of the Bt isolate through solid state fermentation for making the product available at an affordable price. The Bt obtained was formulated as a wettable powder and evaluated against castor semilooper in farmers' fields in Mahabubnagar and Nalgonda districts and in the All India Coordinated Research Project (AICRP), Pigeon pea centers. Data was generated on toxicology, chemistry, bio-efficacy, shelf-life etc. as per the guidelines. The formulation was found to be effective in controlling lepidopteron pests of Castor and Pigeon pea and. IIOR has shared the benefits with BMC Kothakota through Telangana State Biodiversity Board for licensing

DOR Bt-1 formulations to different biopesticide entrepreneurs and to BMC Kothagudem through Andhra Pradesh State Biodiversity Board for licensing DOR Trichodermaviride B-16 formulations to different bio-pesticide entrepreneurs. Further, IIOR is sharing 3% of the license fee charged from entrepreneurs with State Biodiversity Boards. Thus, although much research is being conducted in India relating to Microroganisms very few of these findings are being commercialized and the benefits shared with the society.

7 Conclusion and Way Forward

Microbial diversity is extremely evident for the sustainable development of any Country. However, despite the obvious economic value of microbial diversity, microorganisms have been largely ignored in debates on the conservation and management of global diversity. There is, therefore, an urgent need to persuade policy-maker to be more concerned about the conservation, management and exploitation of microbial diversity. In a State like Kerala which has huge untapped treasures of biodiversity, numerous biodiversity rich areas which have enormous invaluable microbial diversity exist. The identification, sustainable utilization and proper conservation will lead to the discovery of new medicines, and numerous other products from them. As a step towards this, Kerala State Biodiversity Board had started identifying and conserving these areas as local biodiversity rich areas with the support of local communities. Similarly, other States also should identify such biodiversity rich areas as Local Biodiversity Heritage Sites for the better conservation of microbial diversity of our country.

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Methods for Exploring the Microbial Diversity of Western Ghats in India and their Extended Applications in Various Fields

H. Shabeer Ali, P. Prajosh, and A. Sabu

Abstract Western Ghats of India is one of the global biodiversity hotspot that seeks extreme care and conservation. Unique geography and climatic condition in different locations of Western Ghats entertain the existence of a number of endemic species that are under extreme threat. Study of microbial diversity in biodiversity hotspots is a challenging field. The difficulties associated with sample collection, culturing of microbes, identification and metabolite purification renders the microbial pool of biodiversity hotspots untouched. This chapter aimed to summarize the microbial diversity of Indian Western Ghats and their potential applications in various fields. Instead of reviewing the microbial diversity of Western Ghats, this chapter provides a complete guide on the tools and techniques involved in exploration of microflora of any biodiversity hotspots. A number of sampling techniques, microbial enumeration and identification methods have been discussed in which Metagenomics approach and library construction is a turning point in the study of cultivable and non-cultivable microflora of an ecosystem. With the advanced techniques like flow cytometry, microautoradiography and the use fluorescent probes, the metabolic status of the microbial population can be monitored *in situ*. Molecular identification techniques such as Polymerase Chain Reaction (PCR), sequencing and Restriction Fragment Length Polymorphism (RFLP) are useful for the phylogenetic analysis and species identification. Special focus was given to microbial enzymes, industrial pollutants degrading microbes, pesticide degraders, quorum sensing and quorum quenching molecules reported so far from Western Ghats. The pieces of information provided in this chapter signify the applications of unique microflora of Western Ghats and importance of conservation.

Keywords Western Ghats · Microbial diversity · Biodiversity · PGPR

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1 Introduction

Diversity among living organisms from all the elements of ecosystem such as terrestrial, marine and other aquatic environment is termed as 'Biodiversity'. Biodiversity includes variability between and within species and of ecosystems. It encompasses each and every forms of life ranging from viruses to higher level vertebrates including human. There are biodiversity hotspots all over the world which entertain the existence of native endemic species and communities of the intact natural ecosystem.

India with its unique geography and climatic condition that nurture the diverse flora and fauna has been recognized as one of the mega-diverse zone in the world. Biodiversity in India is sustained by two mega-biodiversity zones namely 'North Eastern hill regions' and the 'Western Ghats'. The North Eastern hill regions cover Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland and Sikkim.

Western Ghats on the other hand, runs parallel to the west coast of India from 8° N to 21° N latitudes, 73° E to 77° E longitudes for around 1600 km (covering an area of approximately 1,64,000 km^2) with an elevation range of 300-2700 m, covering southern Gujarat, Maharashtra, Karnataka, Kerala, Goa and Tamil Nadu (Fig. 1a). Gradient climatic conditions in Western Ghats contribute to the existence of diverse ecosystems and life forms which make it as one of the global hotspots of biodiversity (Ramachandra and Suja 2009; Cincotta et al. 2000; Rajasri et al. 2016).

1.1 Climate

The altitudinal gradation and distance from the equator significantly affects the climate of Western Ghats. The climate is humid and tropical in the lower reaches tempered by the proximity to the sea. The geographical variations in different locations of Western Ghats are the sole reason for its varying climates (Fig. 1b, c & d). The north and south regions of Western Ghats with elevations of 1500-2000 m and above have a moderate climate with mean temperature range of 20 °C (south) to 24 °C (north). Some parts of this region have freezing temperature during winter months. The average rainfall in this region is 3000–4000 mm and reaches maximum up to 9000 mm. The eastern region of the Western Ghats receives comparatively less rainfall ranges from 1000-2500 mm. Data from rainfall figures reveal that there is no relationship between the total amount of rain received and the spread of the area. The areas which receive heavy rainfall (Areas to the north in Maharashtra) are followed by long dry season, whereas the regions closer to the equator with less annual rainfall have the rainy season lasting throughout the year.

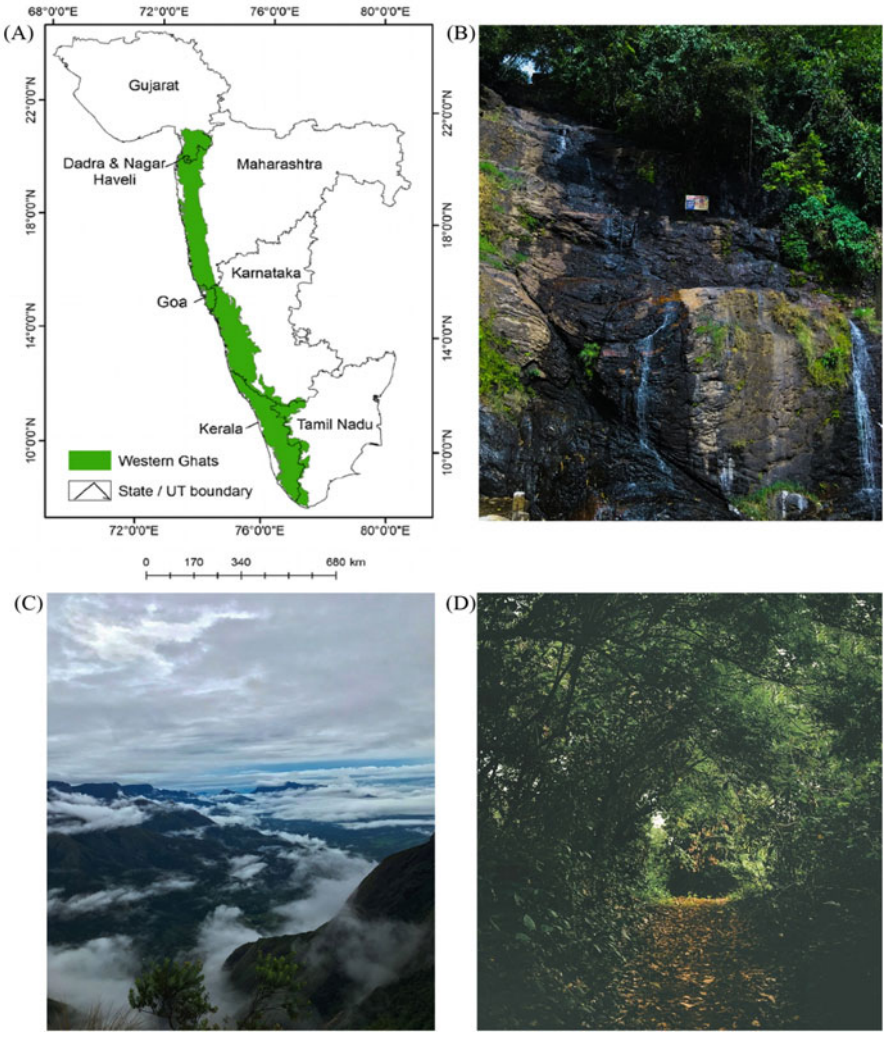

Fig. 1 (**a**) Schematic representation of the distribution of Indian Western Ghats (Adopted from Sudhakar Reddy et al., Jha and Dadhwal 2016, Reproduced with permission). Figures **b**, **c** and **d** show the diverse geography such as Shola (**b**), high altitude hill region (**c**) and Dense forest (**d**) with in the Western Ghats. *Picture Courtesy; Josna Jose (Reproduced with permission).* Source: Fig. 1a. Jha and Dadhwal 2016. Figure 1b, c and d); Josna Jose

1.2 Flora and Fauna of Western Ghats

The major vegetation types of Western Ghats includes tropical evergreen forests, dry deciduous forests, moist deciduous forests, sholas, scrub jungles, savannas, including high rainfall savannas, peat bogs and Myristica swamps. The diverse climate and

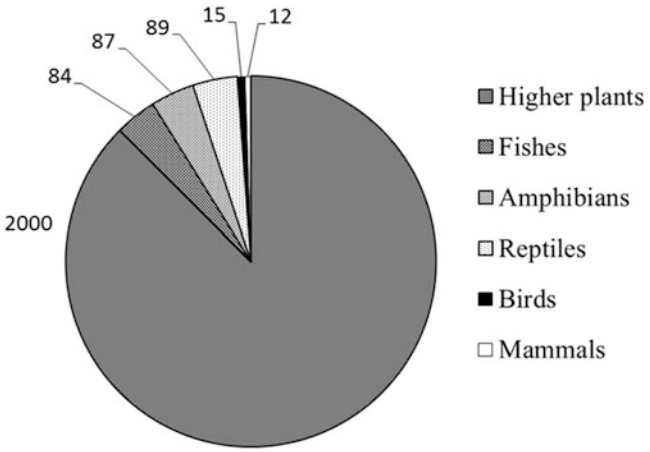

Fig. 2 Diagrammatic representation of the occurrence of different endemic species in Indian Western Ghats. (Diagram was prepared according to the reports of Daniels 1997)

geographical alignment have contributed to significant diversity in living forms. According to the recent reports over 5000 flowering plants have identified so far, of these the gymnosperm flora is represented by *Cycas circinalis* (Cycadales), *Decussocarpus wallichianus* (Coniferales) and *Gnetum ula* and *G. contractum* (Gnetales). In addition to the vegetation, 6000 insects, 334 butterfly, 258 molluscs, 139 mammals, 508 birds and 179 amphibian species have also been identified were identified till now, apart from these at least 325 globally endangered species are the major highlights of Western Ghats that needs to be conserved (https://wwf.panda.org/knowledge_hub/where_we_work/western_ghats/).Beyondthe statistics, there are several unexplored species in each level still awaiting scientific identity. Endemicity is the major attraction of Western Ghats that seeks high priority conservation than any other ecosystem. Earlier survey conducted by Daniels (1997) revealed that vast majority of the species explored in Western Ghats are endemic to it. The endemic class of organisms in the Western Ghats comprises of 2000 species of higher plants, 84 species of fishes, 87 species of amphibians, 89 species of reptiles, 15 species of birds and 12 species of mammals (Fig. 2).

Among the 139 mammal species, nocturnal Malabar large-spotted civet (Fig. 3l) is the critically endangered mammal of Western Ghats. The arboreal Lion-tailed macaque (Fig. 3i) is another mammal seriously at risk of extinction, statistical data has shown that only 2500 of this species are remaining with largest population in Silent Valley and Kudremukh National Park. These hill ranges also entertain the seasonal migration of endangered Asian elephants (Fig. 3e). The Nilgiri Bio-sphere, Karnataka's Ghat, Brahmagiri and Pushpagiri wildlife sanctuaries are the large reservoirs of Asian elephants and Indian tigers (Fig. 3h). Next to Sundarbans, the unbroken forests bordering Karnataka, Tamil Nadu and Kerala together constitutes for the largest population of Indian tigers. The Bandipur National Park and Nagarhole harbors more than 5000 vulnerable Gaur. The endangered Nilgiri langur

Fig. 3 Some of the endangered endemic species of Western Ghats (For picture courtesies please see the acknowledgment section). Source; Fig. 3a (By Rushen@flickr); (**b**) (Gallery360@flickr); (**c**) (Pieter@flickr); (**d**) (Adhikesavan Dhandapani@flikr); (**e**) (Sumankumar R@flickr); (**f**) (Vidjit Vijayasanker); (**g**) (Debrup Chakraborty@flickr); (**h**) (Mridanga Kashyap (Manabjyoti kalita) @flikr), (**i**) (Roshen Alex Jacob@flikr); (**j**) (bibinprk91@flikr); (**k**) (Felicia McCaulley@flikr); (**l**) (Diana@pinterest, astronomy-to-zoology. tumblr.com, Kerala tourism), (**m**) (Adhikesavan Dhandapani@flikr), (**n**) (S.D. Biju 2004, Karthickbala at ta.wikipedia, CC BY-SA 3.0)

(Fig. 3d) is found mainly to the west of Kodagu forests. Indian Muntjac (Fig. 3a) is the dominating populations of Bhadra Wildlife Sanctuary and project tiger reserve in Lakkavalli of Chikmagalur. Karnataka forests in the Western Ghats are the home to largest population of Asian elephant (Fig. 3e), sambar (Fig. 3f), gaur, vulnerable sloth bears, tiger, leopard (Fig. 3b) and wild boars. Significant population of black panther (Fig. 3c), normal variety of leopards and Great Indian Hornbill (Fig. 3g) can be found in Dandeli and Anshi national parks in Uttara Kannada district. Within the Western Ghats, the forests of Mahabaleshwar, hills of Coimbatore, Pulneys, Tirunelveli, Nilgiris, places like Nagarahole, Silent Valley etc. were recognized as hotspot region to hold diverse and endangered life forms both in aquatic as well as terrestrial ecosystem (Ramachandra and Suja 2009). Nilgiri Tahr (Fig. 3b) is endemic to the Nilgiri Hills and the southern portion of the Western and Eastern Ghats of Tamil Nadu and Kerala. The purple frog (*Nasikabatrachus sahyadrensis*) (Fig. 3n) of Indian Western Ghats was recorded as the endangered species and enlisted in the IUCN (International Union for Conservation of Nature) red list of threatened species (Biju 2004). In addition, King cobra (Fig. 3j), the Mugger crocodile, Malabar gliding frog, Pipe snake, Denison's barb (Fig. 3k), Bamboo mushroom and Intrella mollusk are the other endemic species of Western Ghats under severe threat.

1.3 Microflora in an Ecosystem and their Impact on Biodiversity

Like the climate and geographical alignment, the microbial consortia associated with the basic elements of the ecosystem contribute a profound effect on biodiversity. Especially, the microbes associated with the rhizosphere soil, plants and animals possess crucial role. When discussing about the role of microbes in biodiversity, the positive and negative impact of microbial interaction should be addressed with prime importance. There are classical examples which describes the beneficial and harmful effect of microbes to the ecosystem and living forms associated with it. Researchers have already been started to explore the role of microbial interaction on biodiversity with special focus on biodiversity hotspots like Western Ghats. In the ancient times, microbial interaction was the special focus of research, eventually the research had diverged in to different aspects which invested the major interest to explore the mechanism of microbial interaction and their consequences. Antibiotics, the key molecules of microbial interaction are the contribution of such advanced research. Such findings provoked the quest for novel molecules from unexplored sources that can be used as therapeutics, nutrients, cosmetics, food and food additives. Cultivable microbes from biodiversity hotspots were the initial target for the exploration of such novel molecules. Eventually, with the emergence of modern techniques such as metagenomics and biprospecting, the entire microbial consortia associated with an ecosystem were targeted for the same. The unexplored microbial population and the

unexplainable mechanisms of interaction have contributed to the dense vegetation which ultimately attracted human intervention for the purpose of agriculture.

Diverse microbial population can be found in almost all the basic elements of the universe, such ubiquitous nature of microorganisms make it difficult to determine even the rough estimate of existing microbial population. In addition to the ubiquitous nature, genetic instability and increased rate of gene mutation largely entertain enormous diversity of microbial population. Irrespective of the size, each and every group of organism contributes to the existence and diversity of other groups. The microbial diversity within an ecosystem has a profound effect on the flora and fauna associated with it and vice versa. The rhizosphere soil harbors enormous bacterial load with lower diversity, plants are known to exert selective pressure on rhizosphere microbial community, this indicates that differential microbial communities are associated with different plant species. From these statements, it can be inferred that the plant derived metabolites have profound effect on the colonization and diversity of rhizosphere microbial populations (Hacquard et al. 2017; Yang and Wang 2017; Zhang et al. 2017). In reverse, the plant growth promoting rhizosphere bacterial genera (PGPR) such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Acinetobacter, Burkholderia, Arthrobacter*, and *Paenibacillus* (Finkel et al. 2017; Sasse et al. 2018; Zhang et al. 2017) serves to nourishes the plants with growth promoting secondary metabolites.

Interaction between microbe-microbe, plant-microbe, animal-microbe, plant-animal and animal-animal serves as the basis for sustaining the equilibrium of an ecosystem. Microbial colonization in any environment is established by the interaction of microbes with other members of same species or with a suitable higher level host that supports colonization. The underlying mechanisms behind microbial colonization involves chemotaxis, quorum sensing system, biofilm formation, genetic exchange, cellular transduction signaling, conversion and exchange of secondary metabolites and siderophores (Braga et al. 2016). The basic concepts and driving forces of microbial interactions were then translated in to science for the development of therapeutic agents and nutritional supplements. This led to the exploration of microbial populations in the biodiversity hotspots such as Western Ghats. The general examples for the positive and negative interaction with in the microbial communities and also between microbes and other higher classes of organisms includes Symbiosis (Mutualism, Commensalism and Parasitism), predation, Amensalism (competition and antibiosis), Antagonism, etc.

Chemical communication between the interacting partners serves the basis for majority of the microbe-microbe and plant-microbe interactions. In response to the altered gene expressions induced by the interacting microbes, plants release plethora of primary and secondary metabolites that attract more members to the colony which elicit root-root interactions (Mommer et al. 2016), nutrient availability (Rosier et al. 2016; Sasse et al. 2018) and protection from invading pathogens (Bertin et al. 2003; Li et al. 2013). By employing the metabolomics approaches, researchers have started to explore and quantify the metabolites synthesized and secreted by the associating partners. This signifies the importance of microbial interactions and the underlying

communication mechanisms of unexplored microbial populations especially in biodiversity hotspots.

1.4 *Microflora of Western Ghats*

It is a difficult task to bullet point the exact number of prokaryotic and eukaryotic species isolated so far from any of the biodiversity hotspots all over the world. Difficulty in reaching such biodiversity hotspots, unpredictable properties and culture conditions of the isolates are the major challenges associated with the exploration of microbial populations in such environments. It is expected that the microbial population explored so far accounts less than 5% of the total microbial population expected to be existing in the universe. Taking the bacterial population in to account, approximately 5000 prokaryotic species have been identified so far which represents a maximum of only 1-10% of the roughly estimated 3,00,000 to one million prokaryotic species on earth (Stanely Stanley 2002). Of these, only 1% of the explored prokaryotic species can be cultivated in the laboratory. The above statements indicate the complexity associated with the study of microbial diversity in various ecosystems (McInerney et al. 2002). With the introduction of metagenomics techniques, the study of microbial consortia associated with various ecosystems have been advanced.

Diverse population of fungi play vital role in the ecosystem and their close association with vegetation provide several benefits. Rough estimates indicate that out of 1.5 million fungal taxa only 70,000 species have been recognized so far. This indicates, we have been explored only about 5% of the fungal world believed to exist. From the Western Ghats region, nearly 13,000 fungal species have been reported so far. In the Western Ghats itself approximately 300,000 plant species are believed to have mycorrhizae association, but only 130 species of mycorrhizal fungi have been known till date. In addition, fungal population of the Western Ghats aquatic system is the less explored than the terrestrial ecosystem. The fungal population received considerable importance because of their ability to breakdown complex organic substances such as chitin, keratin, cellulose and lignin. In most instances they are capable to degrade petroleum hydrocarbons, plastic and hazardous chemicals and pesticides too (Bhat n.d., Humanity Development library 2.0).

Locality based study of microalgae diversity in the Western and Eastern Ghats of Tamil Nadu, India have been reported 97 microalgae species belonging to 3 taxonomic groups were identified. The 97 species comprises of *Cyanophycea, Chlorophycea* and *Bacillariophycea* species. *Aphanothece microscopica, Chroococcus minutus, Coelospharium dubium, Hydrococcus rivularism, Oscillatoria princeps, Nostoc muscorum, Nostoc puncteforme, Nostoc commune, Gleotricha gausii, Calothrix braunii, Rivellaria sp., Tolypothrix tenuis, Scytonema schmidtii* were the predominant species in Cyanophyceae. The predominant species in *Chlorophyceae* were *Chlorella sp., Scenedesmus sp., Pediastrum duplex, Cosmarium consperum, Euastrum elagans* and *Micrasterias Americana.* The

Bacillariophyceae comprises *Navicula hallophyla, Rhophaldia gebrella, Fragellaria intermedia, Pinnularia virdis, Nitzchia palliate* (Suresh et al. 2012).

Tukaram et al. (2012) have screened 627 soil samples collected from the Western Ghats region of Maharashtra, India to assess the diversity of Cyanobacterial population. They have identified 94 Cyanobacterial species that comes under 38 genera, 14 families and 5 orders. Among the numerous isolates, *Westiellopsis prolifica* Janet and *Nostoc calcicola* Brebsson ex Born. et Flah were found to be the most abundant. Whereas, *Nostoc*, *Anabaena* and *Chroococcus* were the densely populated genera. *Myxosarcina spectabilis* Geitler was the less abundant population.

1.5 Tools and Techniques to Explore the Microflora of Biodiversity Hotspots

1.5.1 Sample Collection

The study of microflora in an ecosystem encounters many challenges especially in the case of uncultivable microbes. Study of microflora starts with a number of sample collection techniques to more complex molecular characterization techniques. A number of specially designed sample collection techniques and devices are available for the collection of soil, water and air are available. High-pressure retaining deep-seawater samplers, sediment traps, bins or grabs for sediment sampling, plankton net (for plankton collection), glass fiber filters for particulate matters and membranes, glass plate and rotating drum (for aquatic surface microlayer). Deep drilling and excavators (geological sample collection), shovel, hand auger (diameter between 1 and 10 cm) or vehicle-mounted hydraulic auger are the common soil samplers (Joux et al. 2015).

1.5.2 Nucleic Acids Extraction and Analysis from Environmental Samples

Since the DNA of organisms has longer life span than RNA, the former is the more preferable biomolecule for the purpose of biodiversity studies. Due to availability of a number of extraction kits, DNA extraction techniques are much simpler now days. However, aging, effect of nucleases and impact of ionizing radiations on nucleic acids present in the samples make the analysis a difficult task. Clay particles and inorganic matrix preserve the nucleic acids in the environment for several years (Frostegård et al. 1999). In contrast to DNA, presence of RNA in an environmental sample indicates the recent synthesis from living cells and precisely is an indication of the expression of corresponding gene.

Followed by nucleic acid extraction, polymerase chain reaction (PCR) helps to amplify the target gene with the help of specific primers and polymerase enzyme. Since the conventional PCR is not directly applicable to RNA, it has to be converted

back to DNA by the methodology known as complementary DNA synthesis (cDNA) with the help of reverse transcriptase enzyme. After PCR amplification of the target gene, the diversity between the different gene fragments can be determined by RISA (Ribosomal Intergenic Sequence Amplification), RFLP (Restriction Fragment Length Polymorphism), T-RFLP (Terminal Restriction Fragment Length Polymorphism), DGGE (Denaturing Gradient Gel Electrophoresis) and TGGE (Thermal Gradient Gel Electrophoresis) and SSCP (Single Strand Conformational Polymorphism) techniques. Eventually, the gene of interest can be integrated in to a suitable vector for storage and further analysis. The entire sequence of the gene can be determined by sequencing methods such as Sanger method (Sanger et al. 1977). Bioinformatics analysis of the sequences is the efficient method to determine the phylogenetic origin of the organisms. The sequences can be compared with the gene sequence of other organisms already present in the database using a computerized approach called "Basic Local Alignment Search Tool" (BLAST).

1.5.3 Metagenomics

Metagenomics is a molecular technique used to study the microbial population present in environmental samples by directly analyzing the DNA present in it without being cultivate them. On the other hand, functional metagenomics allows genomic analysis and correlate with particular function observed in the environment exerted especially by the uncultivable microbes. The two modern techniques 'metatranscriptomics and metaproteomics' emerged from metagenomics allow deep functional analysis of microbial populations. The Metaproteomics technique aims to characterize the proteins synthesized by the microflora (Ghosh et al. 2019). The approach towards the exploration of microflora of biodiversity hotspots such as Western Ghats has been changed dramatically with the introduction of metagenomics technique.

1.5.4 DNA Microarrays

DNA microarray (DNA chips, microchips, biochips, gene chips) is a powerful high-throughput experimental approach that entertains the analysis of complementarity or relatedness between hundreds to thousands of genes at the same time (Schena et al. 1995; Guschin et al. 1997; Dugat-Bony et al. 2012). This methodology works on the basis of nucleic acids hybridization.

1.6 Observation and Enumeration

1.6.1 Cytometry Techniques

It includes a set of techniques used to measure and characterize the physical properties of individual cells and cellular components. Microscopy is the oldest and effective technique in this aspect. Light microscope, Phase contrast microscope, Differential Interference Contrast Microscope, Fluorescence Microscopy, The Confocal Scanning Laser Microscopy and different types of Electronic Microscopes comes under this category.

The laser-based biophysical technology 'flow cytometry' is another technique employed in cell counting, cell sorting, biomarker detection and protein engineering.

1.6.2 Activity Measurement at Cellular Level

Molecular probes (Fluorescent Probes) can be used to examine the physiological state of the cells. Such probes can be conjugated to a fluorophore with different targets (Joux and Lebaron 2000) that can be used to learn the physiological state of individual cells within a population. Nucleic acid specific fluorescent probes are frequently used in microbial ecology for cell counting as well as to analyze the nucleic acid content of individual cells that helps to distinguish between populations. The cells with high nucleic acid content are considered to be more active than those with lower amount of nucleic acids (Lebaron et al. 2001).

Microautoradiography can be used to characterize the metabolic activity of a cell by monitoring the degradation of radioactive labeled substrates. Those cells positive for the use or degradation of the substrate will be radioactive, that can be traced by a photographic emulsion and the precipitation of silver salts around each active cell.

In situ hybridization with fluorescent probes works on the basic principle of pairing between complementary nucleic acid sequences. This technique is useful to identify the presence of desired microbes in collected samples.

1.7 Measurement of Biomass

Microbial biomass can be indirectly ascertained by quantifying the characteristic cellular components or metabolites. If the component to be measured is unique for a particular population, it will be easy to get an indirect measure about the biomass of that population. In order get accurate results, the target compound should be present in the cell at fixed concentration and disappear immediately after cell death. Membrane lipids and lipid components of bacterial cell wall especially phospholipid fatty acids (PLFA) are the commonly targeted molecules for biomass estimation. Muramic acid is another marker used for such purposes. With the help of conversion

factors, microbial biomass carbon can be determined by correlating the average carbon per cell content and the result of cell counts obtained by microscopy or flow cytometry (Joux et al. 2015).

Phytoplanktonic biomass can be quantified by the estimation of pigments such as chlorophyll. In the more advanced version, the optical property of chlorophyll-a of phytoplankton makes it possible for satellite imaging. By absorbing light in the blue region of the visible spectrum, the Chlorophyll-a selectively modifies the photon flux that passes through the photic zone of the ocean. This absorption changes the spectrum of the sunlight reflected from the ocean (reflectance) (Joux et al. 2015).

1.8 Measurements of Heterotrophic and Chemoautotrophic Bacterial Production

Heterotrophic bacterial production can be ascertained by the radioisotopic techniques using thymidine or leucine (3H or 14C) incorporated DNA and protein (Fuhrman and Azam 1980; Kirchman et al. 1985). These techniques can be used for aquatic samples, sediments and soils. Dark [14C] bicarbonate assimilation can be used to determine the prokaryotic chemoautotroph activity (Herndl et al. 2005).

1.9 The Measurement of Bacterial Enzymatic Activities

The ability of microbial communities to enzymatically degrade different carbon substrates be determined using the Biolog EcoPlate™ system (Biolog Inc., CA, USA). The 96-well microplate system contains triplicates of 31 carbon substrates (amino acids, sugars, carboxylic acids, phenolic compounds, polymers) as well as controls in each well. A colorless tetrazolium dye is the essential component in all the wells. Degradation of substrates by the action of microbes can be detected by the formation of purple color due to respiratory activity of cells that reduces the tetrazolium salt. Color development can be quantified using a microplate reader at 590 nm. This method is compatible for different types of environmental samples such as water, soil, sediment and sludge (Zak et al. 1994; Montserrat et al. 2005; Preston-Mafham et al. 2002).

Natural substrate molecules such as amino acids or carbohydrates labeled with fluorescent molecules (methylumbelliferone (MUF) or the 4-methylcoumarinyl-7-amide (MCA)) can be used to determine the bacterial exoenzyme activity. After enzymatic hydrolysis the fluorophore will get detached from the substrate and fluorescence appears. The fluorescence can be quantified by a spectrofluorometer, and the enzyme activity is expressed as units of fluorescence per unit time (Hoppe 1991).

1.10 Use of Microsensors at Interfaces and within Sediments and Biofilms

The specific solutes and solute gradients in a microbial population can be measured with the use of chemical microsensors. For such purposes, the microsensor is introduced with a micromanipulator in a specific direction which moves in a programmed direction and it will stop at regular intervals to record the data.

2 Applications of Western Ghats's Microflora

2.1 Western Ghats Microflora as the Rich Source of Industrially Important Enzymes and Metabolites

Microbial enzymes play significant role in different aspects of product development such as food, cosmetics, beverages, medicines, etc. Such leading industries are currently focusing on engineered enzymes to enhance the catalytic activity to save time and also to improve the quality and quantity of the product. Since the protein engineering or enzyme modification seeks expertise and high cost financial investments, attempts have already been initiated to explore the natural sources such as unexplored microbial populations to find out improved versions of natural enzymes. Because of several scientific reasons, biodiversity hotspots such as Indian Western Ghats are the focus area to explore such populations.

Saravanan et al. (2015) have isolated nearly 32 bacterial isolates from the soil samples collected from the Shola forest, Western Ghats, Kodaikanal region, Tamilnadu, in which 62.5% of the populations were positive for protease, 28.1% for amylase and glutaminase, 65.6% for urease, 68.7% for agarase, 90.6% for pectinase, 12% for lipase and 31.5% for invertase. Logeswaran et al. (2014) have identified diverse enzyme producing bacterial populations in the soil samples collected from the Velliangiri hill of Western Ghats in Tamilnadu. *Bacillus mycoides, Rhodococcus equi, Sphingomonas koreensis, Bacillus subtilis, Clostridium clostridioforme, Bacillus mojavensis, Pseudomonas alcaligenes, and Paenibacillus glucanolyticus* were the identified species that produce industrially important enzymes such as amylase, cellulase, pectinase, proteinase, and lipase, in which, *Pseudomonas alcaligenes* alone produces all the five enzymes.

Extracellular lipase producing *Serratia marcescenes* strain SN5gR isolated from the fecal sample of lion-tailed macaque (*Macaca silenus*) is the another information about the enzyme producing bacteria from Western Ghats of South India (Gupta et al. 2013). Kuppusamy et al. (2012) have identified three different wood rot fungal species namely *Polyporus hirsutus, Daedalea flavida, Phellinus* sp1 from the Western Ghats area, of Tamilnadu and Karnataka with the potential to release ethylene from 2-keto-4-thiomethyl butyric acid by the action of extracellular enzymes.

The enzyme L-asparginase with anticancer potential was reported by Priya et al. (2011) from *Streptomyces* Sp (TA22) isolated from the soil sample collected from Western Ghats, near Thirunelveli district, Tamil Nadu. The metagenomic approach carried out by Vidya et al. (2011) for the isolation and characterization of novel α-amylase from the soil samples collected from silent valley region of Western Ghats encountered the amylolytic activity of clones at wide temperature range 60–80 C. Further 16S rRNA gene sequencing showed 95% similarities with *Exiguobacterium* sp. AFB-11 and AFB 18.

Dhamodharan and Rajasekar (2013) have isolated a pink pigmented *Methylobacterium* from the rhizosphere soil of Western Ghats. The characteristic pink pigmentation is due to the production of carotenoids which believed to protect the bacterium from extreme light and radiation. The pigment produced by the bacterium possesses added industrial benefits.

2.2 *Western Ghats Microbial Isolates with Biocontrol and Therapeutic Potential*

Literatures cited so far revealed the rich source of microbial populations in Western Ghats that can be molded to design and develop biocontrol and therapeutic agents to compete with life threatening infections and diseases. Pieces of researches described here signify the value of unexplored microbial treasure of Western Ghats of India.

Devadass et al. (2016) have isolated few strains of *Streptomyces* such as ERI-15, ERI-17, ERI-14 (*Salinispora arenicola*) and ERI-28 (*Micromonospora echinospora*) from the soil samples collected from soil samples collected from Kanyakumari, Tirunelveli, Dindigul, Coimbatore and the Nilgiri districts of Western Ghats in Tamil Nadu, India, with antimicrobial activity against a number of drug resistant bacterial and fungal pathogens. Shobha et al. (2014) have described the antifungal potential of a pigmented Streptomyces (KSRO4) isolated from Agumbe region of Western Ghats. Activity against skin pathogens such as *T.kannei, T. mentagrophyta* and *Microsporon gypseum* have received considerable attention.

An attempt made by Nair et al. (2015) to counterfeit the plant pathogens *Phytophthora capsici* (infecting pepper) and *Rhizoctonia solani* (infecting chickpea) have revealed the potency of bioactive agents produced by *Actinomycetes, Pseudomonas, Bacillus and Trichoderma* isolated from the western Ghats soil samples of Kerala region. *In vivo* treatment of infected plants with the isolates under greenhouse conditions have revealed significant reduction of the intensity of foot rot disease of black pepper and collar rot of chick pea.

Exploration of Western Ghats microbial diversity to tackle the emergence of multidrug resistant pathogens (Mohandas et al. 2012) have revealed the presence of microbial populations in Silent Valley forest soil producing β-lactamase inhibiting bioactive molecules. It was observed that, extracts collected from 45 isolates had promising inhibitory effect against b-lactam resistant *Bacillus cereus* strain (PL 10).

Among the 45 isolates, two strains were found to inhibit the extended spectrum β-lactamase (ESBL) producing *Klebsiella* ESBL1101 and three were inhibitory to methicillin-resistant *Staphylococcus aureus* (strain MRSA831).

A study conducted by Ramalakshmi and Udayasuriyan (2010) have identified enormous diversity of *Bacillus thuringiensis* in the soil samples collected from Western Ghats regions of Tamil Nadu, India. From 525 soil samples collected from the 14 different spots, they have introduced 316 new *B. thuringiensis* isolates that produce parasporal crystalline inclusions. They have observed seven different types of crystalline inclusions in the new isolates in which cuboidal inclusion was predominant in 26.9% of the isolates. In addition, significant differences were observed in the molecular weight of *B.thuringiensis* crystal proteins, they recorded six different types with molecular weights 135 and 65, 135, 95, 65, 43, and 30 kDa respectively. The observation indicates the diversity and adaptation of the natural biocontrol agent according to the requirement of different locations within the Western Ghats.

Arasu et al. (2009) have isolated *Streptomyces* spp. ERI-3 from forest rock soil sample of Western Ghats region, Tamil Nadu, India. The ethyl acetate extract of the isolate was found to be active against bacteria and fungi with minimum inhibitory concentration of 0.25 mg/ml against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Xanthomonas* spp. and *Candida albicans.*

2.3 *Plant Growth Promoting Microbes Reported from Western Ghats*

It is more common to identify the presence of plant growth promoting bacteria from different ecosystems associated with plants. However, the efficacy of such microbes may vary from place to place, especially when dense and diverse vegetation occurs. Vast array of microbial populations with tremendous plant growth promoting activity can be observed in forest soils than in any other area. The reason behind such microbial diversity is the diverse vegetation and complex interactions between them in the forest environment. Several reports are coming out from the Western Ghats region of India, regarding the improved plant growth promoting activity exhibited by novel microbial strains.

Three new bacterial strains '*Micrococcus* sp NII-0909' (Dastager et al. 2010), a Gram negative, rod shaped "*Pantoea* NII-186" (Dastager et al. 2009) and *Bacillus thioparus* NII-0902 (Kumaran et al. 2010) with plant growth promoting activity. Their efforts to determine the plant growth promoting activity have revealed that all the isolates exhibited phosphate solubilization, 1-aminocyclopropane-1-carboxylate deaminase activity, HCN, auxin and siderophore production. It was observed that the release of organic acids in to the surroundings facilitate the P-solubilizing activity of *Micrococcussp* NII-0909. Differential expression of the described properties by

Bacillus thioparus NII-0902 under wide temperature range (5–40 ° C) make it different from the other two isolates.

Efforts have also made to identify the microorganisms from Western Ghats with plant growth promoting activity as well antagonistic activity against plant pathogens. In this aspect, Western Ghats oriented study conducted by Amanna et al. (2018) further revealed the antagonistic effect of some rhizosphere and endorhizosphere bacterial isolates from niches of Western Ghats, Karnataka against major fungal and bacterial plant pathogens. The endorhizospheric *Pseudomonads*, *Rhizobium* sp. and other isolates were found to produce a series of antimetabolites such as phenazine, phloroglucinol and pyrrolnitrin. In addition, the isolates exhibited varying levels of cyanogenesis, siderophore production, volatile antimetabolites and indole acetic acid production. The molecular techniques revealed that the isolates belonged to 3 major groups namely alphaproteobacteria, gammaproteobacteria and bacteroidetus.

Culture oriented and metagenomic approach made by Kumar et al. (2013) using the rhizosphere soils of *Rauwolfia* spp. collected from Western Ghats regions of Karnataka indicated the prevalence of *Pseudomonas* sp. followed by *Methylobacterium* sp., *Bacillus* sp. and some uncultivable bacteria. From the 58 rhizosphere soil samples, they have identified nearly 200 rhizobacteria from 15 different bacterial genera. Considerable differences in species richness have been identified between different sample collection spots. In summary, out of the 70% of the isolates colonized on tomato roots, 42% were able to produce indole acetic acid, 55% were capable of phosphate solubilization and considerable percent of population were found to produce siderophore, salicylic acid, hydrogen cyanide, chitinase, phytase, cellulase and protease, respectively. Nearly 33% and 53% of the isolates exhibited anatagonistic activity against the fungal pathogens *Aspergillus flavus* and *Fusarium oxysporum* respectively.

Similarly, another *Bacillus* sp. WG4 has been reported from the Western Ghats regions of Kerala with plant growth promoting activity as well as antagonistic activity against the plant pathogen *Pythium myriotylum.* The researchers have claimed the presence of pyrrolo [1,2- a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) as the basis of antifungal activity. In addition, synergistic antifungal effect of *Bacillus* sp. WG4 was observed in combination with standard biocontrol agent *Trichoderma* sp. (John et al. 2016).

2.3.1 Mycorrhizal Association

A survey made by Thangavelu and Udaiyan (2000a) in the Western Ghats region of Southern India using the root and soil samples of plants growing in forest, grassland, scrub, and cultivated land or plantation had revealed that out of 329 plant species examined, 174 species (representing 61 families) were involved in mycorrhizal association. Of these, arbuscular mycorrhizal association was observed in 81 species. Arbuscular mycorrhizal fungal spores of 35 species belonged to *Acaulospora*, *Gigaspora*, *Glomus, Sclerocystis* and *Scutellospora*. According to their findings, the scrub vegetation was rich in arbuscular mycorrizal association, whereas the

agricultural and plantation soil exhibited lower levels of such association. This indicates that the impact of human intervention on such plant-microbe interactions. Another research conducted by the same team (Thangavelu and Udaiyan. 2000b) have shown that 60 out of 71 species of pteridophytes had vesicular arbuscular mycorrhizal association. The spores of nine vesicular arbuscular mycorrhizal fungi belonged to *Acualospora, Glomus, Sclerocystis* and *Scutellospora*.

2.3.2 Microflora of Western Ghats with Environmental Applications

Recent researches have shown the diversity of environmentally important microorganisms in the Western Ghats region. Majority of such isolates have the potential to enhance the bioremediation of variously contaminated land and water bodies. Most of them have attractive functions like degradation of potentially hazardous aliphatic and aromatic compounds such as long chain hydrocarbons, textile dyes, pesticides and so on.

Johnson et al. (2020) have reported a lignolytic *Bacillus cereus* WGB1 from the Soil samples collected from the foothills of Marudhamalai area of Western Ghats. They have claimed that *B.cereus* WGB1 has the potential to degrade methylene blue dye that may cause serious environmental impacts on improper discharge (Balcha et al. 2016). Similar experiment conducted by Selvam et al. (2003) have reported the azo dye decolorization ability of a white-rot fungi *Fomes lividus*, isolated from the logs of *Shorea robusta* in the Western Ghats region of Tamil Nadu, India. According to their findings, the fungi can efficiently decolorize azo dyes such as orange G, congo red, amido black 10B and also remove colors from dye industry effluents. Another experiment conducted by Selvam et al. (2012) have reported the efficacy of two lignin degrading white rot fungi '*Schizophyllum commun'e* and *'Lenzites eximia'* isolated from live and burnt tree of *Tramarindus indica* from the Western Ghats region of Tamil Nadu, India to decolorize azo dyes. It was observed that both the isolates can decolorize the azo dyes congored, methylorange, erichrome black- T and also the dye industry effluents.

Yamuna et al. (2017) have introduced few wood rot fungal isolates namely *Daldenia concentrica, Lepiota sp.* and *Trametes serialis* collected from the Western Ghats region of Tamil Nadu, India that are exhibiting similar properties. Their team had screened the fungal isolates for their ability to degrade the azo dyes such as orange G, methyl orange and congo red. From their findings, *Lepiota* sp. was found to be the most effective in decolourizing azo dyes especially orange G. Whereas, *Daldenia concentrica* and *Trametes serialis* were found to be effective on methyl orange and Congo red respectively.

Stenotrophomonas rhizophila isolated from the oil contaminated soil composts from Western Ghats region of Karnataka had shown its ability to utilize crude oil and poly aromatic hydrocarbons (Praveen Kumar and Manjunatha 2015). Singh and Sedhuraman (2015) have screened a number of endophytic non-streptomycetes groups of actinomycetes from *Hibiscus rosasinensis* leaves from the Western Ghats region for the identification of biosurfactant, polythene, plastic and diesel

degradation properties. They have identified a new strain *Nocardiopsis* sp. *mrinalini*9 with the potential to exhibit the indications of above mentioned properties.

Sunitha and Onkarappa (2018) reported two isolates of *Streptomyces* sp. (SJRO-06 and SJRO-10) from the coffee plantation soil of Western Ghats region of Chikmagalur, Karnataka, India. The isolates were found to degrade the pesticide 'chlorpyrifos'.

2.3.3 Quorum Sensing and Quorum Quenching Phenomenon Reported from Western Ghats

The term quorum sensing implies the regulation of gene expression in response to variations in cell or population density. Based on cell density, the quorum sensing bacteria synthesize and release varying levels of chemical signals (autoinducers). Detection of autoinducers in threshold concentration by the other members of the communicating network will leads to the activation or repression of a specific gene as per the requirement (Uroz and Heinonsalo 2008). Both Gram-positive and Gram-negative bacteria uses quorum sensing signals to regulate a number of events such as symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. Gram-negative bacteria use acylated homoserine lactones as the autoinducer, whereas processed oligo-peptides acts as the communication signal for Gram-positive bacteria (Dong et al. 2000; Miller and Bassler 2001).

In contrast to quorum sensing, quorum quenching activity is the inhibition of quorum sensing molecules and related events. In this aspect, there is an increased demand for the quorum quenching molecules from natural sources to resist quorum sensing related consequences such as bacterial colonization and drug resistance (Barrios et al. 2009; Morohoshi et al. 2013). Degradation of acylated homoserine lactones can be achieved by the action of acylases and lactonases on the acyl side chains and lactone ring respectively (Rashid et al. 2011). In the recent past few findings were reported from the Indian Western Ghats regarding the quorum quenching molecules from natural sources.

Vadakkan et al. (2018) have reported the quorum sensing inhibitory effect of the root extract of *Solanum torvum* collected from the Western Ghats region of Thrissur District, Kerala, India against *Chromobacterium violaceum*. Another experiment conducted by Rajesh and Ravishankar Rai (2014) in Western Ghats region have revealed the N-acyl homoserine lactones degradation by the endophytic bacteria *Bacillus firmus PT18 and Enterobacter asburiae* PT39 present in *Pterocarpus santalinus*. In their experiment, they have observed 80% inhibition of violacein production in a biosensor strain and considerable inhibition of biofilm formation by *Pseudomonas aeruginosa* (Strains PAO1 and PAO1-JP2). In a similar experiment (Shastry et al. 2018), the N-acylhomoserine lactone hydrolytic activity of a Gram-negative endophyte *Enterobacter* sp. CS66 isolated form *Coscinium fenestratum* Gaertn collected from forest of Western Ghats in Karnataka, India. Mani et al. (2017) have isolated a new strain of pigment producing *Streptomyces hygroscopicus* AVS7

(KP732441) from the Western Ghats of India. The pigment was found to be a carboxylic acid derivative with quenching activity against the quorum sensing molecules produced by *Staphylococcus aureus*.

3 Threats and Conservation of Western Ghats

According to the reports of International Union for Conservation of Nature (IUCN), the geography and wildlife of Western Ghats is under high risk due to forest loss, encroachment and conversion. Decades ago, the Western Ghats were covered by dense forests, the increasing human population continuously exerts developmental pressure on forest land and most of it has already been converted. Due to human intervention major part of Western Ghats forest land have been converted to agricultural land or cleared for livestock grazing, timber industry, housing, reservoirs and roads. Mining of metal ore in an illegal way exerts serious ecosystem damage, Western Ghats region of Goa is an example. In Kerala region, sand mining is the leading cause of Western Ghats destruction. As a consequence of sand mining, the people living in that area had victim vulnerable landslides in the last two years that haunted hundreds of lives.

Intrusion of human population into forest land causes human-wildlife conflict which seriously affects wildlife as well as human being. Further, extraction of forest products such as spices, herbal medicines, timbers and so on for commercial purposes is a critical issue that affects the biodiversity of protected areas. Pollution in any sense emerging from the close premises or from the nearby urban areas is of greater threat to the ecosystem. Use of agrochemicals such as chemical fertilizers, pesticides and herbicides for the plantations adversely affect the flora, fauna and microflora of any ecosystem.

The above mentioned activities significantly affect the vegetation which ultimately results in unpredictable climatic changes. Abnormal climatic change in biodiversity hotspots like Western Ghats exerts potential threat on the existence of endangered endemic species. This statement signifies the consequences of developmental pressure on the biodiversity in Western Ghats. Even after the Government has initiated a number of projects and plans to conserve Western Ghats and its biodiversity, this is an alarming situation to take strict measures to protect it further. Protected Area network, tiger reserves and biosphere reserves are the general strategies that the Government has taken to conserve the biodiversity of Western Ghats. As a result, nearly 10% of the total area of Western Ghats is currently declared as protected area.

4 Conclusion

It is summarized that the biodiversity hotspot 'Indian Western Ghats' is a home for a number of endangered endemic species. The microbial diversity of Western Ghats is less explored when compared to higher organisms. The difficulties associated with the study of microbes from environmental samples are the prime reason for the less exploration. Study of microbial diversity in biodiversity hotspots is an important field in many aspects. The microbial interactions and their role to serve the higher organisms and vegetation are the primary reason that demands detailed investigations on microbial diversity in an ecosystem. The microflora of an environment keep on working to contribute soil fertility and healthy environment by recycling minerals and excluding pathogenic invaders. The chemical mediators involved in such interactions also serves as therapeutics, nutrients, food additives, etc. The study of microflora in biodiversity hotspots like Western Ghats without disturbing the natural environment can serve the humanity in many aspects. At the same time, conservation strategies should also be implemented on an emergency basis to conserve the diversity and species richness of biodiversity hotspots.

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Endophytic Microorganisms of Western Ghats: Diversity and Biosynthetic Potential

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Abstract Endophytes are microorganisms that reside within the healthy plant tissues in a symbiotic association. Endophytes can be bacteria, fungi, or actinomycetes. They act as a reservoir of many bioactive secondary metabolites beneficial to the environment, agriculture, and industries such as the pharmaceutical industry. These organisms also play important roles in nutrient cycling, biodegradation, and bioremediation. The endophytic populations are greatly affected by the climatic conditions and the location where the host plant grows. Western Ghats of India represents unique regions of biologically diverse areas called "hotspots" which harbors numerous species of plants, animals, and microbes. A large variety of endophytic organisms are residing in these regions. This chapter is an effort to explore the diversity of endophytic microorganisms of the Western Ghats and their biosynthetic potential.

1 Western Ghats: Microbial Diversity and Biosynthetic Potential

1.1 Western Ghats

The Western Ghats is one of the most unique ecological niches of India, perhaps of the whole world. Flanked between the Arabian Sea in the West and the parts of Deccan plateau and the Eastern Ghats in the East, it spans an area of over 140,000 km^2 transversing the states of Tamil Nadu, Kerala, Goa, Maharashtra and Gujarat. Often called the Great Escarpment of India, it is a UNESCO world heritage site and one among the most recognized biodiversity hotspots in the world. Many areas of Western Ghats are protected by the establishment of 2 biosphere reserves,

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13 National Parks, that helps preserve the ecoregion. The largest protected area in the Western Ghats is the Nilgiri Biosphere Reserve which is comprised of many national parks (e.g. Silent valley, Bandipur) and wildlife sanctuaries (e.g. Sathyamangalam, Wayanad). The magnificent mountain range is considered a 'gene pool' harbouring a huge variety of animal, plant and microbial species. Just under 6% of the total land area of India, it is home to more than 30% of the total plant, fish, bird and mammalian species found in India. The presence of over 7402 species of flowering plants, 1814 species of non-flowering plants, 139 mammalian species, 6000 species of insects and 290 freshwater species (Nayar et al. 2014) make the niche so diverse and unique. Among the numerous species found in the Western Ghats, about 325 species are globally threatened and 1800 species are endemic to this region. The efforts of the ecologist Norman Myers made the Government of India recognize it as an area sensitive to any development and thus was declared as a hotspot in 1988. This is because the complex, species-rich habitats like tropical rainforests are much more adversely affected than any other habitat. Among the various classes of organisms in the Western Ghats, microorganisms seem to be the most diverse group; many species have not been discovered and identified yet.

1.2 Microbial Diversity

Many recent studies such as water sample analyses form various regions of Western Ghats show a high abundance of microbial diversity including species of Proteobacteria (58%), Firmicutes (26%), Actinobacteria (13%) and Bacteroides (3%) (Ruckmani and Chakrabarti 2011). About 300 new varieties of *Bacillus thuringiensis* were isolated from the Western Ghats which produce 7 different types of parasporal crystalline inclusions. Other novel bacterial species/strains identified recently from various samples of mud and soil of the Western Ghats are the *Rhodobacter viridis* [Strain JA737 (T)] (Raj et al. 2013), *Pontibacter niistensis* and *Paracoccus niistensis* (Dastager et al. 2010). Thermotolerant fungal species such as 9 species of Ascomycota were isolated from the Western Ghats which opens doors for the identification of the molecular and physiological mechanism of heat resistance. On examining 329 plant species, about 174 species showed an arbuscular mycorrhizal association. Among this, 81 species which were previously considered non-mycorrhizal, were identified to have mycorrhizal association. Many new species such as *Gliocephalotrichum longibranchium, Gliocephalotrichum bulbilium* were also isolated from this region (Singh et al. 2012). A systemic mapping of the microalgal population in Kerala identified 33 algal taxa and discovered 3 new species (John and Francis 2012).

1.3 Biosynthetic Potential

Soil samples from the Western Ghats are a rich source of Plant Growth Promoting Rhizobacteria (PGPR) like *B.thioparus* (NII0902) *Microcous* sps (Dastager et al. 2010) etc. Several bacterial species coming under *Streptomyces* sp. and, *Exiguobacterium sp are* potential source of industrially relevant enzymes like asparaginase and amylase (Mohana Priya et al. 2011, Vidya et al. 2011). Soil fungi *Tolypocladium* sps. Have potential anti-microbial activity and produce a cyclosporine related compound which can be exploited as an immunosuppressant drug (Jadhav et al. 2007). Several bacteria isolated from the Western Ghats soil are shown to control the growth of phytopathogens, *Rhizoectonia solania* (Islam et al. 2012). Thus, the vast diversity of the Western Ghats presents before us a tremendous opportunity to explore them for human welfare.

2 Endophytic Microorganisms of Western Ghats

2.1 Endophytes

An endophyte is a bacterium or fungus that resides within a plant either for the entire life or during certain stages of its life cycle in a endosymbiotic relation. It does not cause any apparent disease/damage to the host. Endophytes maintain their life cycle in such a way that they enhance host growth, improve the host's ability to tolerate abiotic stresses such as drought, salinity, toxicity due to heavy metals, oxidative stress and to biotic stresses like herbivory, attacks by insects, pests and pathogens etc. Endophytes were first discovered by the German botanist, Johann Heinrich Friedrich Link in 1809. They are transmitted either vertically (through reproduction) or horizontally (among plant individuals). Vertical transmission occurs when fungal hyphae residing the parent plant penetrate the embryo while horizontal transmission occurs through asexual conidia or sexual spores that spread to other plants in a community.

More than one endophyte could be found in nearly every land plant. The areas of high diversity such the Western Ghats have the highest diversity of endophytes with novel and diverse chemical metabolism. There could be approximately one million species of endophytic fungi in the world. Many species belonging to the fungal phyla Ascomycota, Basidiomycota; especially the members of Hyporales and Xylariales of class Pyrenomycetes are found to exist as endophytes. Another interesting group of endophytes, arbuscular mycorrhizal fungi such as Glomeromycota is seen associated with various plants. Bacterial endophytes have been discovered that belong to a broad range of taxa including alpha-Proteobacteria, beta-Proteobacteria, gamma Proteobacteria, Firmicutes etc. There are two different systems of classification of endophytes. The first system classifies them based on the genetics, biology and mechanism of transmission into two categories-Systemic (true) or non-systemic

(transient). The former category includes endophytes that live in the host for their entire lifetime and whose concentration and diversity would not change with changes in the environment, whereas the latter category includes those that vary in the diversity and number as there occur changes in the environment. The second system classifies endophytes based on taxonomy, host range, colonization, fitness as Clavicipitaceous (or Class I) and non- Clavicipitaceous (Include classes II, III, IV). Clavicipitaceous endophytes proliferate within grasses and are transmitted vertically. Most of the species of non-Clavicipitaceous endophytes belong to Class Ascomycota which are associated with nearly all land plants and can switch between endophytic and free-living lifestyles.

Most of the endophyte-plant relations are not well understood yet. The endophytes and their hosts have a mutualistic relationship. Studies have shown that fungi show wedged or flattened growth pattern against plant cells indicating that the hyphae are attached to the host cell wall. It is also noted that all plant-endophyte relation is not strictly mutualistic as some of the transient endophytes may evolve to pathogens as resources get limited or when the host plant is stressed. Endophytes are seen to actively reproduce when the host is under stress or begin to senesce.

2.2 Importance of Endophytic Microorganisms

Endophytes offer a plethora of advantages to host with immense applications in agriculture and medicine. Mutualistic behaviour of the endophytes aid in the health and survival of the host plant that has been affected with pathogens or that grows in an environment with stresses of any kind such as water stress, heat stress, nutrient availability, poor soil quality, salinity, herbivory *etc*. In addition to this, the endophytes that extensively colonize plant tissue can competitively disrupt the activity of any pathogen and can increase the growth and improve overall plant hardiness. The presence of certain endophytes in the meristems and the reproductive tissues can enhance the survival of the host as a result of the influence of secondary metabolites released by the endophytes on increasing the nutrient uptake. Plants rely on their endophytic relation during the circumstances of light-limited conditions. Crops are (Bacon and Hinton 2014) shown to grow faster and more resistant than crops lacking any endophytic relations. *Piriformospora indica* is an example of such a fungus that colonizes plant roots.

Certain attributes to the resistance provided by endophytes are due to the production of secondary metabolites such as the ones produced by *Phoma eupatorii* that inhibit the phytopathogens *Phytophthora infestans* (de Vries et al. 2018). Some of the secondary metabolites produced are non-edible and toxic to consumers and thus protects the host plants from herbivory. One such example is the protection of ryegrass from Argentine stem weevil by endophytes AR1 and AR37 (Le Cocq et al. 2017) and protection of grasses against insects due to the release of toxic alkaloids. Endophytes enhance the uptake of land limited minerals such as Phosphorus and Nitrogen and thus make them accessible to the plants. The Endophytic

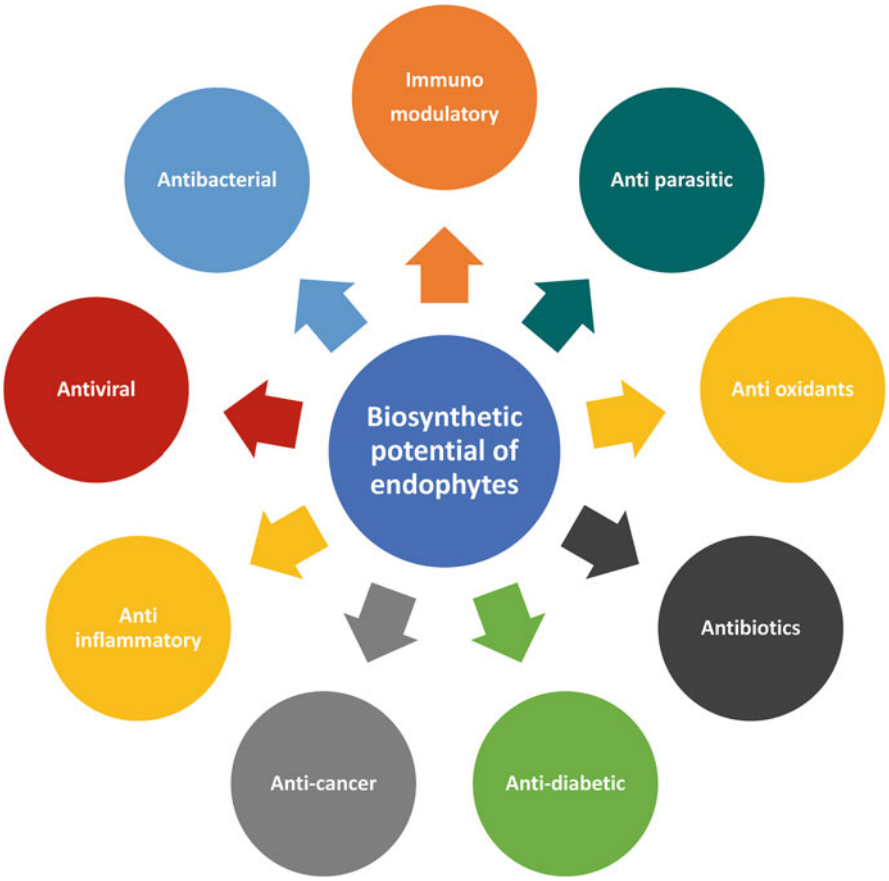

Fig. 1 Biosynthetic potential of endophytic microorganisms

bacterium, *Paenibacillus polymyxa* have roles in fixing atmospheric nitrogen into accessible forms. Several species of endophytes can produce organic materials like polyenes, leptons. Many fungi have also demonstrated to digest polyurethane to some degree (Russell et al. 2011). Endophytes produce some compounds which can be used as insecticides and fungicides in Integrated Pest Management. The biosynthetic potential of endophytes that can be utilized in medicine and pharmaceutical industries are summarized in Fig. 1.

3 Endophytic Fungi of Western Ghats and their Biosynthestic Potential

Plants growing in the biodiversity hotspots such as the Western Ghats and having ethnobotanical utility are often explored for the presence of endophytic fungi with bioactivity potential. Several compounds extracted from such endophytic fungi of the Western Ghats are useful as anti-bacterial, anti-fungal, immunosuppressants, anti-cancerous agents etc. Endophytic fungus living on angiosperm species of the Western Ghats, *Nothapodytes foetida, Hypernicum mysorense, Hypernicum japonium* does not show any host preference or species dominance. Most of these had antimicrobial activity but among them, the prominently noted species was *Bionectaria ochroleuca* (Samaga et al. 2014). The fact that many Aspergillus species in the Western Ghats are good source of antifungal compounds like heptanone and furfural derivatives make them a potential source of developing antimicrobial drugs. Phomochromone A, Phomochromone B and Phomotenone are the metabolites extracted from the endophytic fungus Phomopsis sps which shows good anti-fungal, anti-bacterial and anti-algal activity (Ahmed et al. 2011). Xylaria endophytes in Western Ghats produce several industrially relevant enzymes like cellulase, laccase and lipase which could be suitable for biotechnological utilization. The anti-parasitic activity was observed in the endophytes living in tree barks of Western Ghats (Kaushik et al. 2014). Endophyte fungi belonging to genus Alternaria, Fusarium, Xylaria, Penicillium, Trichoderma can be explored further for developing anti-malarial drugs.

Fusarium species represent an interesting class of endophytic fungi found in the Western Ghats that has anti-microbial and anti-cancerous potential. *Fusarium oxysporoum* produce vinblastine and vincristine which are potential anti-cancerous drugs (Kumar et al. 2013). The taxol drug, paclitaxel from *Fusarium solani*, and Camptothecin 2, a quinolone alkaloid from the *Entrophospora infrequent* are potent anticancer compounds from fungal endophytes of Western Ghats. Similar chemical products are extracted from various endophytic fungi that have enormous applications in the treatment of various diseases such as cancer (Chakravarthi et al. 2008, Madhusudhan et al. 2015, Kharwar et al. 2011). 9-Deactoxy fumigaclavine C 11 isolated from *Aspergillus fumigatus* an endophyte obtained from the healthy stem of *Cynodon dactylon* showed high cytotoxicity against human leukaemia cells whose action is similar to that of Doxorubicin Hydrochloride, a drug currently used in treating leukemia (Ge et al. 2009). *Chaetomium globosum* HYML55 from *H.mysorense* produce, Chaetoglobosin F, an anti-inflammatory and anti-neoplastic compound (Samaga et al. 2014) and *Tragopogon graminivorous* produce a wound healing compound Luteolin (Bayrami et al. 2018).

Another important category of products is the one that is involved in the biosynthesis of Silver Nano particles (AgNPs). They exhibit anti-oxidant and anti-disease characters. AgNPs biosynthesized from Cladosporium cladosporiodes—an endophyte of *Loranthus micranthus* seen in the Western Ghats—has antioxidative capability comparable to that of ascorbic acid (Popli et al. 2018). The Nanoparticles

Table 1 Endophytic fungal species of Western Ghats and its potential application

S. No	Fungal endophyte	Host	Uses	References
1.	*Entrophospora infrequens*	*Nothapodytes foetida*	Camptothecin 2 synthesis to treat ovarian and lung	Kharwar et al. (2011)
2.	*Cladosporium cladosporiodes*	*Loranthus micranthus*	Silver Nano particles (AgNPs) exhibiting anti-oxidant and anti-disease characters	Popli et al. (2018)
3.	*Tragopogon graminifolius*	Many plant species	Wound healing compound Luteolin synthesis	Bayrami et al. (2018)
4.	*Chaetomium globosum* HYML55	*Hypericum mysorense*	Chaetoglobosin F production	Samaga et al. (2014)
5.	*Aspergillus fumigatus*	*Cynodon dactylon*	Cytotoxicity against human leukemia cells	Ge et al. (2009)
6.	*Pestalotiopsis microspora*	*Taxus wallichina*	Taxol drug production against cancer	Madhusudhan et al. (2015)
7.	*Fusarium oxysporum*	*Rhizophora annamalayana*	Taxol drug production against cancer	Elavarasi et al. (2012)
8.	*Fusarium mairei* UH23	Many species	Taxol drug production against cancer	Dai and Tao (2008)
9.	*Fusarium solani*	*Taxus celebica*	Paclitaxel production against cancer	Chakravarthi et al. (2008)
10.	*Fusarium oxysporoum*	*Juniperus recurva, Catharanthus roseus*	Vinblastine and vincristine, Podophyllotoxin	Kumar et al. (2013)
11.	*Bionectaria ochroleuca* NOTL33	*Nothapodytes foetida*	Anti-bacterial and anti-fungal properties	Samaga et al. (2014)
12.	*Alternaria longipes*	*Cllicarpa tomentosa*	Anti-bacterial properties	Raviraja et al. (2006)
13.	*Phomopsis* sps	Many species	Anti-bacterial and anti-fungal properties	Ahmed et al. (2011)
14.	*Xylaria* sps.	*Psychotria bisulata*	Anti-bacterial properties	Govinda Rajulu et al. (2013)
15.	*Nigrospora oryzae*	*Macaranga peltata*	Anti-plasmodia	Kaushik et al. (2014)

also possess anti-diabetic effect as it inhibits the enzymes α-amylase and α–glucosidase responsible for the hydrolysis of complex carbohydrates. This also shows anti-cholinesterase activity, which can be utilized to inhibit cholinesterase enzyme as a remedy to treat Alzheimer's disease. Important endophytic fungal isolates of Western Ghats and their potential applications are shown in Table 1.

4 Endophytic Bacteria and their Biosynthetic Potential

The Western Ghats is rich in endophytic bacterial diversity. Natural products of endophytic bacteria possess bioactivities such as anti-fungal, anti-carcinogenic, anti-oxidant properties. Bacterial endophytes of the Western Ghats are usually members of the genus Bacillus and Streptomyces. Several biosynthetic compounds have been identified and isolated from the endophytic bacteria of these regions. *Bacillus megaterium* isolated from *Garcinia indica* produces a lassopeptide similar to paeninodin that inhibits the growth of *Escherichia coli* (Webster et al. 2020). *Enterobacter asburiae* isolated from *Coscinium fenestratum* produces a thiopeptide that is useful against *Escherichia coli* and many other bacteria. Bacillus sps isolated from *Pterocarpus santalinus* produce lantipeptide and bacteriocin BGC. *Serratia marcescens* isolated from *Memecylon malabaricum, Coscinium fenestratum* possesses anti-cancerous and anti-malarial compound prodigiosin (Khanam and Chandra 2018). Identification of potent Plant Growth promoting Rhizobacteria and biocontrol agents from these regions promise the exploration potential of endophytic bacteria in Western Ghats (Bashan et al. 2008). Endophytic bacterium *Gluconacetobacter diazotophicus* from various tropical and sub-tropical plants of the Western Ghats show high Indole acetic acid production, Phosphorous and Zinc solubilization etc. (Madhaiyan et al. 2004).

Streptomyces that have been isolated from the region produce compounds having anti-microbial, anti-cancer (Mohana Priya et al. 2011), anti-malarial (Sankarganesh and Joseph 2019), and anti-diabetic potential (Akshatha et al. 2014). Anti-oxidant compound Gancidin was isolated from *Streptomyces paradoxus* VITALK03 (Ravi et al. 2017). An anti-fungal compound like Ketoconazole was produced by *Streptomyces thermocarboxydus* strain (Passari et al. 2017). Important endophytic bacterial isolates of Western Ghats and their potential applications are shown in Table 2.

5 Conclusions and Future Directions

The vast diversity of endophytes and the immense biosynthetic potential of their secondary metabolites make the Western Ghats "Treasure House" for the scientists. The search for novel anti-cancer agents as well as compounds derived from nature is critical. Serious side effects and high cost of the present therapeutic agents underscores the importance of using such natural compounds. However, it is interesting to note that, only a single novel anti-cancer agent—paclitaxel—was identified from endophytic sources during 1990–1995 but their numbers hit 75 during 2006–2010 that included compounds such as podophyllotoxin, hypericin *etc*. As chemists turn their attention to endophytic fungi, the number of new compounds isolated should increase over the next few years. A fungal source of the anti-cancer agent is much valued as it is virtually an inexhaustible source of metabolites and if budget-friendly, they provide a promise of drugs that could be affordable even to the poor for disease

Table 2 Endophytic bacterial species of Western Ghats and its potential application

S. No	Endophytic bacteria	Host	uses	reference
1.	*Bacillus tequilensis, Chryseobacterium indologenes, Bacillus aerophilus, Pseudomonas entomophila, pseudomonas hibiscola, macrococcus caseolyticus, Enerobacter ludwigii*	*Aloe vera*	Anti-microbial and anti-oxidant activity	Akinsanya et al. (2015)
2.	*Aureimonas pyllosphaerae* DSM25024 and *Aureimonas jatrophae* DSM25025	*Jatropha curcas*	Anti-disease properties	Madhaiyan et al. (2004), Vasundhara et al. (2016)
3.	*Bacillus aryabhattai*	*Pterocarpus santalinus*	Anti-fungal activity *Pectobacterium carotovorum*	Webster et al. (2020)
4.	*Bacillus megaterium*	*Garcinia indica*	Lassopeptide production that inhibited the growth of *Escherichia coli.*	Webster et al. (2020)
5.	*Bacillus thuringiensis*	*Aphanamixis polystachya* and *Madhuca indica*	Bt toxin production	Webster et al. (2020)
6.	*Enterobacter asburiae*	*Coscinium fenestratum*	Thiopeptide production against *Escherichia coli*	Webster et al. (2020)
7.	*Gluconacetobacter diazotophicus*	Various tropical and sub-tropical plants	Indole acetic acid production, phosphorous and zinc solublization	Madhaiyan et al. (2004)
8.	*Lactobacillus* sps.	*Adathoda boddemei*	Anti-cancerous	Swarnalatha and Saha (2016)
9.	*Pseudomonas asphenii*,	Many sources	A potent plant growth promoting Rhizobacteria	Bashan et al. (2008)
10.	*Serratia marcescens*	*Memecylon malabaricum, Coscinium fenestratum*	Anti-cancerous and anti-malarial compound prodigiosin	Webster et al. (2020), Khanam and Chandra (2018)
11.	*Streptomyces* sp. strain BPSAC101 and *Streptomyces thermocarboxydus* strain BPSAC147	*Rhynchotoechum ellipticum*	Anti-fungal	Passari et al. (2017)
12.	*Streptomyces* sp. strain BPSAC121	*Rhynchotoechum ellipticum*	Anti-cancerous and anti-microbial	Passari et al. (2017)
13.	*Streptomyces* BJSG4			

(continued)

Table 2 (continued)

S. No	Endophytic bacteria	Host	uses	reference
		Kalanchoe pinnata	Anti-malarial activity	Sankarganesh and Joseph (2019)
14.	*Streptomyces longisporoflaxis* and *Streptomyces* sp. JQ26174	*Leucasciliata* and *Rauwolfia densiflora*	α-Amylase production	Akshatha et al. (2014)
15.	*Streptomyces paradoxus* VITALK03	Many species	An anti-diabetic and anti-oxidant properties	Ravi et al. (2017)
16.	*Streptomyces* sp.TA22	Various sources	Anti-cancer enzyme L-asparagine production	Mohana Priya et al. (2011)

treatment. Further scientific exploration of the Western Ghats will surely help identify and commercialise novel compounds for disease management and cure and thus improve the quality of human life.

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Lichen Flora in Western Ghats of Kerala, India: A Source of Innovation

Biju Haridas, Sabeena Aliyarukunju, and Shiburaj Sugathan

Abstract Lichens are symbiotic plant-like organisms composed of a fungal partner (mycobiont) and one or more photosynthetic partners (photobiont). The fungal partner may belong to Ascomycetes, Basidiomycetes, or rarely Deuteromycetes, and the photobiont is often green alga or cyanobacterium. They are classified based on the fungal component along with the fungal system. The Western Ghats in India are rich in biological diversity and vital to their endemism. More than 325 globally threatened plant and animal species are found in the region. Situated along the southwest corner of India, bordered by the Lakshadweep Sea, the State of Kerala, with an area of 38,863 km^2 is considered one of India's peaceful and cleanest states. The varied topographical features, high rainfall, and geologic conditions have favored different ecosystems, from shola forests on the mountain valleys to the mangrove forests along sea coasts and estuaries.

Globally 20,000 species of lichens are known. While in India, it is 2907 species representing 14.8% of the world's flora. These are distributed under 406 genera and 79 families. Unfortunately, only fragmentary work has been done to characterize Lichens in the Western Ghats of Kerala state. Presently, the Western Ghats of Kerala account for over 800 species of lichens. This chapter discusses lichen diversity in the Kerala part of the Western Ghats, the history of lichenological investigation, the medicinal and economic importance of lichens in the region, and their conservation problems.

Keywords Biodiversity · Kerala · Lichenized fungi · Western Ghats

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1 Introduction

Lichens are one of the critical components of the Indian flora. The vast topographical and climatic diversity of ecologically interesting areas in India has gifted with a rich lichen flora, both in variety and abundance. The study of Lichens is relatively neglected throughout India. The total number of recorded species in India is relatively small compared to the world average. Despite the intense exploration and survey efforts during the last five decades, our knowledge about lichens from different India's floristic regions is incomplete. Many areas are still unexplored for their lichen wealth. The rapid destruction of habitats, increasing atmospheric pollution, over-exploitation, grazing, frequent forest fires, and other anthropogenic interferences are responsible for a decrease in the population of lichens worldwide and the Western Ghats. In the rich ecosystem of the Western Ghats of Kerala, there is a lack of knowledge regarding lichen diversity, ecology, and distribution patterns. Therefore, inventorization is highly valuable to understanding the status of lichens in the Western Ghats. Lichens are a rich resource of novel bioactive compounds mostly not reported in other plant groups. Most of the secondary compounds produced by lichens have antibiotic properties. Recent research shows that our knowledge of lichen bioprospection is still minimal and exploration of lichens is may bring many more new species. The country's vast and diverse topographical area exhibits rich diversity of lichens, which provided many scopes to utilize these organisms for their potent biomolecules. The lichens may be a good source of unique phytochemicals; however, not much work has been done for medicinal bioprospection and chemistry in Kerala, probably due to their non-availability in bulk and slow growth rate in nature.

2 International

In most regions of the world, lichens are poorly studied, and their decline is often not documented. There are about 400 genera and 20,000 species of lichens recognized globally. They are the most exciting organisms occupied by diverse and varied ecological habitats with different morphological thalli and life forms. The concern about lichens exploration goes back to the nineteenth century. Still, much emphasis on conservation, utilization, and ecological studies has begun only after the Rio earth summit on biological diversity. The present state of knowledge on lichens is mainly concentrated on systematic exploration, ecology, patterns of distribution, commercial application, and as a tool for monitoring atmospheric pollution. Loppi et al. (2000) found heavy metals such as Cr, Zn, and Cd, contamination in epiphytic lichen *Parmelia caperata* at a municipal solid waste incinerator in central Italy. The lichens are also studied as indicators of forest health in the USA (McCune 2000).

A review article by Huneck (1999) highlights the significance of lichens from Germany and their metabolites. He has reported numerous lichen metabolites, the

lichen substances that comprise cycloaliphatic, aromatic, and terpenoid compounds. Lichens and their metabolites have a broad biological activity like anti-viral, anti-microbial, antitumor, allergenic, plant growth inhibitory, antiherbivore, and enzyme inhibitory. Usnic acid very active lichen substances and is used in pharmaceutical preparations and perfume industries. Clear vertical distribution patterns of epiphytic species and life-forms were found by Cornelissen and Steege (1989). Special attention was given to the two categories of species: sun epiphytes and the pioneers. This study on the host preference and host specificity on two *Eperua* species adds a tropical example to the known pairs of congeneric trees that harbor different cryptogamic vegetations. Hyvärinen et al. (1992) measured the influence of stand age and structure on the epiphytic lichen vegetation in the middle-boreal forest of Finland. They concluded that the response of single lichen species to changes in the environment seems to vary considerably, including differences in competitive ability and ecological strategy between the species. The ecology and distribution of lichens in tropical deciduous and evergreen forests of Thailand were studied by Wolseley et al. (2002). They concluded the paper saying that the high diversity, combined with the presence of species and genera associated with a cool-temperate forest, is evidence that these forests are relicts of the extensive temperate flora of the cretaceous and early tertiary before the breakup of Gondwana land. Lücking (2003) compared Takhtajan's floristic region of the world based on vascular plant distribution to foliicolous lichen biogeography. Nascimbene et al. (2006) studied the epiphytic lichens in three forest types (1) late-successional stands with several large trees, (2) pioneer stands on abandoned pastures without large tree, and (3) open and grazed stands of treeline forest in Central Eastern Italian Alps.

3 Work Done in India

Bélanger (1838) was the first person studied the lichens of the Western Ghats. He enumerated 40 species of lichens from Pondicherry and Coromandel Coast. Montagne (1842) enumerated several lichens from the Nilgiri hills of South India, collected by Peroltet. A rigorous collection of Lichens from the Western Ghats was carried out during the middle of the nineteenth century. Bélanger and Bory (1846), Babington (1852), Nylander (1860), Stirton (1876, 1879), Hue (1898, 1899, 1900a, b, 1901), Jatta (1903, 1905, 1911), Smith (1926), Choisy (1931), Santesson (1952), Moreau and Moreau (1952) were the significant lichenologists who studied lichens of Western Ghats. During the twentieth century, expeditious improvement was made in the studies of Indian lichens. Hue (1900a), Prain (1905), Zahlbruckner (1911), Quraishi (1928), Chopra (1934) made the foundation for the Indian Lichenology. Awasthi (1965) and Singh (1964) have compiled lichens described by various researchers till the 1960s, including that of Western Ghats.

The 'Father of Indian Lichenology,' Prof. D.D. Awasthi systematically initiated the study of lichens. The school of Lichenology at Lucknow University contributed a lot to the lichens of Nilgiri and Palni hills (Singh 1984). The lichenological

investigations in the Western Ghats were strengthened during the 1960s with the setting up of the school of Lichenology at the Agarkar Research Institute (then Maharashtra Association for Cultivation of Sciences), Pune by Patwardhan and his associates. Patwardhan and Kulkarni (1976, 1977a, b, c, 1979a, b), Patwardhan and Makhija (1978, 1980a, b, 1981a, b, c) are some of the important publications. Later the work was extended to the Andaman Islands and other parts of the country. Lichenology Laboratory of National Botanical Research Institute, Lucknow, and Botanical Survey of India have compiled vast information on the lichens of Western Ghats. Singh (1980) consolidated the lichenological investigations during 1966–1977. Upreti (1992, 1995, 1997a, b) and Upreti and Nayaka (2000, 2003) updated the developments in Indian Lichenology. Catalog of the lichens from India, Nepal, Pakistan, and Ceylon (Awasthi 1965) enlists around 250 taxa of Western Ghats lichens. Two hundred twenty-five taxa were added to the lichens of Western Ghats during 1966–1977 (Singh 1980). Estimation by Patwardhan (1983) and Singh and Sinha (1997) recorded 800 lichen species in the Western Ghats. The publications of Awasthi (2000a), who has brought out a *Handbook on lichens* and another book on *Lichenology in the Indian Subcontinent* (Awasthi 2000b), has listed the lichen occurring in India. He prepared a key for the common species occurring in India. Nayaka and Upreti (2005) enumerated the occurrence of 949 taxa of lichens from the Western Ghats with a high percentage of endemism (26.7%), which is highest for any lichenographic regions of India. In India, the corticolous (growing on bark) exhibit their dominance followed by terricolous (soil-inhabiting) and saxicolous (rock-inhabiting) lichens. Khare et al. (2009) recorded 65 lichen genera on soil from India under 22 terricolous families. In Goa, the cursory collection available from Bondla and Bhagwan Mahavis WLS (Nayaka et al. 2004), while Cotigao WLS is intensively explored by Randive et al. (2017a, b). Foliicolous lichens from the Western Ghats region of Goa state were further surveyed by Randive et al. (2017a, b). Gujarat state is fairly well explored, resulting in up to 95 species of lichens (Nayaka et al. 2013). Fifty hectares permanent plot of Mudumalai Wildlife Sanctuary in Tamil Nadu district was surveyed by Ingle et al. (2016) and recorded 66 species of lichens belonging to 27 genera and 16 families while Mishra et al. (2017) enlisted 58 species of lichens belonging to 27 genera and 17 families from 7 localities of Bhimashankar Wildlife Sanctuary, Maharashtra, in the Western Ghats. Sinha and Gupta (2017) reported 266 species of microlichens from the State of Sikkim.

4 Work Done So Far in the Kerala Part of Western Ghats

In Kerala, the listing of lichens has not received much attention in comparison with other lower groups of plants and only fragmentary work has been done in the study of lichens from this part of Western Ghats. There are several problems confronted regarding the identification of lichens, which is a difficult group to work with (Kumar and Sequiera 1997a). Vohra et al. (1982) collected 77 species from Silent

Valley National Park in Kerala, of which 11 were new additions to India. Kumar and Sequiera (1997a) reported 20 species of macrolichens as new records from Silent Valley National park. Later, Kumar and Sequiera (1997b) in an appraisal of the lichen flora of Western Ghats, reviewed work done till date and enumerated 315 taxa of lichens under 75 genera, based on published work. Mukherji et al. (1999) published a book on lichens with notable contributions from different authors on different aspects of lichen taxonomy and ecology of other phytogeographic regions of India. In the KFRI research report, Kumar (2000) reported 253 macro lichens from Kerala with many new records. Subsequently, Kumar and Sequiera (2001, 2002, 2003) enumerated lichens from New Amarambalam Reserve Forest in Malappuram district, Chembra in Palghat district, and Thirunelly in Wayanad district. Easa (2003) documents nearly 300 lichen species found in Kerala. Later, the montane forests of Kerala part of Western Ghats were surveyed by Biju and Bagool (2009) and listed 242 species of lichens belonging to 62 genera and 29 families. Biju et al. (2010) delineated 10 lichen species belonging to the Parmelioid lichen group new to Kerala's lichen biota. Singh and Sinha (2010) in "Indian Lichens: An Annotated Checklist" recorded 564 lichens from Kerala. Divakar et al. (2010) erected the new genus *Remototrachyna* dividing a core group of 15 former *Hypotrachyna* species occurring in Kerala into monophyletic groups re-evaluated morphological and chemical characters. Joshi et al. (2010) described a new species, *Phlyctis karnatakana* S. Joshi & Upreti, from Thekkadi in the Idukki district of Kerala state, while Mishra et al. (2011) described seven species of *Phyllopsora* from Kerala. Biju et al. (2012) described eight lichen species of the family Graphidaceae from Kerala State, while Pandit and Sharma (2012) described *Lobaria adscripta* (Nyl.) Hue collected from Munnar road, Idukki, Kerala. Biju et al. (2014a, b) enlisted 136 species of lichens representing 45 genera and 25 families from 6 major forest areas of the Idukki district. Biju et al. (2014a, b) also described six graphidaceous lichens new to Kerala's Western Ghats. In addition to this, Biju et al. (2015) rediscovered two endemic and little-known species of *Pyxine* from the Western Ghats of Kerala after a gap of five decades. Joshi et al. (2012a, b) described 7 species of the genus *Chapsa* while Joshi et al. (2012a, b) further added 12 species of the genera *Leucodecton* and *Myriotrema* from the Western Ghats of Kerala based on earlier collections. Sharma et al. (2012) additionally recorded six more Graphidaceous lichens *viz. Fissurina cingalina* (Nyl.) Staiger, *Fissurina comparimuralis* Staiger, *Fissurina indica* B. O. Sharma, Khadilkar & Makhija, *Fissurina nitidescens* (Nyl.) Nyl., *Fissurina submonospora* B. O. Sharma, Khadilkar & Makhija from Idukki, Wayanad, and Palghat districts of Kerala state. Singh and Singh (2012) described *Pyrgillus idukkiensis* Kr. P. Singh & Pushpi Singh, a new calicioid lichen species of the family Pyrenulaceae from Myladumpara of Idukki district while Logesh et al. (2014) collected *Bactrospora intermedia* Egea & Torrente from Malappuram district of Kerala state.

Rai and Upreti (2014) reported *Pseudocyphellaria ceylonensis* H. Magn. from Munnar, Kerala. Joshi et al. (2016) added five more lichens to the lichen wealth of Kerala. Bajpai et al. (2017) reckoned the occurrence of 14 species of the genus *Cryptothecia* from Kerala. Bajpai et al. (2018) described 12 species of *Lepraria* and

Leprocaulon viz. Lepraria achariana Flakus & Kukwa, *Lepraria caesioalba* (B. de Lesd.) J.R. Laundon, *Lepraria cupressicola* (Hue) J.R. Laundon, *Lepraria elobata* Tønsberg, *Lepraria incana* (L.) Ach., *Lepraria isidiata* (Llimona) Llimona & A. Crespo, *Lepraria leuckertiana* (Zedda) L. Saag, *Lepraria lobificans* Nyl., *Leprocaulon adhaerens* (K. Knudsen, Elix & Lendemer) Lendemer & B.P. Hodk., *Leprocaulon coriense* (Hue) Lendemer & B.P. Hodk., *Leprocaulon pseudoarbuscula* (Asahina) I.M. Lamb & A.M. Ward and *Leprocaulon textum* (K. Knudsen, Elix & Lendemer) Lendemer & B.P. Hodk.) from various districts of Kerala state based on earlier collections by other lichenologists. Joshi et al. (2018) recorded 34 thelotremoid Graphidaceaeous members from Kerala. Sinha et al. (2018) reported 7 lichen species from the Kerala part of Western Ghats, and a study made by Zachariah et al. (2018, 2019, 2020) added 38 species to the lichen biota of Kerala state, of which 2 were new to India and 36 were new records to the lichens of Kerala state. Purushothaman et al. (2021) enumerated 5 lichen species from the Goodrical Reserve forest in the Pathanamthitta district of Kerala state as new to Kerala while Biju et al. (2021) reported 15 graphidaceous lichens from the Western Ghats of Kerala, of which 6 were new to India, 1 was new to Peninsular India and 8 were new records for Kerala. Anilkumar et al. (2022) added seven new records of macrolichens from Mathikettan shola National Park in the Western Ghats of Kerala state of which two species were new to Peninsular India and five were new additions to the lichen biota of Kerala. Recently, Sequeira et al. (2022) described *Parmotrema sahyadrica*, a new species of parmelioid lichen from Southern Western Ghats. At present, the State of Kerala represents ca. 800 species of lichens which were made through various publications and contributions.

Most of the forest areas in Kerala has not been discovered methodically for the lichen study; it is obvious from the data that the State of Kerala, which occupies only 1% of the geographical area of India, possess enormous abundance in lichens, especially the macrolichens when compared to the other State of India. While going through the literature, it has been observed that there are many lacunae in the lichenological studies from this part of the phytogeographic region. Most of the studies were concentrated mainly on macro lichens diversity, but not in an exhaustive survey except Sequiera (2003). Only fragmentary studies were done for the inventory of corticolous, saxicolous, and foliicolous microlichens from Kerala. Many of our protected areas remained unexplored for the lichen inventory either for macro lichens or for microlichens. If we pay more attention to the exploration of unexplored areas, it will give more species from these diverse habitats of Kerala.

5 Reasons for Undertaking the Present Work

Dr. Kasturirangan utilized nicely the remote sensing and GIS-based information for demarcating ecologically sensitive areas. Needless to say, they were mainly dependent on flowering plants and animals. Unfortunately, such studies are not available for non-flowering plants, which are considered as indicators of environmental

qualities. Lichens, although being a well-known bioindicator in India, there exists a significant gap in the proper utilization of their potential. As a mega diversity centre with a high level of endemism in both flora and fauna, India is distinctive in its biota and one of the most varied in the world, ranging from the cold arctic zone of the Himalayas to the tropical areas of the southern Western Ghats. The countless diversity in this region may be due to the enormous geographical location extending over many degrees of latitude, varied topography, climatic zones, and country position at the junction of many biogeographic regions and subregions (Nair 1991). There are about 2000 species of lichens described from the temperate and subtemperate areas of Himalayas, areas of the Western Ghats and Eastern Himalaya. Among these, Western Ghats holds about 1600 species of lichen, including both micro and macro forms. Among south Indian states, Tamil Nadu has received much attention for the study of lichens. Lying on the lap of the Western Ghats and hottest of 'hotspots' of endemism, Kerala also grasps rich lichen in its diverse habitats. Unfortunately, in Kerala, the accounting of lichens has not received much attention when comparing with other branches of botany. Only a scrappy work has been done from this part of the region. Kumar and Sequiera (1999) enumerated 771 lichens from the Western Ghats, which include the states of Karnataka, Kerala, Maharashtra, and Tamil Nadu, based on a survey of all the available literature on the lichens of these areas and based on macro and microlichen keys of Awasthi (1988, 1991). A detailed account of the lichens and their special habitats is very much essential for understanding the lichen flora of a particular area and their diversity. It is, therefore, vital to explore the unexplored and less-explored areas to know the diversity and to develop suitable conservation measures as well as for sustainable management. To fulfill this objective and to fill up the gaps, an intensive study of both macro and micro lichens were undertaken in the Kerala part of Western Ghats and discussed here.

6 Area: Kerala Part of Western Ghats

Kerala is one of the world's exceptional natural beauty areas with a diversity of ecosystems, species, and vast genetic resources. With an average of 10,000 km^2 patches of original tropical forests, Kerala holds about 10,035 species of plants all together in vascular, non-vascular, and lower groups in its pristine habitats (Nayar 1997). Being placed in a 'hottest' of hotspots of endemism in India, Kerala has three endemic 'hotspots' such as Agasthyamala, Anamalai—High ranges, and Silent Valley—Wayanad Plateau. Besides these, Kerala has 2 Biosphere Reserve, 5 National Parks, and 16 Wildlife Sanctuaries to its credit.

6.1 Location and Topography

Situated in the southwest corner of peninsular India, Kerala lies between 8°18′ and 12°4′ North latitude and between 74°52′ and 77°22′ East longitudes. The entire region covers an area of about 38,863 km^2 and has 14 political districts. The State is limited by the Western Ghats Mountains in the east and the Arabian Sea in the west. The altitude of the area ranges from sea level to the highest mountain peak of Anaimudi (2694 m) in Idukki District. Besides these, there are series of mountain ranges sloping on the windward side but with steep gradients on the protected side. Topographically the State is also divisible into four zones, viz. coastal belt, the midlands, hilly uplands, and the highlands.

6.2 Climate

The humid tropical climate of Kerala with irregular wet and dry periods are intimately associated with the high precipitation South West Monsoon and scattered rainfall of North-East Monsoon. These two Monsoons together form the State's rainy year in full swing for about 6–8 months. The average annual rainfall is about 3000 mm. The rain sometimes extends even up to 7000 mm. The highest relative humidity varies from 100% in June to 80% in December and the lowest from 78% in July–August to 48% in January. Temperature predominantly ranges from a monthly mean maximum of 33.6–27.7 °C and a monthly mean minimum of 26.5–21 °C.

6.3 Vegetation

The forest areas of Kerala falls under the Hooker's (1907) Malabar Botanical regions classified based on the specific content of the families under each botanical province. Diverse vegetational types are met within Kerala, depending on the altitude and rainfall and temperature differences. Thus, the area underpin Tropical rain forests, Tropical moist deciduous forests, Tropical dry deciduous forests, Shola forests, Grasslands, and Riparian forests.

7 Ecology of Lichens

Climate play a vital role in the ecology of lichens. The availability of water, sunlight, temperate to cold climate, unpolluted atmosphere, wind condition, and the type of substratum are the substantial factors responsible for the ideal growth of lichens. The

lichen thalli are very sensitive to polluting gases like sulfur dioxide and oxides of nitrogen.

(a) *Water*

The requirement of water varies significantly in the different species of lichens. Recurrent rains are of great advantage for their growth as the metabolic activities are dependent predominantly on moisture content in the thallus. In the absence of rain, dew and a humid atmosphere can relatively satisfy the moisture requirements owing to their ability to absorb moisture from the atmosphere. The Southern Western Ghat of Kerala receives an average rainfall of 3000 mm of per annum disseminated over 8 months. Even when the rain is less, the relative humidity is very high. These factors play an important role in the growth and distribution of lichens in this region's evergreen and montane forests.

(b) *Sunlight*

The requirement of sunlight is an essential but much variable factor responsible for distributing various species of lichens in this area. Most of the grey colored foliose taxa and *Usnea* sp. occur in highly exposed places where there is bright sunlight. Dark-colored taxa like *Collema, Leptogium, Lobaria, Sticta* and *Pseudocyphellaria* are adapted to grow in covered areas. The majority of lichens, however, occur in transitional situations where there is moderate exposure between the two extremes. So trees in the ecotone regions, grasslands, upper canopy of trees in the evergreen and montane forests possess a greater number of lichens. It is fascinating to note that trees facing midday and afternoon sun harbours many lichens in their upper canopy branches.

(c) *Temperature*

Temperature plays a vital role in the growth and distribution of lichens. A moderate to a cold climate with an average temperature of 20–25 °C is suitable for the development of lichens. Montane forests of Chembra in Wayanad, Silent Valley National Park in Palghat, Eravikulam National Park in Idukki, and Agasthyamala in Trivandrum average temperature of 21 °C harbours a large number of macro lichens in their habitat. About 100 different species of macro lichens are observed in these forests. Species like *Everniastrum cirrhatum, Heterodermia leucomela* subsp. *boryi*, *Usnea* sp. are abundantly seen along with these forests.

(d) *Wind*

Normal wind currents are essential for the proper growth of lichens. Trees in the open grasslands inhabit a large number of lichens. Grasslands with the rocky area also hold many foliose and some fruticose forms. Crustose and horizontally growing foliose thalli are not affected by high wind currents. But erect and pendulous fruticose forms are liable to be damaged or broken apart unless suitably adapted.

(e) *Substratum*

For successful anchoring, the lichen thallus needs a substratum. The substratum chemistry and the texture play a crucial role in the type of lichen, which it

supports. Generally, the lichen taxa have a preference for a particular substratum. The nature of the substratum forms a basis for the classification of lichens.

8 Conservation

Lichens are important for their ecological importance, aesthetic value, and usefulness to humanity. Lichen is one of the most influential unions in nature. It can be observed as a community rather than an organism since it consists of at least one photobiont species and a species of mycobiont. The algal portion of the lichen thallus is susceptible to atmospheric pollution. However, the fungal part usually makes up the bulk of the thallus, thereby moderating the polluting gases effect to some extent.

Moreover, the fungal portion is drought-resistant and can resist periods of hot and cold temperatures and dryness. This creates the lichen as a useful monitor for both pollution and atmospheric moisture level. In the past, lichens were not considered as flora to be conserved. At present, there is no legislation relating to the conservation of lichen in any State. However, conservation policies in some States include non-vascular plants (including lichens) along with vascular plants. The knowledge of lichen distribution depends mainly on data in herbarium records and also based on recent collections by lichenologists. From this, it can be assumed that lichens found in one or at the most a limited localities should be viewed as rare. If the habitat to which it is limited is threatened, it follows that the species can be treated as endangered.

Fire is the greatest threat to lichens irrespective of the substratum on which they grow. Lightning or man-made fire can annihilate a lichen population. Air pollution in cities, highways, and around factories clear off these areas of sensitive lichens. Sensitivity of lichens to air pollution originates from the capacity to concentrate air or water-borne compounds in the plant body, potentially to toxic levels. Sulfur dioxide, fluoride, lead, and zinc is common reasons of death in lichen populations. Aerial application of fertilizers, fungicides, and insecticides is also likely to influence the non-vascular flora considerably. Clear-felling practices abolish the whole lichen habitats, causing an alteration of the microclimate in the surrounding vegetation as drier conditions prevail. Quarrying areas where lichens cover the surface results in the eradication of their entire population that occurs on the rocks. Lichens are the store houses for a wide range of unique secondary metabolites. These substances are being studied for new pharmaceuticals and agrochemicals, as well as commercially valuable enzymes for use in biosensors, biotransformation reactions, and diagnostic kits. Recent works have recognized compounds with marked anti-tumor properties (terpenes), anti-amoebic activity (fatty acids), and nematocidal activity. Collecting specimens for scientific studies may be a threat when an over-enthusiastic collection of a particular species could place; especially when local populations or rare species are at risk. Strict vigilance is necessary to protect this nature wealth.

The factors involved in the loss of lichen diversity in India (Upreti 1995) are, the change in the ecological environments, forest cover, loss of habitat, and increase of

the urban and industrial areas. The anthropogenic pursuits in montane areas such as agriculture, mineral extraction, tourism, hydro-electric, and road building projects are leading to the fast decline of lichen-rich habitats. Excessive utilization and selective removal of economically important lichens by local people causes a serious threat to the lichen wealth of any state. Collected from the high altitude areas of Kerala, lichens are used indigenously and also are traded to many neighboring countries. Few folkloric groups in certain areas of Western Ghats collect these plants. Lichen population of India has considerably reduced in its diversity and occurrence, in the past few years. The lichens weigh very little when dry and the raw material necessary for the various uses of lichens is relatively high. Thus, a vast bulk of these plants are required. Three hundred twenty tons of lichens are annually used for different purposes in Nepal and adjoining regions of India (Moxham 1986). Approximately 800 metric tons of lichens are collected annually from different regions of India; of this, about 50–80 metric tons are exported (Shah 1997). On the other hand, quantitative studies have not been accomplished in the Kerala part of Western Ghats to measure the quantity of lichen collected, utilized, exported, and its effect on particular lichen species or whole lichen diversity of this region.

9 Uses

The lichens have been used as domestic items of Indians since ancient times as medicines and in various cultural events (Kumar and Upreti 2001). “Shipal” in Atharvaveda (1500 BC) is the first record of lichen used as medicine. The vernacular name “Charilla” is widely used in Ayurveda system of medicine. “Shailya” and “Shila Pushp” (Shila—rock, Pushp—flower) are the Sanskrit synonyms of lichens. The main use of lichens is based on the secondary metabolites, which are often produced abundantly in the lichen thalli. These metabolites have been utilized by in cottage industry for making dyes, medicines, and perfumes. Lichens containing specific substances were extensively used for dyeing purposes in Europe in the 16th–18th century. In China, Nepal, and few European countries, some species of lichens are still being used in the traditional medicine system. Recent medical research has confirmed the antibiotic effect of a large number of species. Usnic acid, which is produced by many yellowish lichens (e.g. *Usnea* spp.), has been used to cure ulcers. Recent research has focused more on the effects on tumor activity and immune response. French perfume industry utilizes tons of lichens annually collected from oak trees in Southern Europe and North America (Richardson 1975). Lichens are industrially processed in Nepal, where *Parmotrema tinctorius* (Nyl.) Hale is used for the extraction of lichens resinoids. *Heterodermia diademata* (Taylor) Awas. is used as an antiseptic and healing agent for cuts and wounds in Sikkim (Saklani and Upreti 1992) and the western Himalayas (Negi and Kareem 1996). *Parmotrema sancti-angelii* (Lynge) Hale is burned to ash and used to cure fungal skin disease in Central India (Lal and Upreti 1995). *Everniastrum nepalense* (Taylor) Hale ex Sipman is used in Nepal to treat tooth-ache, soreness of throat, pain

(Kumar et al. 1996), and numerous other diseases. Foliose lichens like *Parmotrema tinctorum*, *P. grayanum*, *P. reticulatum*, *P. sancti-angelii* and *Everniastrum cirrhatum* are mixed together and used as flavoring agent and spice in meat and vegetable preparations in Central and South India. In the local markets of Kerala, this is available as "Biriyani Phool" which acts as a seasoning agent in the preparation of Biriyani.

10 Medicinal and Economic Importance of Lichens

The medicinal values of Indian lichens have a long historical backdrop. A constituent of spices as well as folklore drug Charilla; *Parmelia* sp. and *Parmelia nepalense* have their usage as aphrodisiac treatment of toothache and sore throat (Lal and Upreti 1995; Kumar and Upreti 2001; Kumar et al. 1996). While *Thamnolia vermicularis* (Schwartz) Ach. (Icmadophilaceae) from Western Himalayas, is used as antiseptic (Negi and Kareem 1996); and *Heterodermia diademata* (Talyor) D.D. Awas., (Physciaceae) was used for cuts and wounds (Saklani and Upreti 1992). Many reviews have conversed the pharmaceutical potential and biological activities of lichen substances (Huneck 1999; Müller 2001). Some lichens were engaged in the treatments for coughs, jaundice, rabies, and restoring lost hair. *Heterodermia leucomelos* was found powerful against human as well as plant pathogenic fungi (Shahi et al. 2001). Broad-spectrum antifungal properties at 80 μL/mL were evident in the aqueous extract of *Parmelia cirrhatum* against some human and plant pathogens (Shahi et al. 2003). The phenolic compounds and their derivatives in lichen have been proved to be harmful for pathogenic microbial fauna. In a study focusing on a large number of lichens and their cultured symbionts for their antibacterial and antioxidant activity, concluded that mycobiont and photobiont culture of *Usnea ghattensis* and *Arthothelium awasthii* have high antioxidative and antibacterial potential (Behera et al. 2008). On a global basis, lichens can be regarded as a source of multifold utility herbal compound.

11 Threats to Lichen Diversity in Kerala

Lichens are subtle organisms. Their reaction to environmental change may include changes in their diversity, abundance, morphology, physiology, accumulation of pollutants etc. (Nimis et al. 2002). The main threats that apply to biodiversity in general are also true for lichens, which revolve around enlarged population, e.g. urbanization, industrialization, cultivation, tourism, forestry, habitat obliteration and fragmentation, hydroelectric projects, mining, air pollution, climate change etc. Habitat degradation and loss is the most serious threat to biodiversity in general (Groom et al. 2006) and in lichens in particular (Wirth 1976, 1999). Natural forests were found to host a characteristic lichen flora that is not found in secondary forests

or fragmented forest landscapes (Bergamini et al. 2005). Habitat disintegration leads reduced population size in lichens and in heterothallic species, the absence of a compatible partner precludes sexual reproduction. This State might be aggravating in small populations, populations that have gone through genetic bottlenecks during extended time periods with small population sizes, or newly founded populations (Scheidegger and Werth 2009). The agricultural practices not only destroy natural vegetation but also lead to eutrophication and reclamation due to usage of fertilizers and pesticides. The grazing is another agriculture associated problem which is detrimental for soil inhabiting lichens. The fire as a forest management practice to grow fresh grass in sanctuaries cause serious threat to terricolous, saxicolous as well as corticolous lichens growing over nearby trees in Kerala forests.

A severe increase in the demand for herbal products, spice and condiments the non-timber forest products (NTFPs) are being been over exploited throughout India and Kerala in particular. Unregulated harvesting of lichens has become a serious hazard to biodiversity in Himalayas and Kerala part of Western Ghats (Upreti et al. 2005a, b). In a study conducted in Uttarakhand, few macrolichens (*Parmelia* spp., *Everniastrum* spp., *Usnea* spp., and *Ramalina* spp.) represented the most heavily traded species of NTFPs. In Karnataka, large amount of lichens are collected by villagers and sold to the mediators or nearest dealers at the rate of Rs. 35–45 per kg.

Further, these lichens were dried, processed, graded, and were sold in the market at Rs. 200–230 per kg. In this way, every year, about 8–12 tons of lichens are being harvested from the WG forests. Any herbal preparation needs a large amount of raw material, and lichens weigh very low, especially after drying. Lichens are also a slow-growing organism, and their annual growth ranges from few millimeters to centimeters. Hence, once if they are detached from their natural habitat, they take several years to re-establish. Another apprehension coupled with lichen harvesting is the collection of co-occurring lichens along with preferred ones. Mostly crustose and smaller foliose lichens belong to this category; sometimes, they may be rare or threatened. Such twigs or bark, after removal of desired lichens, will be either burnt or thrown, leading to the destruction of lichen diversity. The overexploitation can lead to a reduced propagule source. The productivity of the lichen community is reduced while the demand for the resource remains constant. Because these, lichens are an important part of a large human population's cultural life, increasing market prices will, in all likelihood, not regulate the demand for this resource (Scheidegger and Werth 2009).

Yet another threat which is specific to lichens and other cryptogams which lack the ability to maintain the water content is air pollution, which has led to the severe decline of numerous species. Lichens exhibit different levels of sensitivity to pollution. The fruticose lichens are more sensitive, followed by foliose, while crustose are much tolerant species. Upreti et al. (2009) reviewed air pollution monitoring studies with lichens in India. Loss of the lichen diversity and change in their community due to air pollution, urbanization, and changed climate was witnessed in Bangalore (Nayaka et al. 2003) and Kolkata cities (Upreti et al. 2005a, b). Unfortunately, such studies are not available for any localities in the Western Ghats of Kerala.

Among all the threats, climate change is likely to dramatically affect the distribution and abundance of lichen populations (Ellis et al. 2007). The lichens at montane forests of Kerala would be affected more with migration species to the cooler elevation and invasion of alien species at warmer regions, hence altering the lichen community's structure and eventually extinction of sensitive species.

12 Lacunae in Lichenological Studies in Western Ghats of Kerala for Conservation

Conservation biology targets at upholding self-sustaining populations of rare and endangered species. In India, the conservation strategy mostly involves the protection and restoration of habitat or an ecosystem. Such a strategy usually assumes safeguarding a larger area will take care of other organisms within that ecosystem. However, cryptogams, like lichen, demand specific conservation strategies and approaches. Lichens often have particular habitat requirements mostly decided by microclimate and are not generally shared by other organisms. Habitats rich in a threatened organism such as birds, mammal, and the vascular plant may not also be rich in threatened lichen taxa. The management techniques and conservation measures for these organisms may be contradictory to lichens (Brown et al. 1994). Some of the threats like climate change and air pollution would have greater effects on lichens than vascular plants and animals. Therefore, an ecosystem approach of conservation seldom leads to positive results in these inconspicuous organisms. Therefore, the modern conservation concepts have expanded to include conventionally neglected groups of organisms, and it has become more grateful that understanding and maintaining the biodiversity of taxa other than vascular plants and vertebrate animals must be addressed explicitly (Will-Wolf and Scheidegger 2002). Further, the bioindicators such as lichens would have much importance as Article 7 in the Convention on Biological Diversity necessitates signatory countries to identify mechanisms of biological diversity important for long-term conservation and sustainable use of biodiversity.

13 Discussion

The hilly State of Kerala lies secluded from the Deccan Plateau by the mountainous belt of the Western Ghats. It covers a geographical area of 38,864 sq. km. In fact, the State is a narrow strip of 32–120 km in width widening for about 565 km along the Malabar Coast on the western side of Peninsular India. Lying between the coordinates 8°18′ and 12°48′ N latitude and 74°52′ and 77°22′ E longitude, the boundaries of the State are the Lakshadweep Sea in the west, Tamil Nadu in south and east, and Karnataka in the north. Rainfall is very high and ranges from 125 to 500 cm per

annum. The State gets the advantage of both southwest and north-east monsoons. The southwest monsoon begins in June and continues till the middle of August with irregular intervals while the north-east monsoon is spread over to October to December. The area of the State is 1.18% of the total area of the country and is governmentally divided into 14 districts. Due to the long tract of Western Ghats along the eastern side and the Arabian Sea along the western side, the physiography of the State is highly varied. The State has a complex topography with mountains, valleys, ridges, and scraps. The altitude varies from sea level to 2695 m above MSL. The high land region constitutes important areas with regard to biodiversity. These mountain ridges are continuous from the north to the south except for the Palghat gap of 30 km. Nestled in the verdant forest of the Western Ghats, in Kerala, there are 16 Wildlife Sanctuaries, 5 National Parks and 2 Biosphere Reserves. Among them, the largest is the Periyar Tiger Reserve, and the smallest is the Peppara Wildlife sanctuary.

The principal part of the forests of Kerala are in highlands and midlands. It is obvious that the State's floristic diversity, like that of the Western Ghats, has an early lineage. Such an early flora is not just a record of botanical antiques but also a dynamic biological cause where speciation occurs at an accelerated speed (Ashton 1977). The most distinguished feature of the State is the formation of tropical rain forests along the Southern Western Ghats windward side, which is lying parallel to the west coast (Sasidharan 2004). The woods along the Western Ghats extended uninterrupted from the northern to the southern end and extended down, particularly on the Western side, almost up to the seashore. Kerala's forests presently cover 28.63% (11,125.5 sq. km) of the total geographic area (State Biodiversity Strategy and Action Plan for Kerala 2005).

Chandrasekharan (1962) suggested the following classification for the Kerala forests (1) Tropical wet evergreen forests (2) Tropical moist deciduous forests (3) Tropical dry deciduous forests (4) Montane subtropical forests and (5) Montane temperate forests. As per the resource survey (Chandrasekharan 1973) the montane temperate shola forests of Kerala cover an area of 14 sq. km. These are met within a altitudinal zone between 1000 and 1700 m on the hills of South India and also above 1000 m on the higher hills of central India (Champion and Seth 1968). This type of forests occurs as a continuous expanse of the evergreen vegetation. They are cooler and wetter than the lower vegetation. Scientifically speaking, "Sholas' are Tropical montane forests" located in the higher mountain tracts of Western Ghats which are interspersed with rolling grasslands. The forests of tropical mountains are mostly divided into upper and lower montane formations on the basis of their physiognomy, which varies with altitude. The southern Western Ghats montane forests are the most species rich ecoregion in the Peninsular India. In Kerala, it extends as a long and narrow strip with from Wayanad, where the habitat makes a change from the drier forest in the north to the more moist forest in the south. The heights of endemism in these montane forests are truly surprising.

It is clear from the observations in the present study that lichens have substrate preferences. However, some species exhibit their ability to grow on a wide range of substrates. The substrate may have a direct effect on the thallus morphology. The

identification keys are often based on substrate classification and lichen communities may be saxicolous, corticolous, lignicolous, muscicolous, terricolous and foliicolous, referring to the species found on rocks, bark, wood, bryophytes, soil and leaves respectively.

Some lichens are adpressed to their substrates only vaguely while others such as certain crustose species are entirely immersed in the substrate, be it rock or bark. Most of the substrate-specific species may in fact, derive certain essential and specific nutrients from the substrate. Certain very ancient species characteristically occur on one substrate have disjunct populations living on entirely different substrates. This type of vicariism apparently arose due to a gradual removal of suitable habitats in the stranded area. Many of these species have not changed morphologically despite their disjunctions and substrate differences (Brodo 1973). Rough surfaces usually bear a richer lichen flora than smooth surfaces. The availability of moisture has been recognized as the prime factor in the distribution of lichens. Lichens, sometimes do form extensive growths over large areas of bark. Thick growth of *Ramalina* and *Physcia* has been reported to interfere in the development of adventives shoots in tea plants, mostly through competition for available sunlight (Asahina and Kurokawa 1952).

Altitude plays a significant role in the distribution of any lichen taxa. In our studies, it has been witnessed that some of the lichen species have worldwide distribution, some are restricted to certain areas the country, some are localized within the Western Ghats region and few species are found to be endemic. The species having vegetative propagules exhibit their regional distribution. Propagules like isidia and soredia being heavier in weight do not disperse too far while the apothecia bearing species which produce ascospores lighter in weight disperse to far off distances.

The moist forests in the temperate regions are rich in lichens. The tall tree trunk of the low land forests may well contain more lichen than is generally thought because of their best sites are in the canopy (Nair and Daniel 1986). The tree trunks of the evergreen forests harbours with crustose lichens. Foliaceous lichens are common on the upper side of the evergreen leaves of shrubs and lower branches of trees where ever moisture and shade are available (Awasthi 1977). The micro-climatic conditions as well as arboreal elements in the area influence the distribution of lichens in the region (Patwardhan 1983). Subtropical regions of Kerala form the richest home for many lichens. Based on climatic conditions, the lichen flora of Kerala can be divided into temperate, subtropical and tropical zones.

The lichens in temperate region (above 1500) m prefer mostly the bark of trees or rocks for their attachment. In the Sub tropical and tropical forests (700–1000 m) the foliose and fruticose lichens and crustose forms of the families Thelotremataceae, Graphidaceae, Trichotheliaceae and Lecideaceae predominate in these high hill ranges. In deciduous forests, particularly in moist and shady places of this region, foliose lichen genera like *Myelochroa, Phyllopsora, Pseudocyphellaria, Phaeophyscia, Coccocarpia* seen associated with crustose lichen genera of the families Arthoniaceae, Pyrenulaceae, Bacideaceae, Graphidaceae and Pertusariaceae.

Both the tropical and subtropical zones exhibit dominance of crustose lichens. The tropical and subtropical humid zone has a well-developed lichen flora other than dry zone. The rain forest with high humidity provides a appropriate habitat for many pyrenocarpus and graphidaceous lichen taxa to colonize. The foliicolous (leaf colonizing) lichens also find the best development in such habitats. The majority of foliicolous lichens are epiphyllous as they grow on the upwardly exposed leaf surface. The dead leaves either still attached or already fallen show a lichen flora quite different from that of living leaves. The colonization of lichens on trees depends largely on the pH of the bark, the thickness, smoothness, hardness etc. In the rain forests with cooler temperature *Sticta*, *Pseudocyphellaria*, *Pannaria* and other parmelioid genera are common and sometimes they dominated the flora.

It was noteworthy that the evergreen forests of Kerala had the domination of corticolous species while the saxicolous and terricolous species were less represented. Ecotone regions supports many species of lichens. Factors like wind, humidity and sunlight are at excellent level as far as lichens are concerned. Rocks and trees of grasslands also hold many lichens. Majority of the them are saxicolous in nature.

14 Analysis of the Lichen Flora

It is assessed that there are about 2907 species of lichens present in India and Western Ghats contain around 1300 lichen species whereas Kerala part of Western Ghats delineates about 800 species belonging to 171 genera and 51 families. It accounts almost 29% of the total lichens in India. Among them, about 90 species are endemic to Western Ghats of Kerala. Among different districts, Idukki have the maximum number of lichens followed by Palghat, Wayanad Thiruvananthapuram and Pathanamthitta respectively. The lichen flora of Kerala is dominated by microlichens (leprose and crustose forms) with 463 taxa. Among macrolichens, foliose forms are represented by 240 taxa (including squmulose forms) and fruticose by 97. Graphidaceae with 203 species belonging to 38 genera is the dominant family in the Kerala part of Western Ghats while both *Parmotrema* and *Usnea* are the leading genus with 37 species each. Kerala has more number of Pyrenocarpous (perithecia bearing) lichens followed by Graphidaceous and Thelotremataceous lichens. The distribution of lichens as in other cryptogams are highly influenced by the microclimatic factors of the particular region. In the case of Kerala, the four major ecological features that produce several microclimatic niches for the growth of lichens are substrate, vegetation, climate and altitude. The area provides a wide range of substrates for the growth of lichens and hence, out of the total lichens found here, 562 are corticolous, 110 are saxicolous, 96 are terricolous, 13 are foliicolous and 8 are muscicolous. The optimum growth of lichen diversity in Kerala is found to between 700 and1500 m. Wide variation in the rainfall pattern and the complex topography of the region is liable for a wide range of vegetation types in the Western

Ghats of Kerala and the lichen communities in each vegetation type differs to a great amount.

15 Family-Wise List of Lichens with No. of Genera and Species Occurred

Sl. no.	Families	No. of genera	Genera with no. of species
1.	Arthoniaceae	38	*Arthonia* (3 sp.)
			Arthothelium (4 sp.)
			Cryptothecia (23 sp.)
			Herpothallon (5 sp.)
			Tylophoron (3 sp.)
2.	Arthopyreniaceae	8	*Arthopyrenia* (8 sp.)
3.	Ascomycota	1	*Kalchbrenneriella* (1 sp.)
4.	Baeomycetaceae	1	*Baeomyces* (1 sp.)
5.	Brigantiaeaceae	2	*Brigantiaea* (2 sp.)
6.	Byssolomataceae	2	*Calopadia* (2 sp.)
7.	Caliciaceae	25	*Acolium* (1 sp.)
			Buellia (5 sp.)
			Diplotomma (1 sp.)
			Hafellia (1 sp.)
			Pyxine (17 sp.)
8.	Candelariaceae	1	*Candelaria* (1 sp.)
9.	Catillariaceae	1	*Catillaria* (1 sp.)
10.	Chrysotrichaceae	1	*Chrysothrix* (1 sp.)
11.	Cladoniaceae	27	*Cladonia* (26 sp.)
			Pilophorus (1 sp.)
12.	Coccocarpiaceae	3	*Coccocarpia* (3 sp.)
13.	Collemataceae	32	*Collema* (4 sp.)
			Enchylium (1 sp.)
			Lathagrium (1 sp.)
			Leptogium (21 sp.)
			Physma (1 sp.)
			Scytinium (4 sp.)
14.	Gomphillaceae	1	*Bullatina* (1 sp.)
15.	Graphidaceae	203	*Acanthothecis* (1 sp.)
			Allographa (15 sp.)
			Asteristion (2 sp.)
			Astrochapsa (1 sp.)
			Austrotrema (1 sp.)
			Chapsa (4 sp.)
			Crutarndina (1 sp.)

(continued)

Sl. no.	Families	No. of genera	Genera with no. of species
			Diorygma (4 sp.)
			Diploschistes (5 sp.)
			Dyplolabia (1 sp.)
			Fibrillithecis (1 sp.)
			Fissurina (14 sp.)
			Glaucotrema (1 sp.)
			Glyphis (2 sp.)
			Graphina (7 sp.)
			Graphis (36 sp.)
			Hemithecium (4 sp.)
			Leucodecton (8 sp.)
			Myriotrema (7 sp.)
			Nitidochapsa (1 sp.)
			Ocellularia (27 sp.)
			Pallidogramme (3 sp.)
			Phaeographina (4 sp.)
			Phaeographis (11 sp.)
			Phaeotrema (1 sp.)
			Platygramme (3 sp.)
			Platythecium (4 sp.)
			Pseudochapsa (2 sp.)
			Reimnitzia (1 sp.)
			Rhabdodiscus (3 sp.)
			Sanguinotrema (1 sp.)
			Sarcographa (3 sp.)
			Stegobolus (2 sp.)
			Thalloloma (1 sp.)
			Thecaria (1 sp.)
			Thelotrema (16 sp.)
			Topeliopsis (1 sp.)
			Wirthiotrema (3 sp.)
16.	Hymeneliaceae	1	*Ionaspis* (1 sp.)
17.	Lecanographaceae	1	*Alyxoria* (1 sp.)
18.	Lecanoraceae	14	*Glaucomaria* (1 sp.)
			Lecanora (13 sp.)
19.	Leprocaulaceae	3	*Leprocaulon* (3 sp.)
20.	Letrouitiaceae	2	*Letrouitia* (2 sp.)
21.	Lobariaceae	19	*Dendriscosticta* (1 sp.)
			Lobaria (3 sp.)
			Pseudocyphellaria (5 sp.)
			Ricasolia (2 sp.)
			Sticta (8 sp.)
22.	Lopadiaceae	4	*Lopadium* (4 sp.)

(continued)

Sl. no.	Families	No. of genera	Genera with no. of species
23.	Malmideaceae	1	*Malmidea* (1 sp.)
24.	Megalosporaceae	2	*Megalospora* (2 sp.)
25.	Monoblastiaceae	4	*Anisomeridium* (4 sp.)
26.	Mycosphaerellaceae	1	*Stigmidium* (1 sp.)
27.	Naetrocymbaceae	2	*Naetrocymbe* (2 sp.)
28.	Nectriaceae	1	*Cylindromonium* (1 sp.)
29.	Ochrolechiaceae	1	*Ochrolechia* (1 sp.)
30.	Opegraphaceae	5	*Opegrapha* (3 sp.)
			Sclerophyton (1 sp.)
			Zwackhia (1 sp.)
31.	Pannariaceae	14	*Erioderma* (1 sp.)
			Fuscopannaria (2 sp.)
			Leioderma (1 sp.)
			Lepidocollema (2 sp.)
			Pannaria (3 sp.)
			Parmeliella (2 sp.)
			Pectenia (1 sp.)
			Psoroma (2 sp.)
32.	Parmeliaceae	141	*Bulbothrix* (5 sp.)
			Canoparmelia (3 sp.)
			Cetrariopsis (1 sp.)
			Crespoa (2 sp.)
			Eumitria (2 sp.)
			Hypogymnia (2 sp.)
			Hypotrachyna (18 sp.)
			Melanelia (1 sp.)
			Menegazzia (1 sp.)
			Myelochroa (6 sp.)
			Parmelina (1 sp.)
			Parmelinella (5 sp.)
			Parmelinopsis (2 sp.)
			Parmotrema (37 sp.)
			Parmotremopsis (1 sp.)
			Punctelia (1 sp.)
			Relicina (4 sp.)
			Remototrachyna (11 sp.)
			Usnea (37 sp.)
			Xanthoparmelia (1 sp.)
33.	Patellariaceae	1	*Patellaria* (1 sp.)
34.	Peltigeraceae	2	*Peltigera* (2 sp.)
35.	Pertusariaceae	16	*Lepra* (2 sp.)
			Pertusaria (14 sp.)
36.	Phaeosphaeriaceae	1	*Didymocyrtis* (1 sp.)

(continued)

Sl. no.	Families	No. of genera	Genera with no. of species
37.	Phlyctidaceae	2	*Phlyctis* (2 sp.)
38.	Physciaceae	48	*Cratiria* (2 sp.)
			Dirinaria (6 sp.)
			Heterodermia (20 sp.)
			Hyperphyscia (3 sp.)
			Leucodermia (2 sp.)
			Phaeophyscia (4 sp.)
			Physcia (4 sp.)
			Physciella (1 sp.)
			Polyblastidium (6 sp.)
39.	Porinaceae	23	*Clathroporina* (1 sp.)
			Porina (21 sp.)
			Trichothelium (1 sp.)
40.	Pycnoraceae	1	*Pycnora* (1 sp.)
41.	Pyrenulaceae	58	*Anthracothecium* (19 sp.)
			Pleurotrema (2 sp.)
			Pyrenula (33 sp.)
			Pyrgillus (3 sp.)
			Sulcopyrenula (1 sp.)
42.	Ramalinaceae	36	*Bacidia* (2 sp.)
			Bacidiopsora (1 sp.)
			Polyozosia (1 sp.)
			Phyllopsora (15 sp.)
			Psorella (1 sp.)
			Ramalina (15 sp.)
			Schadonia (1 sp.)
43.	Roccellaceae	9	*Bactrospora* (2 sp.)
			Chiodecton (1 sp.)
			Dichosporidium (1 sp.)
			Enterographa (1 sp.)
			Mazosia (3 sp.)
			Roccella (1 sp.)
44.	Sphaerophoraceae	1	*Bunodophoron* (1 sp.)
45.	Sphinctrinaceae	1	*Pyrgidium* (1 sp.)
46.	Stereocaulaceae	9	*Lepraria* (8 sp.)
			Stereocaulon (1 sp.)
47.	Strigulaceae	6	*Strigula* (6 sp.)
48.	Teloschistaceae	2	*Gyalolechia* (1 sp.)
			Teloschistes (1 sp.)
49.	Trypetheliaceae	14	*Astrothelium* (3 sp.)
			Bathelium (1 sp.)
			Bogoriella (2 sp.)
			Laurera (4 sp.)

(continued)

Sl. no.	Families	No. of genera	Genera with no. of species
			Trypethelium (4 sp.)
50.	Vahliellaceae	1	*Vahliella* (1 sp.)
51.	Verrucariaceae	7	*Dermatocarpon* (1 sp.)
			Hydropunctaria (1 sp.)
			Normandina (1 sp.)
			Parmentaria (3 sp.)
			Verrucaria (1 sp.)

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Plants and Microorganism Bio-compounds on Agriculture Applications by Nanotechnology

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Abstract The demand for food increases with the growing world population, new agricultural exercises have been modified to improve food production by employing more effective pesticides and fertilizers. However, the long-term applications of chemistry insecticides resulted in residues accumulating in different environments. Moreover, chemistry insecticides affect ecosystems, human health, and organisms non-target. Therefore, bio-insecticides "Green pesticides" have posted another way to synthetic insecticides in crops and public health. Scientific globally focuses on the prospects of plant extracts, phytohormones, and plant growth-promoting rhizobacteria (PGPR) as bio-insecticides for insect pest management. Many bio-compounds have plant growth promoting, antifeedant, attractive, antifungal, insecticidal, and repellent activities against a broad spectrum of microorganisms with some selectivity. The bio-compounds have been evaluated at different concentrations and show the significant effect of controlling a pest; nevertheless, nanotechnology can potentiate their properties. The nanotechnology field opens up novel potential applications for the next generation of agriculture. Nanotechnology is already being explored and used in medicine and pharmacology, but interest in crop protection is just starting. The present contribution describes several advances in using bio-compounds from plants and microorganisms as a source of development

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nanotechnology on agricultural science with control activity of phytopathogen, including the own experiences in this area of research.

Keywords Bio-compound · Plant extracts · Phytohormones · Plant growth-promoting rhizobacteria · Nanotechnology

1 Introduction

The sustainability of agro alimentary manufacturing is a worldwide demand, and in emerging nations, it challenges more consequences due to technological weaknesses and climate change tendencies. Especially in Latin America and the Caribbean, considerable awareness is attracted to the waste and food factor, mostly in postharvest procedures (Aguilar-Veloz et al. 2020). Estimates show that crop or food damages caused by plant pests can make up to 40% of worldwide food yields and correspond to economic damage higher than 220 billion dollars per year (United Nations 2018). Indiscriminate use of pesticides has generated difficulties, such as pest resistance, the resurgence of pests, ecotoxicity, and the elimination of natural enemies. These glitches attract the scientific world's attention to developing an eco-friendly and human-safe alternative pest control method (Duhan et al. 2017).

Nanotechnology is an interdisciplinary investigation field. For example, works and research have been completed to develop agricultural yield through extensive investigation in nanotechnology, as shown in Fig. 1. On the other hand, the green innovation caused in blind pesticides and chemical fertilizers changing using green pesticides or biopesticides considered safe, target-specific, biodegradable, and eco-friendly (Singh and Singh 2016).

Nanoparticle-mediated material release to plants, phytohormones, plant growth-promoting rhizobacteria, and biosensors for cultivation is potential by nanoparticles or nanochips. Nanoencapsulated traditional herbicides, fertilizers, and insecticides aid in the gradual and continuous delivery of micro and macronutrients and agro-chemicals, causing the plants an exact dose (Duhan et al. 2017). The current review focuses on advances in agronomical nanotechnology which now performs a vital role in detection control, preventing and developing the detection limit, easy operating, and cost-efficiency of with pathogens in many crops worldwide.

2 Nanotechnology in Agriculture

Nanotechnology is the research and experiment of controlling and manipulating materials at a nanometric scale (1–100 nm); it delves into the model, description, and use of modern methods and techniques at this same scale. This technology has taken a rise in recent years, so it has a wide range of tools, techniques, and potential applications, an example of these can be in medicine, biology, engineering, agriculture, among others (Shrivastava and Dash 2009; Yarlagadda et al. 2019). Moreover,

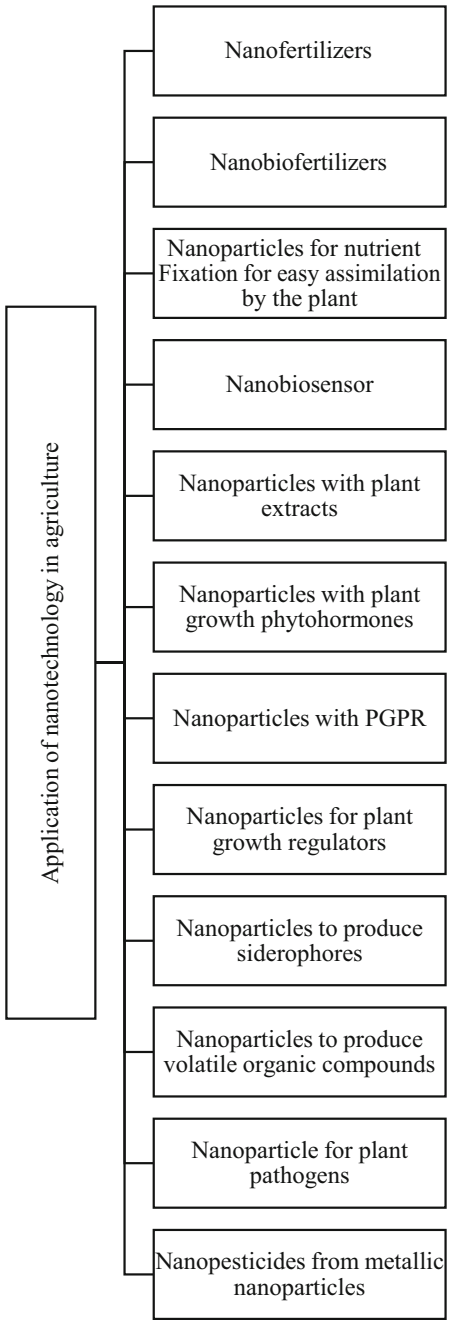

Fig. 1 Diagram description of functions of nanotechnology in agriculture

the application of nanotechnology over conventional systems has different advantages, to name a few: higher dissolution level, increased solubility, generates faster action mechanisms, increased bioavailability, and in living areas decrease the dose of the active principles, so it is expected that the expansion of its applications will become widespread in the following years (Rangasamy 2011).

This technology shows enormous virtues that will revolutionize and improve agriculture. Furthermore, population levels are rising, creating food supply difficulties in the following decades. Therefore, nanotechnology can help improve the yields and efficiency of agricultural production. All this coupled with the fact that the application of this technology in this sector increased economic rates and income in the market by almost 25%, and it is expected for 2020 that the incorporation of nanotechnology in the agricultural sector will boost global economic growth to about 3 trillion dollars (McKee and Filser 2016; Pandey 2018).

3 Nanotechnology in Plant Pathology

More than a few microorganisms, such as bacteria, fungi, insects, nematodes, viruses, and parasites, can produce plant diseases. Many methods are used for its control, among which agrochemicals and biological control stand out. However, its application still has various flaws, which is why new alternatives are currently required to control these pathogens (Agrios 2005). The application of nanotechnology in crop protection can generate positive change, increasing yields and reducing damage. Different types of nanoparticles or nanosystems are used to control phytopathogens, such as metallic nanoparticles and polymeric nanoparticles (Chhipa 2019; Kah et al. 2018).

3.1 Nano Pesticides from Metallic Nanoparticles

Metallic nanoparticles are the nanostructures currently have the most significant application in different industries since they are simple to develop and shape and size-control. Generally, the most studied are titanium, silver, copper, and zinc nanoparticles in the agricultural industry. Several studies have demonstrated the efficiency of these nanosystems against phytopathogens. The application of this type of nanostructures has succeeded in inhibiting the growth of pathogens such as *Alternaria alternata*, using titanium dioxide nanoparticles (TiO_2NPS) at a concentration of 100 ppm at the laboratory level and in Greenhouse conditions significantly decreased the damage caused, compared to the absolute control (El-Gazzar and Mahmoud 2020).

On some occasions, the effect of nanoparticles can be potentiated in combination with conventional phytopathogenic control products; as confirmed by Malandrakis (2020), he evaluated the combined effect of silver nanoparticles with

benzimidazoles, methyl tryptophan, and carbendazim against resistant isolates of *Monilia fructicola*. The results suggested that applying these particles as individual treatment and, in combination with conventional fungicide, provides the means to counteract resistance to benzimidazole.

Nanosystems developed from polymers of natural origin, trying to reduce the damage that metallic nanostructures can generate. An example of this and, in comparison, to way, Dananjaya (2017) to realize an analysis of the result of chitosan nanoparticles (CNP) and nanocomposites of silver chitosan (CAgNC) against a complex of *Fusarium oxysporum* species. This test and use of analytical techniques and microscopy verified that both nanosystems managed to induce damage on the membrane and the surface of the mycelium of the phytopathogenic agent, and specifically, CAgNC inhibited radial growth. They concluded that both systems function as antifungal agents against *Fusarium oxysporum*.

3.2 Nano Fertilizers

Overpopulation has generated an excessive request for nutrition, so the application of synthetic fertilizers that improve the progress of crops developed an excessive culture of application of these, which is continuously lost by leaching or volatilization, which has caused contamination of soils (FAO 2017). Therefore, nanotechnology for the application of fertilizers can reduce costs and damage to the environment since nanofertilizers can act as micronutrients or can be developed as nanocapsules that allow the release of nutrients when required (Usman et al. 2020).

Some of the virtues of nanofertilizers are the ability to penetrate the seed covers without effort, growth the accessibility of nutrients, expand the entree and reach of nutrients to the leaves and branches, which together is replicated as an intensification in development and higher plant yield (Pandey 2018; De la Torre et al. 2013). The expansion of nanofertilizers from micronutrients and macronutrients such as iron (Fe), calcium (Ca), potassium (K), manganese (Mn), magnesium (Mg), Nitrogen (N), phosphorus (P), and sulfur (S), achieved thanks to the combination of these themselves with nanomaterials. An example of this is hydroxyapatite, which generates a controlled and precise release, reducing costs and losses generally caused (Zulfiqara et al. 2019; Monreal et al. 2016).

3.3 Nanobiofertilizers

Biofertilizers are compounds or formulations with microbial agents and compounds that stimulate plant growth or fixation of atmospheric nitrogen. Therefore, nanobiofertilizers can be defined as incorporating into the nanostructure inside it with an active molecule that improves plant growth, so they use polymeric nanoparticles with natural or biodegradable matrices sought (Simarmata et al. 2016).

Polymeric nanoparticles are colloidal particles that, in their content, can load active compounds, which manage to supply the content in a slow and sustained manner. These systems' advantages over the conventional application of compounds: the high bioavailability, a controlled content release, and excellent protection of the active principles. This type of system can be made from various polymers, both synthetic and natural (Acharya and Pal 2020).

The nanoencapsulation of stimulating biocomposites in the development of plants begins to have a higher interest in the scientific and industrial fields. For example, studies show that the nanoencapsulation of compounds such as phytohormones in nanoparticles of natural matrices such as chitosan presents a more significant increase in tomato plants' development than the induction or exogenous application of these hormones in a conventional way (Andrade et al. 2020).

3.4 Nano Biosensor

Nanobiosensors are nanosystems built to analyze data at an atomic scale, store data, identify metabolites, and detect analytes and microorganisms. These nanobiosensors mixed in conjunction with "restrained bioreceptor" analyses are selective molecules of the objective analyte. The development and application of these presented as another neglected contribution to the monitoring and collecting data on the integrity of the soil and plants (Rai et al. 2012; Pandey 2018).

Various mechanisms and systems for developing nanosensors; however, carbon nanotubes and graphene have been the most widely studied and used, increasing the development of electrochemical sensors to detect plant diseases. Several studies in which Multiwalled carbon nanotubes were developed and evaluated to detect phytopathogens, an example of this may be the case of the study of quinoline as a secondary metabolite in oil palm leaves, attacked by Ganoderma boninense (Isha et al. 2019).

4 Plant Extracts Applications in Agriculture

Phytopathogenic fungi, bacteria, viruses, and others; cause considerable destruction to numerous cost-effectively essential crops. Chemical products have been used successfully to regulate phytopathogenic microorganisms' disease. Nevertheless, due to the widespread use of synthetics controls, numerous fungicides and bactericides developed fewer active because many plant pathogens improved resistance. Also, pesticides produced adverse results on non-target microbes, such as plant growth-promoting rhizobacteria (PGPR) and environmental and human health concerns (Shuping and Eloff 2017). Since early times, plants have been identified as a natural resource of numerous bio compounds with extensive biological activities. These compounds have been utilized for numerous fields such as cosmetology,

pharmacy, perfumes, the food industry, tinctures, dyes, agronomical sectors, among others (Ghnaya et al. 2016). About 75% of the world's population relies on plant extracts as a treatment for numerous applications. We can obtain essential oils, plant extracts, herbal teas, salves, infusions, and others contained by natural products. Natural extracts are complex combinations of bio compounds with characteristic biological derivatives generally from the leaves, stems, fruits, or roots. The biological activities presented involve the antibiotic, antioxidant, antiparasitic, antifungal, antihelmintic, herbicidal effects against germination and seedling growth (Armendáriz-Barragán et al. 2016; Ghnaya et al. 2016). Plant extracts with antimicrobic properties and includes a variety of secondary metabolites like alkaloids, quinones, terpenoids, saponins, glycosides, flavonoids, and tannins are a point of study and research focus (Rodino et al. 2017). The intensity of these bioactive compounds differs in some ecological conditions, pathosystems, solvents extraction, and others (Shaheen and Issa 2019).

Moreover, for getting extracts usually, organic diluents (e.g., dichloromethane, ethyl acetate, ethanol, hexane, methanol, and others) are employed. Therefore, the decisive vehicle of extracts is obtained to prevent their direct use in organisms or systems from being evaluated. Another hand, the security, conservation, and aim delivery of plant extracts are a new experiment to overcome their possible use for controlling pathogens (Rubió et al. 2013). Currently, exploration is interested in the constitution of plant extracts, whereas mixtures that make possible the inexpensive, efficient, security, and direct usage of natural products should be focused on. One of the most remarkable techniques to apply natural extracts consists of nanoparticles (Armendáriz-Barragán et al. 2016). Nanomaterials are materials with dimensions fluctuating between 1 and 100 nm; nanoscale-sized particles exhibit distinctive structural, chemical, magnetic, mechanical, biological properties, and electrical estates.

For this reason, of their size and specific physicochemical features of nanoparticles can be generated formulations with more than a few advantages, such as encapsulation of active agent of chemical nature in the preparation (a combination of actives), directing of target structures (low toxicity), easy removal of organic solvent through the advance of the nanoparticles (active purification actions), maintenance and shield of the encapsulated compounds (enzymes damage, environment, and others), and accurate delivery of incorporated active (Patra et al. 2018; Armendáriz-Barragán et al. 2016). To potentialize the variety of biological compounds of plant extracts and the benefits presented by the nanoparticles, scientists have been progressively more involved in designing formulations of nanoparticles from several polymers by encapsulated plant extracts, essential oils, and bio compounds. Table 1 can appreciate the use of several bioactives of nanoformulations by the agro-industrial and food industry.

The global requirement for healthy and safe nutrition with the minimum synthetic process is growing day by day, mainly by the health and ecologically concerned purchasers. In this perspective, scientific knowledge on the groundwork mode of nanocapsules plant extracts products and how to use them is relatively rare and incomplete. Therefore, it is essential to optimize the extraction and manufacturing

Table 1 Nanoformulates based on different extracts, essential oils, and bio compounds of plants and their evaluation effects on the agricultural and food industry

Extract plant	Nanoformulate	Bioactivity reported	References
Guabiroba fruit (*Campomanesia xanthocarpa* O. Berg) Methanolic extract	Nanoencapsulation, with Poly(D,L-lactic-co-glycolic) acid (PLGA) functionality. An emulsion-evaporation technique manufactured the nanoparticles charged by phenolic extract of Guabiroba. Their physicochemical and operational characteristics were examined at two lactic to glycolic acid ratios (50:50 and 65:35)	Effective inhibition of *Listeria innocua,* with lower concentration ($P < 0.05$). Calculations suggested that PLGA nanoparticles can be employed as a release system for phenolic compounds at reduced levels than necessary initially for enhanced functional characteristic	Pereira et al. (2017)
Garlic cloves were obtained from the community marketplace in Porto Alegre, Brazil, and combined with distilled water at the 1:1 (w/w) ratio. Nisaplin® was prepared with 0.01 M HCl to acquire a 12.5 mg/mL stock solution	Co-encapsulated into phosphatidylcholine nanoliposomes. Correspondingly, the diameter and zeta potentials were 179 nm and −27.7 mV, with entrapment effectiveness of about 82% and 90% for nisin and garlic extract	The effectiveness of available and encapsulated nisin and garlic to manage the growth of *Staphylococcus aureus, Escherichia coli*, *Salmonella Enteritidis,* and *Listeria monocytogenes* was assessed over time in whole milk at 37 °C	Pinilla and Brandelli (2016)
Oregano (*Origanum vulgare* L.); Carvacrol and thymol	Nanocapsules (by emulsification of soybean oil in a water solution of Arabic gum + Tween 20) included in chitosan flexible plastic film covering	Regulated delivery leads to the growth inhibition of a broad spectrum of microorganisms such as *Listeria innocua, Staphylococcus aureus, Escherichia coli, Aspergillus niger*. and *Saccharomyces cerevisiae*	Guarda et al. (2011)
Xoconostle (*Opuntia oligacantha C. F. Först*). acids phenolics and flavonoids	Nanoemulsion with essential oil of *Citrus sinensis*	Nanoemulsion increases total phenolic compounds (409.37 ± 22.14 mg EAG/mL), thus enhancing antioxidant activity (357.85 ± 29.26 mg EAG/mL). The *Colletotrichum gloesporoides* was influenced by the nanoemulsion matched by a control (17.1 and 21.25 mm)	Solís-Silva et al. (2018)

(continued)

Table 1 (continued)

Extract plant	Nanoformulate	Bioactivity reported	References
Garlic essential oil (purity >85%, containing three components, around 35% of diallyl disulfide, 42% of diallyl trisulfide, and 16% of diallyl sulfide), saved at 4 °C	The nanoparticles were carried out utilizing the melt-dispersion technique. Consequently, the oil-loading effectiveness may reach 80% at the optimal ratio of essential oil to PEG (10%)	The control effectiveness against *T. castaneum* continued over 80% later than 5 months, probably due to the slow and persistent release of the active elements from the nanoparticles. The control of free garlic essential oil at a similar concentration (640 mg/kg) was 11%	Yang et al. (2009)
Ethanolic extract of propolis 30% was manufactured from Remedios Herbolarios Rosa Elena Dueñas, S.A de C.	Nanoformulations were developed by evaluating the percentage of the components such as chitosan solution, chitosan nanoparticles, propolis, glycerol, and canola oil. Strengths of formulations were 20%, 30%, and 40%, and the control contained Czapeck-dox agar medium	The nanoformulations show the antifungal action of the formulations and the manufacture of aflatoxins of the Aspergillus flavus. The highest impact was found in spore germination, at 97%, equivalent to chitosan-propolis nanoparticles-chitosan nanoparticles-propolis extract at the highest concentration of 40%. At this same concentration, the manufacture of aflatoxins was 100% inhibited with the treatment with chitosan at 1%	Cortés-Higareda et al. (2019)
Passion fruit (*Passiflora edulis* Sims). Phenolic extracts	Co-precipitation with biodegradable polymers, involving poly(DL-lactide-co-glycolide) (PLGA)	The encapsulation with both PLGA significantly enhanced the antimicrobial activity of both extracts as it reduced up to 95% the concentration of extracts needed to inhibit microorganisms improving the delivery of antimicrobials to the microorganisms in an aqueous environment and could be used as replacements to chemical antiseptics to controller pathogen better as *L. innocua*	Oliveira et al. (2017)

(continued)

Table 1 (continued)

Extract plant	Nanoformulate	Bioactivity reported	References
The essential oil is obtained by steam distillation from dry plant material (fresh and mature lemongrass leaves)	Nanocapsules formulated by the interfacial deposition of the preformed polymer once the solvent displacement method. The quantity of the surfactants Tween 80 (77 and 154 mg) and Span 60 (77 and 154 mg), and the quantity of lemongrass essential oil (100 and 200 μL). The amount of poly (lactic acid), ketone, and deionized water were continuous	They have determined in vitro antifungal activity against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* with a MIC dose of 0.1% (v/v) for phytopathogens	Antonioli et al. (2020)
Origanum vulgare essential oil	The antibacterial polyelectrolyte carboxymethyl xylan/chitosan films with halloysite nanotubes and *Origanum vulgare* essential oil	The antimicrobial activity of the prepared nanocomposites was analyzed by the colony count method against *E. coli* and *S. aureus*. These results of all prepared nanocomposites exhibited the distinctive antibacterial potential against both bacteria	Yousefi et al. (2020)
Neem and *Rendle* (Citronella) oil	Encapsulating oils in oil-in-water (O/W) emulsions or nanoemulsions using low or high energy techniques. The study employs different nanoemulsions of crude neem and citronella oils with a surface-active agent	Neem nanoemulsion and citronella nanoemulsion were active against *R. solani* (ED 5013.67 and 25.64 mg L^{-1}) and *S. rolfsii* (ED5014.71 and 20.88 mg L^{-1}).	Osman Mohamed Ali et al. (2017)

process, stabilize these preparations, and analyze the effects of delivery systems application in crops and fields to potentialize their effect. Furthermore, although nanotechnology is one of the hopeful instruments for successful delivery, the toxicological qualities of most of the nanocarriers and their molecular objectives site are yet poorly investigated. Hence, the physicochemical relations of nanoencapsulated extracts plants together with harvests a food method and their toxicological effects need to be explored prior to their industrial purpose.

5 Plant Growth Phytohormones Applications in Agriculture

Phytohormones or plant growth regulators are compounds of low molecular weight that take action at small concentrations. They adjust plants' different cellular processes and response mechanisms to ecological conditions and stress situations (Wani et al. 2016; Fahad et al. 2015). These compounds are of vital importance since because plants cannot move, phytohormones regulate and control the mechanisms to develop strategies to grow sufficiently and defend themselves (Kim et al. 2020).

The plant hormones that have been investigated the most involve abscisic acid (ABA), auxins (Aux), cytokinins (CK), ethylene (ET), jasmonates (JA), and gibberellins (GA). This type of compound can also produce by microorganisms in high concentration levels, which alternate the physiological behavior of plants (Karadeniz et al. 2006). Therefore, the study and production of phytohormones presented a promising tool for their proper application to improve crop yields and resistance mechanisms (Curia et al. 2018).

5.1 Auxins

The auxins are molecules generated from tryptophan in plants, bacteria, and algae. They have a structure very similar to melatonin in animals. They are synthesized, specifically of indoleacetic acid, the most abundant auxin, by two main synthesis routes: one dependent on tryptophan and the other independent from tryptophan but derived from a precursor thereof (Matthes et al. 2018; Mashiguchi et al. 2011). These molecules are formed in several plant tissues and cells, although their production levels are highest at shoot tips, young leaves, and flower buds. They are distributed throughout the plant through the phloem (Brumos et al. 2018). The auxins can intervene in the cellular differentiation of organs, roots, stems, and leaves; in the same way, they can give rise to these. These hormones can regulate the formation and elongation of stems, in calluses, together with cytokinins, which promote cell division (George et al. 2008).

5.2 Cytokinins

Cytokinins have diverse molecular structures; they are synthesized by the enzyme's isopentenyl transferase and a lonely guy. In contrast, the conjugation of cytokinins is carried out mainly through the enzymes cytokinin oxidase, and from there, they pass to receptors that initiate a phosphorylation signaling cascade and subsequently activate response regulators (Wybouw and De Rybel 2019). These hormones are manufactured in several kinds of cells, both in roots and in shoots, not as previously

thought that they only produced in the roots. They are transported locally and remotely through the xylem from the root to the shoots and through the phloem inversely (Kudo et al. 2010). Cytokinins regulate many functions in plant growth and regularly play a role in conjunction with additional hormones, mainly auxins. One of the most significant roles they adjust is cell proliferation by affecting cell division and diversity (Schaller et al. 2014). Various studies have shown that plants that generate high levels of auxin and cytokinin proliferate massively, giving rise to calluses, the latter stimulated by high levels of cytokinins, which induce shoot regeneration (Schaller et al. 2015). These hormones oversee regulating the phyllotaxis of the leaves, developing the female gametes and the vascular cambial. In the same way, they are in charge of inhibition roles since they manage to impede the growth of the root, avoiding its branching and thus helping to design the optimum architecture of the same (Kieber and Schaller 2018; Wybouw and De Rybel 2019).

5.3 Gibberellins

Gibberellins (GA) are a class of phytohormones with a basic molecular structure of 20 carbons; they are derived from 4 isoprenoid units that form a diterpenic structure with 4 rings. Being dipertenic acid, they derived from the isoprenoid biosynthetic pathway, formed mainly from methylerythritol 4-phosphate in terrestrial plants (Lichtenthaler et al. 2006; Hernández et al. 2020). There are more than 130 gibberellins identified in plants and microorganisms; nevertheless, GA1, GA3, GA4, and GA7 have a biological effect as hormones, though the rest function as metabolites or precursors of those already mentioned (Binenbaum et al. 2018). Generally, gibberellins develop plant tissue, specifically in the continually growing parts, such as leaves, roots, and flowering. They have also been shown implicated in the regulation of development, response mechanisms, and induction germination of seeds. Precisely, gibberellic acid controls flowering and extension of the nodal segments, allowing cell extension in response to light circumstances (Hernández et al. 2020; Gupta and Chakrabarty 2013; Alcantara et al. 2019).

5.4 Abscisic Acid

ABA is a plant growth-regulating hormone derived from the precursors of carotenoids in plants and photosynthetic organisms. It is a terpenoid derived from isopentenyl pyrophosphate synthesized from a carotenoid in plastids. It is produced in almost all plant cells and is transported by xylem and phloem (McAdam and Sussmilch 2020; Jordán and Casaretto 2006). ABA was previously considered to be generally a plant growth inhibitor as it could stop the process of germination and plant development. However, various studies have shown that it supports the development of roots and shoots (Humplík et al. 2017). By regulating stomatal

closure, it presented as a water regulator adaptation of plants in different abiotic stress environments (Colebrook et al. 2014). Also, it can regulate the dormancy in the seeds and the process of their maturation (Alcantara et al. 2019).

5.5 *Ethylene*

Ethylene is a phytohormone in the gaseous state; it is colorless and odorless. It is present in angiosperms and gymnosperms. It has a fundamental role in the response of plants to outside stimuli to develop the modification and efficiency of agricultural systems. The concentrations required to initiate its biological effect are deficient, between ppm or ppb. Being a gas, its distribution is high-speed since it is not necessary to transport it (Iqbal et al. 2017; Dubois et al. 2018). Ethylene biosynthesis is identified as the methionine or Yang cycle, which is of high significance since it can recycle methionine as a supply of sulfur (Jordán and Casaretto 2006).

It has a wide selection of effects on plant development regulates various processes such as seed germination, sex determination, fruit ripening, growth, ripening, and plant senescence. This phytohormone plays a crucial role in the abiotic stress response of plants (Kazan 2015; Khan et al. 2020). It is related to synergistic and antagonistic processes when combined with different hormones since they induce and potentiate the application of auxins, abscisic acid, and cytokinins in development and maturation processes. On the contrary, in combination with gibberellic acid and jasmonic acid, they are inhibited by the combination with ethylene (Alcantara et al. 2019).

5.6 *Jasmonic Acid*

In animals, jasmonic acid is a phytohormone of lipid origin with a molecular structure like prostaglandins. It performs as a indicator molecule for the responses of plants to several stress situations and participates in various growth and development processes. Chemically speaking, it is a cyclopentenone with a pentenyl chain and a carboxylic chain (Carvalhais et al. 2017; Ting et al. 2014). It is synthesized from the octadecanoic pathway from the α-linoleic acid released from the membranes of chloroplasts (Wasternack 2007). It is generally found in peak molar concentrations per gram of fresh weight in plant tissue, and its concentration increases about external stimuli or conditions (Laredo et al. 2017).

In plants, jasmonic acid regulates different physiological processes such as growth, cell development, embryonic organs, seed germination, root development, fruit ripening, and tuber formation (Gomi 2020; Chávez et al. 2012). In the same way, this acid is produced after the attack of some pathogens, such as microorganisms or insects. In addition, it is present in the regulation of compounds during

situations of abiotic stress, such as droughts, injuries, exposure to ozone (Chunmei and Zhi 2013; Laredo et al. 2017).

6 Plant Growth-Promoting Rhizobacteria Uses in Agronomy

Plant growth-promoting rhizobacteria (PGPR) demonstrates a crucial position in the environmental agriculture business and positively affects biotechnology and nanotechnology (Khan and Rizvi 2014; Vejan et al. 2016). The expression "plant growth-promoting bacteria" refers to bacteria colonizing the rhizosphere and incrementing plant growth. The rhizosphere is the soil habitat where the plant root is available and is defined as a region of the upper limit microbial activity, causing a rich nutrient combination in which essential macro- and micronutrients are acquired (Vejan et al. 2016). The microorganisms present in the rhizosphere are different from their habitats due to the survival of root exudates that offers nutrients for microbial development (Burdman et al. 2009).

PGPR has been developed to increase harvest yields by enabling plant growth across direct or indirect systems. The ways of PGPR involve varying hormonal and nutritional equilibrium, producing resistance for plant pathogens, and solubilizing nutrients for environmental uptake by plants (Vejan et al. 2016). Therefore, an ideal PGPR should possess some of the characteristics presented in Fig. 2 (Nakkeeran et al. 2005; Grobelak et al. 2015; Nazari and Smith 2020).

At the same time, PGPR and nanomaterials may thus be a promising approach for managing plant growth and production. Moreover, applying nanomaterials such as silver, titanium, zinc oxide, silica, gold, and others with PGPR possesses exceptional potential (Nayana et al. 2020).

6.1 Nanoparticles for Nutrient Assimilation for Simple Uptake by the Plant

The demand for a good quality PGPR biofertilizer was highlighted last year. The inclusion of nanoencapsulation understanding has been essential to the uprising of today's PGPR biofertilizers' design (Vejan et al. 2016). The productivity of nanofertilizers matched to conventional fertilizers has been demonstrated to minimize nitrogen deficiency due to leaching, releases, and long-term application by soil organisms (Liu et al. 2006). However, the PGPR procedure as fertilizer by traditional techniques is not functional as 90% is lost to the air in the application, they are sensitive to the environment (UV radiation, heat, and others), and run-off requires application prices to the dairy farmer. Nanoencapsulation knowledge could be used as a flexible and adaptable tool to protect PGPR, increase their time and spreading in

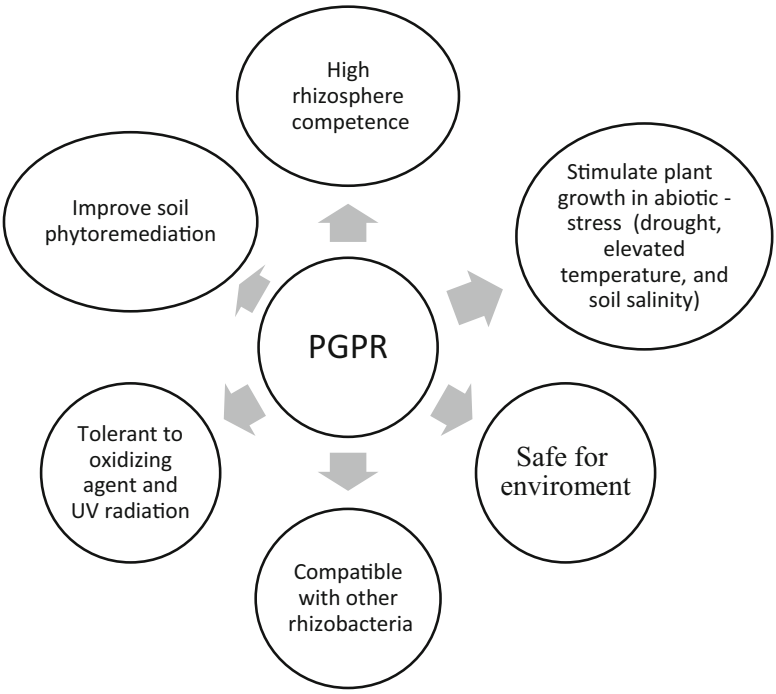

Fig. 2 PGPR ideal characteristics for plant growth promotion

fertilizer preparation, and allow the managed release of PGPR (Vejan et al. 2016). For the sample, a PGPR, separated or combined, silica can substitute conventional fertilizers and be employed as a beneficial agent for biofertilizer improvement (Ramprasad et al. 2015; Suriyaprabha et al. 2012).

6.2 Nanoparticles for Plant Growth Regulators

Phytohormones or plant growth regulators are organic materials created organically in higher plants, controlling development or other physiological tasks at a site isolated from its formation point and working in minute amounts (Suman et al. 2017). Phytohormones are usually applied to plant development, and recent studies revealed the effect of PGPR and nanoparticles in growing phytohormone production. Khan and Bano (2016) reported that treatment with silver nanoparticles and PGPR stimulates abscisic acid (ABA) concentrations by 34%, indole acetic acid (IAA) to 55%, and gibberellic acid (GA) to 82%. On the other hand, silver nanoparticles are now an effective instrument to improve phytohormones to a better level (Nayana et al. 2020). Meanwhile, gold nanoparticles increased IAA production by *P. monteilii* when it is present with gold nanoparticles (Panichikkal et al. 2019).

6.3 Nanoparticles to Produce Siderophores

PGPR promotes plant growth by non-volatile compounds, like auxins, cytokinins, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, decreasing plant ethylene concentrations; siderophores enable root assimilation of nutrients (Liu and Zhang 2015). Siderophore increases Fe accessibility inland, although its help to iron transport to several plant segments is not investigated until now (Sah et al. 2017). Siderophore represents an option to synthetical fertilizers by confronting salt-stress effects and Fe restriction in salted lands (Ferreira et al. 2019). An example is the result of siderophore generated by *Pseudomonas* for iron purchase in the existence and non-existence of Fe. On the other hand, ZnO nanoparticles demonstrate a dose-dependent improvement in the siderophore manufacture of microorganisms (Haris and Ahmad 2017). An ecologically pleasant dosage of Zn nanoparticles, besides PGPR, can very well transform the agricultural science area (Nayana et al. 2020).

6.4 Nanoparticles to Produce Volatile Organic Compounds

Certain PGPR strains can emit microbial volatile organic compounds (mVOCs). VOC production is a distinctive estate of various soil microorganisms, even though the uniqueness and volatile actives emitted several among varieties (Liu and Zhang 2015). Compounds consist of several complex mixtures of actives, as well as alcohols, alkenes, alkanes, esters, sulfur, ketones, and terpenoids (Cappellari et al. 2020). Compounds have been presented the capacity to stimulate plant growth and induce systemic resistance (ISR) by microorganism phytopathogenic (Cappellari et al. 2020). PGPR VOCs also induce salinity and stress tolerance and increase S and Fe in nutrition plants (Liu and Zhang 2015). Previous studies determined that the directly application of PGPR and confrontation to VOCs produced by these rhizobacteria improve the biosynthesis of bioactives and enhance biomass manufacture in several aromatic plants (Cappellari et al. 2020). Though there are several types of research around the activities of mVOCs produced by rhizobacteria on the bioactives profit of aromatic plants below abiotic stress circumstances, revisions associated with VOCs emission of with biological action rhizobacteria is an original area appealing collective attention (Cappellari et al. 2020). Moreover, the research of nanoparticles to produce VOCs is not studied to be a new research area.

6.5 Nanoparticle for Plant Pathogens

Developing the conjugative method of nanotechnology and PGPR has a vast capacity to develop both yields and opposition to plant disease. Bioinsecticides

preparation in nanoscale is reasonably lucrative because, with a small quantity, more efficiency could be realized. However, the critical point is to decrease adverse impacts on plants and land, so it is eco-responsive and can be used in organic farming systems (Kasmara et al. 2018). A clear case is the employment of titania nanoparticles. Titania nanoparticles increment the linkage of helpful microorganisms to the roots of oilseed rape and protect the plant from *Alternaria brassica* (Palmqvist et al. 2015). On the other hand, titania nanoparticles offer an efficient stage for PGPR establishment on the plant and subsequently can be employed as a possible vehicle for PGPR improvement for environmental agriculture (Nayana et al. 2020). Laboratory analysis by EDS, SEM, SDS-PAGE, CLSM, and fluorescence lengths corroborated the nanoparticle-mediated establishment, which eventually improved microorganism biomass (Palmqvist et al. 2015).

6.6 Nanoparticles Toxicity in PGPR

Some microorganisms can generate nanoparticles in the substratum (Gurunathan et al. 2009; Khan and Rizvi 2014). However, understanding the possible toxicity of nanoparticles simultaneously with microorganisms is crucial for improving their purpose. In such circumstances, the application of nanoparticles obtained from biopolymers such as proteins and carbohydrates can approach a considerable part (Nayana et al. 2020). Besides, the toxicity of nanoparticles over the PGPR also must be evaluated.

7 Conclusion

Plant pathogens decrease midpoint crop yield by10–20 %, resulting in billions of dollars of damages to food production in the world. Although infection managing possibilities exist for many crops, all options possess substantial shortcomings except for host resistance. The necessity for increasing food manufacture and the tasks caused by a warming climate emphasizes the demand for new infection management techniques and procedures. The employee of natural agents such as plants, phytohormones, and plant growth-promoting rhizobacteria is an alternative ecological technology to manage pathogens in pre, until and postharvest practices. Among developing, tools, skills, technologies, films, edible coatings, and nanoencapsulation techniques show advances and safeguard the development of the treatment efficiency. Nanotechnology has been shown excellent potential in agriculture of precision. Nanoparticles can be simply manufactured from different biological suppliers and employed in agronomy. The nanotechnologies tools and methods can develop the way; agriculture is seen and has a hopeful possibility in the imminent age of agricultural innovation. Nanoparticles possess potential as

nanocarriers loaded with bactericides, fungicides, herbicides, nutrients, micronutrients, nucleic acids, fertilizers, and targeting exact plant tissues to deliver their charge on the needed section of the plant. Biotechnological developments on necessary techniques fast and more exact diagnostic tools employ nanomaterials because they present an essential and hopeful future for modern agriculture, like delivery exactitude of several bio compounds by treatment and disease analysis at an initial phase. Therefore, attention must be paid to the nanotoxicological properties, mechanisms of action against pathogens, and the effect on the products' organoleptic properties. All these qualities will encourage the productive employment, distribution, and commercialization of nanoagrotechnology, but a cost-benefit connection and ecotoxicity evaluation will be essential to comply with this technology's sustainability.

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Implications of Plant Invasion on the Soil Microbial Diversity and Ecosystem Sustainability: Evidence from a Tropical Biodiversity Hot Spot

T. K. Hrideek, M. Amruth, Suby, P. Aswaja, and A. Sabu

Abstract Invasive plants are a major threat to the conservation of biological diversity and ecosystem sustainability across the globe with the associated socio-economic consequences. In addition to this, anthropogenic climate change is mounting new challenges to conservation professionals in controlling invasive plant populations and in mitigating the impacts of invasions on ecosystem stress. While ecosystem restoration depends heavily on the management of the edaphic environment, especially on soil microbiota, which fulfills an important share in ecosystem process, regeneration, and resilience. However, the effects of plant invasions on soil microbial populations and how ecosystem sustainability is impacted are not well explored. This chapter presents a review of scholarship on the role played by soil microbial populations in ecosystem processes and sustainability. This is a synthesis of observations from various ecosystems on the changes in root-zone microbial diversity in the context of plant invasions. The insights from the review are brought forth to discuss a case of plant invasion in forest tracts in the Western Ghats—a global biodiversity hotspot—in Peninsular India, to draw insights on the shifts in soil root zone microbial populations and its possible consequences on the ecosystem sustainability.

Keywords Alien invasive plants · Invasion · Allelochemicals · Allelopathy · Detoxification

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1 Introduction

Range expansion of plant species, either with the aid of human or natural agency or through a combination of both, leading to modification of the plant community structure to a near-total domination by any successful alien species of plants, by inflicting undesirable impacts on the ecosystem and economy is designated as plant invasion. Impacts of the plant invasions on biodiversity are reported from across the world from diverse ecosystems. Plant invasion involves processes and consequences which are not always readily visible unless they are systematically explored. Shifts in soil quality and augmented belowground competition inflicted by invasives on ecosystem resources are some such processes that aid the invasive species to out-compete and exclude the local species.

The past two centuries have seen an increased incidence of range expansion by various organisms purposefully or inadvertently aided by increased transportation and material exchanges owing to imperial expansions and globalization. While range expansions of some of these species are intentional and benign to human society, a majority are unintended and incur a heavy economic cost. According to an estimate, monetized values for global damage inflicted by invasive species total about US $1.4 trillion, which is about 5% of the global economy in 2012. In the USA where more than 100 million acres are affected due to biological invasion, the estimated cost was about 120 billion dollars annually (Anonymous 2012). Yet another estimate for the USA presents a similar figure for the cost of damages amounting from 79 species of harmful invasives is about 185 billion US dollars, which is roughly 1.4% of the gross domestic product (GDP) of the USA in 1993. The estimates provided by Marbuah et al. (2014) of the damages due to invasives in India, Brazil, South Africa, and 5 other countries are to the tune of 12% of their GDPs, which are highest among the countries studied.

Some of the all-time disruptive alien plant invasions identified at the global level by Richardson (2001) are range extension by genera Acacia, Hakea, and Pinus in South Africa; rapid invasion of *Miconia calvescens* in Society and the Hawaiian Islands; spread of *Bromus tectorum* in Great Basin in North America and *Mimosa pigra* invasion in the Northern Territory of Australia. These invasions have pushed hundreds of plant species to extinction, altered the fire, streamflow, and nutrient cycling regimes; the physiognomy of native woodlands altered, and have rendered local wetlands to impenetrable thickets with serious consequences to the local subsistence economy.

To explain the competitive advantage enjoyed by the invasive species over the native species, Callaway et al. (2000) and Callaway and Ridenour (2004) proposed the Novel Weapon Hypothesis. According to this hypothesis, the trait that enables the invasive species' dominance may be considered a 'Novel Weapon'. These traits can be either single or a combination of traits such as high fecundity, dispersal ability, lack of predation, the extraordinary ecological adaptability of the species, and their ability to competitive exclusion of native species from their habitats. These traits are part of the unique survival strategies evolved through co-evolution and

natural selection. Among these traits is the ability to produce certain special metabolites called allelochemicals, which are produced and released to the soil environment by the invasive species which create unfavourable edaphic conditions for the survival of the native species. This trait of deployment of allelochemicals is termed allelopathy.

The 'Novel Weapon' may enable an invasive in its early and faster establishment, and proliferate the habitat while bestowing in species the ability for accelerated growth, greater dispersal, adaptability to a range of ecological conditions, etc. The brunt of the range expansions of the invasives are borne by native species and this is manifested as their poor survivorship and reduced population size. The native animal and plant communities with their reduced population size and distribution are very often pushed into extinction vortices in the wake of a wave of invasion. As the biological invasions are persistent, more investigations into such not so obvious determinants, strategies, and consequences of invasions are warranted. One such area requiring deeper investigation is shifts in soil microbiome consequent to plant invasions aided by allelopathy and the subsequent impacts of this on the ecosystem sustainability and resilience.

Before we discuss the aspects of plant invasion and its impacts on soil microbial diversity, it is necessary to elaborate on the role of the soil microbiome in the ecosystem process.

2 Soil Microbial Populations in the Ecosystem Process

Microbial populations have a dominant presence in the soil environment. While studies on certain categories of soil microbiota are disproportionally advanced than many other lesser explored groups, the knowledge of interactions among these and their role in larger ecosystem processes is still in a fledgling stage. Nevertheless, there are sufficient clues to indicate that studies on the soil microbiome are highly challenging due to the complexity of interrelations and that studies could be highly rewarding due to the tremendous scope and consequences this knowledge has on the ecosystem restoration for improved ecosystem services (Coban et al. 2022). Soil microbes play a crucial role in maintaining the vital edaphic process in the ecosystem resilience such as soil nutrient cycling, maintenance of consistency and structure of the soil, regulation of pathogenic microbial population, and supporting plant biomass production.

2.1 Patterns in Microbial Diversity in Soil

Biological diversity in plant communities is a manifestation of the continuing process of co-evolution which is closely guided by diverse factors at equally diverse levels of interactions viz. climatic, edaphic, inter and intraspecific interactions

among the microbe and host plants besides the powerful factor called human agency. The act of human agency is guided by the perceptions of the utility of the biodiversity elements in the socio-cultural and economic realms.

As in the case of aboveground plant communities, a variety of life forms are found in the belowground environment, which determines the fertility and characteristic of the soil for plant growth. Soil organisms are broadly classed into macro, meso, and micro based on their size and into epigeic, endogeic, or anecic, based on the niches they occupy within their habitats (Swift 1976).

The term macrobiota is designated for those above 2 mm in diameter in the size, and these can be viewed unaided by the human eye. Macrofauna such as invertebrates inhabit and feed in the soil and surface litter includes earthworms to cicadas, ant-lions, and earwigs to snails, spiders, scorpions, wasp larvae, etc.

Organisms with diameters varying between 0.1 and 2 mm are classed as mesobiota and include microarthropods such as springtails, pseudoscorpions, protura, diplura, mites, myriapods such as symphylan and pauropoda and worms such as enchytraeids. While these organisms possess only limited burrowing ability, they occupy soil pores and gather nutrition by feeding on microflora and organic matter.

Microbiota is the smallest organism of size class below 0.1 mm which are more abundant and diverse than other classes. These include microflora and microfauna. The microflora includes bacteria, cyanobacteria, algae, archaea, fungi, yeast, actinomycetes, myxomycetes. These are capable of decomposing most detrital materials. Protozoa, tardigrades, turbellarians, nematodes, rotifers, etc. constitute the soil microfauna. These groups are present in the soil water and find their nutrition by feeding on microflora and plant roots.

Above-ground plant communities have a significant role in determining the structure of soil microbial communities. The abundance and the activity of microorganisms in soil follow horizontal and vertical patterns along the soil profiles. Various groups of microorganisms follow different spatial patterns (Constancias et al. 2015). The spatial heterogeneity in the distribution of the microbiota may range from millimetre-scale up to hundreds of meters. Various studies have compared microbial patterns along the gradients in soil properties such as texture, aggregation, bulk density, moisture, pH, percentage of organic matter in the soil, oxygen concentration, inorganic N availability, precipitation levels, and vegetation dynamics. Most of these properties are found to be significant at the microscopic scale, while others are found to act only over larger distances. In some cases, relatively less disturbed systems have shown a high degree of Spatial heterogeneity (Constancias et al. 2015).

A major review of the role played by the soil microbiome in land restoration by Coban et al. (2022) has suggested three groups of beneficial microorganisms be distinguished with respect to their impact on nutrient cycling, plant growth, and soil structure, which include plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria, together with ectomycorrhizal fungi (EMF). The authors have included cyanobacteria in biological soil crusts (BSCs) as a potential fourth group that is capable of increasing the

availability of soil nutrients either by enriching the soil with nitrogen by fixing atmospheric nitrogen or by improving the surface soil structure.

2.2 *Importance of Faunal and Microbial Populations in Determining Soil Quality*

Soil formation is the net outcome of a complex interaction of biological, chemical, and physical processes. Soil fauna plays a critical role in physical conversion and nutrient release from plant residues. According to Hole (1981), soil macrofauna modifies the soil structure through several activities such as mixing, mounding, forming and backfilling voids, forming and destroying peds, circulation of water and air in the soil, regulating soil erosion, decomposition of organic matter mainly plant and animal litter and thus by aiding nutrient cycling; soil fauna also alters soil structure by constituting new material to soil by regurgitation, mixing of excreta, saliva or mucus with soil existing matter. These activities of macrofauna influence mineralization and humification of soil organic matter, rates of soil turnover, soil texture, and hydrological aspects of soils such as total porosity, water infiltration, and soil water retention in tropical ecosystems.

Soil microbes are responsible for crucial biological transformations and drivers that form pools of carbon (C), nitrogen (N), and other nutrients which are stable and stable (Schulz et al., 2013). Thus, the diversity of microorganisms in soil is considered an important measure of soil health (Garbeva et al. 2004).

2.3 *Plant-Microbe Interaction*

Just like various aspects of the biological diversity of any given biotic community, the diversity of microbial communities is also expressed at different levels of variability and complexity; such as genotypic diversity and variability within the species populations, species richness in terms of the number of species, a relative abundance which is the measure of evenness of distribution of taxons and variability in the constitution of functional groups or guilds within the communities (Torsvik and Øvreås 2002). Little is known about how an entirely new ecosystem change will be induced in plant-microbe interactions when plants are introduced and spread in new locations of varied soil microbiomes.

Studies have amply indicated that the plant-associated microbes may inhabit plant tissues as well as plant surfaces or organs such as the leaves and roots (Berendsen et al. 2012; Bulgarelli et al. 2012). Sanon et al. (2009) has proposed that the microbial communities may have a crucial role in triggering ecosystem-scale changes to soil biochemistry induced by the invasive plants. It is not the function of the ecosystem alone that is affected due to shifts in the composition of the

microbial community in soils in the wake of colonisation by the invasive plant species, but the composition of the native plant community is also greatly affected. Ehrenfeld (2003), has demonstrated that soil carbon, nitrogen, salinity, moisture, and pH are altered in the aftermath of plant invasion. The inoculation, colonisation, and transmission of endophytes across plants are enabled by multiple factors which include co-evolutionary pathways determining the plant resources allocations and adaptive faculty of the endophyte to colonize plant tissues. In any plant organ, the microbial composition is influenced by a range of biotic and abiotic factors. These factors may include soil pH, salinity, soil type, soil structure, soil moisture, and soil organic matter and exudates (Fierer 2017), which are most relevant for belowground plant parts, whereas factors like external environmental conditions including climate, pathogen presence and human practices (Hardoim et al. 2015) influence microbiota of above- and below-ground plant parts. Host and compartment-specific assembly indicate a strong functional relationship between the plant and its above-ground microbiota, however, more research is still required to understand this relationship. Endophytes as well as above-ground microbiota are well known for their potential to promote plant growth, improve disease resistance and alleviate stress tolerance (Stone et al. 2018; Hardoim et al. 2015).

According to Richardson et al. (2000), the invasive ability of plants is strengthened by complex feedback loops of plant-soil microbe interactions. It has been observed that the allelopathic effects of *Polygonella myriophylla* are mediated by microbial and non-microbial oxidative transformations of soil allelochemicals (Weidenhamer and Romeo 2004). Very often than not the functioning of the ecosystem and native above-ground plant community composition is altered greatly by shifts in microbial community composition of soils consequent to colonisation by invasive plant species. Some microbes such as rhizobacteria are linked to the rhizosphere due to certain genetic factors (e.g. those with genomes that have the potential for producing a higher amount of chemotaxis and carbohydrate metabolism) that reflect an adaptation to plant association. Microbial traits in the rhizosphere are often shaped by the evolutionary history of the host-microbe associations, including that of symbiosis, where a diverse set of carbon-rich root exudates (e.g. compounds originating from the photosynthesis, sugars, amino acids, organic acids, phenolics, fatty acids) or root debris are supplied to encourage colonisation of microbes to the plant roots or rhizosphere by a process called rhizodeposits (Williams and de Vries 2020).

Soil microbes are chemotactically attracted to the materials such as plant root exudates, volatile organic carbon, and rhizodeposition. This nutrient-rich rhizosphere caters to an extremely active and dense microbial community which may prove to be a competing arena for microbial strains and traits leading to the selection of symbiotic traits with increased survivorship to the host plant. The evolution of soil microbiome in the rhizosphere with Plant growth-promoting bacteria (PGPB) is an instance of this process (Lugtenberg and Kamilova 2009). In a mechanism that runs contrary to this, the invasive plants can be producing allelochemicals, distinct organic acids, and hormones, that disrupt the structure of soil microbial communities in the rhizosphere and alter the patterns of nutrient cycling that favour the invasive

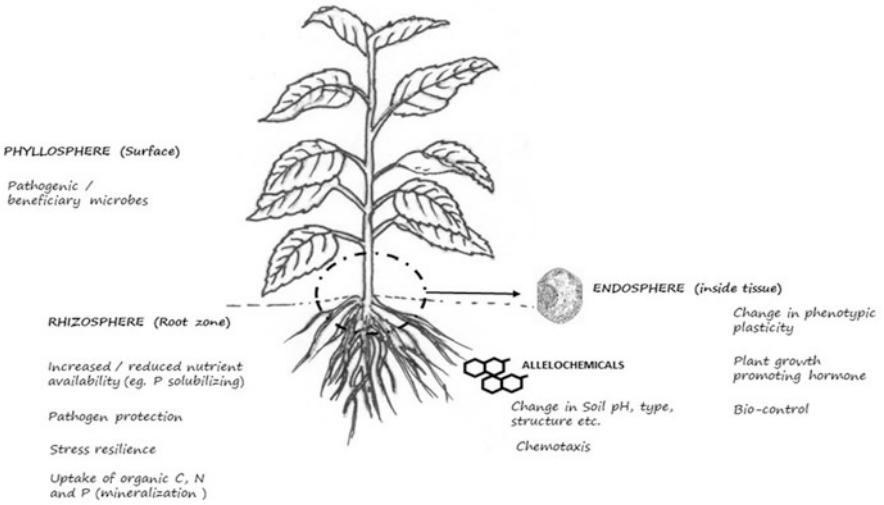

Fig. 1 Plant microbiome interaction

plants (Kourtev et al. 2002; Blank and Young 2004; Caldwell 2005; Lankau 2012; Morris et al. 2016). Roots of invasive plants may either enhance or reduce their mutualistic associations with different mycorrhizal fungi or Nitrogen-fixing bacteria (Jin et al. 2004; Reinhart and Callaway 2006; Pringle et al. 2009; Vogelsang and Bever 2009; Sun and He 2010), which potentially create feedback to plant invasion by enhancing nitrogen uptake of invaders (Pringle et al. 2009) or by lowering the dependence of plant invaders on Arbuscular Mycorrhizal Fungi (AMF) compared to the native plant species (Vogelsang and Bever 2009). Plant root exudates may vary among the plant species, creating characteristics across the rhizosphere microbiomes among specific plant species (Rovira 1969). This 'rhizosphere effect' is created due to characteristic microbial composition fostered around roots when exudates such as 'organic acids, amino acids, fatty acids, phenolics, plant growth regulators, nucleotides, sugars, putrescine, sterols, and vitamins are released into the rhizosphere (Mendes et al. 2013; Hartmann et al. 2008), Fig. 1.

Thus, plant growth and survivorship depend heavily on the nature of plant-microbe relationships as it has a bearing on the ability of the plant to influence its neighbours (Bulgarelli et al. 2013; Mikici'nski et al. 2016). Similarly, another class of chemicals called phytohormones (which includes jasmonic acid, ethylene, and salicylic acid) have been known to selectively impact bacterial phyla in a microbial community (Pieterse et al. 2012; Carvalhais et al. 2014). Conversely, the soil microbes are capable of influencing plant signalling and metabolism by producing certain secondary metabolites. Some scholars consider these microbes as constituting what is called the "plant secondary genome" of the plant hosts (Rout and Southworth 2013). Thus, some of the plant growth-promoting bacteria promote plant growth directly either by facilitating the required compounds or by modulating

levels of plant hormones. Some such bacteria indirectly effect plant growth by reducing the severity of pathogenicity by acting as bio-control agents (Glick 2012).

2.4 Soil Microbes in the Context of Plant Invasion

The diversity of soil microbial community and their composition are significant factors of plant health as the increase in microbial diversity causes an overall increase in survivorship of plant species by, imparting abiotic and biotic stress tolerance. Invasive plants manipulate the fundamental ecosystem process such as the cycling of nutrients and flow of energy in their favour in the ecosystems they invade. Litter decomposition is a crucial aspect of nutrient cycling in most ecosystems where soil enzymes play a crucial role. Soil microbial community and their networks influence the action of soil enzymes (Allison et al. 2004). When invasive plants dominate a habitat, the soil networks and activity of the soil enzymes, which determine the soil fertility and soil maintenance, are also disrupted and modified (Aon and Colaneri 2001; Flory and Clay 2010). This means that while altering the community structure and composition of native vegetation the above-ground, invasive species also inflict changes in soil microbial communities belowground. These create positive feedback for the invasive plant species and negative feedback for the native plant species (Callaway et al. 2004; Kulmatiski et al. 2008). As the quality of litter is altered while increasing the prevalence of invasive plant species, a corresponding change in the microbial community that decomposes the litter is affected.

Plant–Soil Feedback (PSF) studies help understand if the invasive plants interact differently with soil microbes so that invasives dominate the native plant population (Dawson and Schrama 2016). Such studies help determine if the soil microbes play a decisive role in the invasion by providing a competitive advantage to the exotic over native plant species. Results from PSF studies have provided instances where plants in exotic soil environments are often subjected to less negative effects from soil biota than their native environments on the one hand and suffers less in exotic soil environments in comparison with their native counterparts in the same soil (Reinhart et al. 2003; van der Putten et al. 2007; Kulmatiski et al. 2008). For instance, an exotic savanna grass (*Cenchrus biflorus*) may prove to be invasive due to the lack of a soil-borne pathogen in an exotic soil environment, provided that the other conditions are conducive (van der Putten et al. 2007). PSF studies have helped to prove that differences in plant-soil interactions are related to invasion success. This brings us to one of the leading explanations of the invasive ability of an exotic species-the Enemy Release Hypothesis-ERH (Keane and Crawley 2002; Liu and Stiling 2006). According to which specialist soil pathogens of the exotic plants such as fungi and oomycetes that keep these plants in check in their native range could be absent in an introduced range. This provides them a competitive advantage over the native species that have specialist enemies limiting their growth and performance. The Evolution of Increased Competitive Ability hypothesis or EICA (Blossey and

Notzold 1995) is an extension of ERH. According to EICA hypothesis 'due to release from above or below-ground enemies, selection should favour genotypes of exotic species that invest less in unused defence and more into growth, therefore increasing competitiveness against natives'. Some of the studies that corroborate the EICA hypothesis also demonstrated interesting patterns. For instance, TeBeest et al. (2009) found that there is a shift favouring increased biomass allocation in *Chromolaena odorata* to stems in exotic range soils as compared to the individuals of the species grown in their native range which provide them with greater competitive ability in the exotic environments. However, there are also reports contradicting these patterns (Zheng et al. 2015), genotypes of the same species from the exotic range were seen affected less negatively by soil fungi than native range genotypes, but the biomass of exotic genotype was lower than that of the native ones overall. According to Hierro et al. (2005), in order to fully assess the role of 'pathogen release' in invasions require biogeographic comparisons between the exotic and native ranges. In summary, despite the abundance of hypothetical propositions about the patterns of corresponding shifts among aboveground plant communities and belowground microbial populations, the conclusive observational correspondence is not always available (Wardle et al. 1999). Though, it is suggested that the upper trophic levels might be more severely affected by plant diversity variations than lower trophic levels such as microbes (Mikola et al. 2001), forecasting the effects of the aboveground plant community on higher trophic levels of a soil community is felt to be a complex affair. According to Mikola and Setala (1998), this is because communications between trophic levels involve both bottom-up and top-down interactions.

3 Above-Ground and Below-Ground Diversity in the Western Ghats

The Western Ghats is one of the eight 'hottest hotspots' of biodiversity in the world owing to an exceptionally high level of biological diversity and endemism. The Ghats has around 30% of all faunal and floral diversity of India contained in less than 6% of the land area of India. Though explored scantly and inadequately, to refer to the Western Ghats as the "Treasure House" of microbial biodiversity cannot be an overstatement (Madhavan Nampoothiri et al. 2013). Ruckmani and Chakrabarti (2011) reported bacterial species of 21 different genera—Proteobacteria (58%), Firmicutes (26%), Actinobacteria (13%), and Bacteroidetes (3%)—from samples of spring waters in the Western Ghats. The composition of these bacterial groups is indicative of the richness of microbial life in the region. Similarly, an exploration of the plant associations with arbuscular mycorrhiza in the Western Ghats region has revealed that more than 50% of the surveyed species had arbuscular mycorrhizal associations.

Some studies have shown that soil microbes from a 'monsoon evergreen broadleaf forest (MEBF)' are resilient to invasive plants. Some of the novel bacteria, fungus, and actinomycetes isolated from the Western Ghats region and their functions are given in Tables 1, 2, and 3.

4 Biological Invasion: A Case Study from India

This is a case of tree invasion in the Wayanad Wildlife Sanctuary, a humid forest tract, where the composition of soil microbiota has been monitored against the invasion of *Senna spectabilis*. The wildlife sanctuary with an extent of 344.44 sq. km is contiguous with Nagarhole, Bandipur, and Mudumalai National parks across the state boundaries making it a valuable and most extensive habitat of tigers and Asiatic elephants in peninsular India. The sanctuary is an integral part of the Nilgiri Biosphere Reserve and is located in the Western Ghats mountain ranges, one of the eight "hottest hot spots" of global biological diversity and a UNESCO world heritage site. The forest tracts have a high degree of biological richness, habitat diversity, floristic endemism, and rarity. The largest and most prominent peninsular River Kaveri, which feeds the lion's share of the irrigation projects and urban and rural drinking water projects has its perennial tributaries originating from the forested catchment of this protected area network.

The eastern leeward side of the larger landscape of the PA network is relatively drier tracts have only a short wet season followed by a prolonged dry season, Whereas the western windward slopes enjoy a relatively prolonged wet season followed by a short dry season. The summer months witness a seasonal migration of the herbivore population, followed by predators, in the westward direction. This very often triggers a heightened incidence of human-wildlife conflicts in the Wayanad region which has densely settled human habitations and associated agricultural lands. Over the past decades, there is a pattern of intensifying human-wildlife conflicts in the region which is proving to be increasingly expensive to manage. It is feared by conservation managers that additional stress on the habitats due to biological invasions could prove catastrophic as it would escalate human-wildlife conflicts while putting the survival of hundreds of species of endemic flora and fauna at risk.

An appraisal made in the year 2019 reported the occurrence of 67 invasive plant species from within the territories of Wayanad Wildlife Sanctuary. Predominant Invasive Alien Species (IAS) reported include *Chromolaena odorata* and, *Lantana camara, Senna spectabilis, Maesopsis eminii*, *Mikania micrantha* etc. Among these, *Maesopsis eminii* and *Senna spectabilis,* the two tree invasives, are found to be altering vegetation structure and reducing habitat viability. While *S. spectabilis* belongs to the family Fabaceae (subfamily Caesalpiniaceae), *M. eminii* belongs to the family Rhamnaceae (Figs. 2 and 3.). *Senna* and *Maesopsis* are native to Tropical America and Central Africa respectively, and both grow up to 20–25 m in height with a canopy spread of 15–20 diameter.

Table 1 Details of the common bacteria in the Western Ghats and their significance in ecosystem function

Species/genus	Significance	References
Endophytic bacteria		
Gluconacetobacter diazotrophicus C1	Phosphate solubilizing ability, zinc-solubilizing ability	Madhaiyan et al. (2004)
Gluconacetobacter diazotrophicus R3		
Gluconacetobacter diazotrophicus B3		
Gluconacetobacter diazotrophicus Co2		
Rhizobacteria		
Pantoea isolate NII-186	Production of indole acetic acid, HCN, P-solubilization, and siderophore. Potential plant growth-promoting strain and potential bio-inoculant in agricultural environments	Dastager et al. (2009)
Endophytic bacteria		
Bacillus subtilis PXJ-5	Production of Camptothecine (CPT) and 9-methoxy CPT (9-MeO-CPT)	Shweta et al. (2013a)
Bacillus sp. CPC3		
Bacillus cereus strain ChST		
Lysinibacillus sp.		
Stenotrophomonas rhizophila (PM-1)	Natural biosurfactant producer, degradation potency for various hydrocarbons	Praveen Kumar and Manjunatha (2015)
Rhizobacteria		
Bacillus amyloliquefaciens NII 167	Wide spectrum antimicrobial activity against various fungi and bacteria, inhibitor against wide spectrum βlactam resistant strains	Mohandas et al. (2012)
Streptomyces yunnanensis NII 716	Wide spectrum antimicrobial activity against various fungi and bacteria	
Soil bacteria		
Bacillus sp. NII 713	Wide spectrum antimicrobial activity against various fungi and bacteria	Mohandas et al. (2012)
Bacillus sp. B4	Antagonistic activity against phytopathogens *Phytophthora capsici* and *Rhizoctonia solani*	Ramkumar et al. (2015)
Bacillus sp. B5		
Bacillus sp. B12		
Pseudomonas fluorescence P66		
Pseudomonas fluorescence P1		
Rhodobacter viridis sp. nov. strain JA737^T	Bioactive production	Shalem Raj et al. (2013)

(continued)

Table 1 (continued)

Species/genus	Significance	References
Micrococcus sp. NII-0909 (Novel)	Indole acetic acid, siderophore and HCN production	Deepa et al. (2010); Dastager et al. (2010)
Bacillus thioparus NII-0902		
Serratia nematodiphila NII-0928	P-solubilization efficiency, indole acetic acid production, HCN, siderophore production and growth in nitrogen free medium	Dastager et al. (2011)
Chryseobacterium sp., WG4A	Bioactive: aromatic compound	Kumar et al. (2011)
Pseudomonas fluorescence sp.	Enzymes and antimicrobial metabolites	Megha et al. (2007)
Serratia marcescenes strain SN5gR	Enzyme: lipase production	Gupta et al. (2012)
Methylobacterium sp. ERI-135	Cytotoxic (A 549) and antimicrobial effects	Balachandran et al. (2012)
Bacillus subtilis C9	Anti-phytopathogenic	Islam et al. (2012)
Exiguobacterium sp. AFB-11	Amylolytic enzyme	Vidya et al. (2011)
Exiguobacterium sp. AFB 18		

Table 2 Details of the common fungi in the Western Ghats and their significance in ecosystem function

Species/genus	Significance	References
Endophytic fungi		
Alternaria alternata FC39BY	Antimicrobial activity against clinical pathogens and antioxidant properties by fungal extracts	Mani et al. (2015)
Alternaria citrimacularis FC8ABr		
Curvularia australiensis FC2AP		
Fomitopsis sp. *P. Karst* (MTCC 10177),	Produces Camptothecine (Camptothecin, CPT), a quinoline alkaloid, is a potent inhibitor of eukaryotic topoisomerase I, 9-MeO-CPT and 10-OH-CPT in mycelial mats	Shweta et al. (2013b)
Alternaria alternata (Fr.) Keissl (MTCC 5477) and *Phomposis* sp. (Sacc.)		
Trichoderma harzianum WL1	Laccase activity by ABTS oxidation	Sadhasivam et al. (2008)
Thelephora sp.	Dye decolourization in industrial effluents	Selvam et al. (2003)
Fomes lividus		
Fusarium solani	For anticancer	Sudheep and Sridhar (2012)
Phellinus sp1.	Production of ligninolytic enzymes	Selvam et al. (2012)
Polyporus hirsutus		
Daedalea flavida		
Tolypocladium spp. Ti-1	Antimicrobial activity	Ramachandran et al. (2007)
Tolypocladium spp. Ti-2		
Tolypocladium spp. Ti-3		

Table 3 Details of the common actinomycetes in the Western Ghats and their significance ecosystem function

Species/genus	Significance	References
Actinomycetes		
Streptomyces spp. ERI-26	Antimicrobial activity against some Gram-positive bacteria and fungi. Resistant to streptomycin. Produce enzymes such as amylase, cellulase and catalase	Arasu et al. (2008)
Streptomyces spp. ERI-3	Active against bacteria and fungi, a broad-spectrum antibiotic-producing actinomycetes	Valan Arasu et al. (2009)
Streptomyces sp. ERINLG-51	The Streptomyces sp. extract, ERINLG-51, has potential usefulness in identifying new molecules with potential activity against pathogenic microorganisms and cancer.	Balachandran et al. (2015)
Streptomyces sp. RAMPP-065	Antimicrobial and antioxidant potential	Manasa et al. (2012)
Streptomyces sp. S1A	The isolates possess activity against Gram-positive MRSA bacteria, Gram-negative bacteria, and fungal pathogens. Exhibited potent scavenging activity against DPPH and ABTS radicals suggesting their antioxidant potential	Saket et al. (2020)
Streptomyces sp. SS4		
Streptomyces sp. SS5		
Streptomyces sp. SS6		
Streptomyces sp. SCA35		
Nocardiopsis sp. SCA11		
Nocardioides sp. SCA13		
Endophytic actinomycetes		
Streptomyces longisporoflavus A023	α-Amylase inhibitory activity and the ability to augment glucose uptake—antidiabetic agents	Akshatha et al. (2014)
Streptomyces sp. A071		
Actinomycetes		
Streptomyces spp. ERI-04	Strong broad spectrum antifungal activity, potent activity against fungi and dermatophytes, antifeedant activities of extracellular metabolites	Valanarasu et al. (2010)
Streptomyces aurantiacus AAA5	Quinine-related antibiotic resistomycin	Vijayabharathi et al. (2011)
Streptomyces sp	Inhibition of extended spectrum β-lactamase (ESBL)	Mohandas et al. (2012)

Senna and *Maesopsis* share a certain set of features that enable them to proliferate the habitats which include their early reproductive maturity, production of large quantities of viable seeds, wide dispersal ability, etc. They both demonstrate faster rates of biomass accumulation and the ability to reproduce vegetatively. They

Fig. 2 Invasive plant *S. spectabilis*

Fig. 3 Invasive plant *M. eminii*

Table 4 Species richness in forests with IAS and without IAS

		Forest not invaded by *Senna spectabilis*		Forest invaded by *Senna spectabilis*	
Site		2015	2018	2015	2018
Total no of species		112	121	66	19
Native species		107	107	54	13
Habit of native plants species	Herb	49	49	31	6
	Shrub	20	20	8	5
	Climber	12	12	11	0
	Tree	26	26	4	2
Status	Endemic	10	10	2	0
	Vulnerable	2	2	1	0

regenerate from stumps and roots by coppicing when cut. Seeds stay viable in the soil seed banks for up to 3 years (Irwin and Barneby 1982). These trees spread and dominate the habitat at the cost of native species. On comparing datasets of the years 2014 and 2018, featuring the overall biological richness between two, 1-ha plots—out of which one is infested with *S. spectabilis* and the other with no presence of *S. spectabilis*. The species richness was found to be almost double in the plot which has no *S. spectabilis* infestation compared to that of the plot infested. These results indicate that both *M. eminii* and *S. spectabilis* are reducing the diversity and richness of the vegetation. The details are given in Table 4.

The figures in the table clearly point towards a trend in the reduction in species richness in all recorded plant life forms in the plot infested by the invasive tree *Senna spectabilis*. Among the plant species which have disappeared from the study plot includes two species that are endemic to the Western Ghats and one species recorded as vulnerable by IUCN. Whereas the plot that was not infested by the invasive has more or less improved its species richness if not maintained the status.

4.1 Impact of S. spectabilis *and* M. eminii *on the Rhizosphere Microbiota*

The two tree invasives, *M. eminii* and *S. spectabilis,* were found to have a considerable impact on the rhizosphere soil microbial community constituted by fungus, actinomycetes, and bacteria. The number of fungal isolates and species richness of fungi was found to be higher in Invaded Forest Soil (IFS) in the study area when compared to that of Un-invaded Forest Soil (UFS). Seventeen clearly distinguishable fungal isolates are obtained from the IFS sample, while the numbers of isolates are less in samples of UFS. Among the species identified, a few were common in both the soil samples.

The IFS samples are had double the number of Actinomycete population than that in the UFS samples, i.e. plant invasions have provided more niches for

Actinomycetes. Morphological analysis of the actinomycetes indicated that the IFS isolates have more species (richness) of actinomycetes (Anjusha et al. 2019; Athira et al. 2019).

In the case of the bacterial community, the load of pathogenic bacteria is found to be low in the IFS samples compared to that isolated from UFS. Morphological examination of bacterial community suggested that UFS and IFS samples have even distribution of both gram-positive and gram-negative bacilli with the numbers of 'cocci' lower than bacilli in both the samples. It appears that a diverse category of microbial species is attracted to the rhizosphere of these invasive plants.

These observations corroborate the hypothesis that a species could be exerting allelopathic effect on other plant life through an intermediary soil or rhizosphere microbial medium. The allelopathic effects exerted by the invasive species are attributed to the changed composition of the bacterial community in the rhizosphere. The majority of fungal, bacterial, and actinomycetes isolates obtained from the rhizosphere of the invasive were capable of controlling plant pathogenic fungi such as *Sclerotium rolfsii, Pestalotiopsis maculans and Fusarium oxysporum* (Prajna et al. 2019; Aneesha and Hrideek 2018). So, invasive species might be deriving some kind of competitive advantage over the native plant species owing to the presence of these bacterial strains which might provide protection to the invasive from naturally occurring pathogenic fungi in the soil. It is possible that complex mechanisms and metabolites are involved in the process, which are yet to be investigated in detail Krishnapriya et al. (2018).

5 Conclusion

It is important to note that there is an interactive reciprocal response between soil microorganisms and the release of allelochemicals by plants. Diverse groups of soil microbiota play a major role in regulating the concentration of allelopathic chemicals released into the soil, which in turn affect the composition of rhizospheric microbial community or rhizospheric microbiome; on the other hand, the soil microbial community is capable of buffering and modifying allelopathic responses among plants and the soil environment to a certain extent. The role played by the soil pathogenic microbes in control of native plant population as well as the invasive plants and the potential role of allelochemicals working in tandem with soil pathogenic microbes to aid or restrain plant invasion are complex outcomes, the prediction of which requires in-depth understanding to the soil macrobiome functionality. The impact of plant invasion on the aboveground vegetation alone is most often visible to the untrained eyes, while the consequences of the same on the soil microbial community are very little understood let alone its magnitude and relevance are not widely recognised. The glimpses provided by the case studies and discussions in the chapter suggest that in order to garner a larger picture of the ecosystem resilience and sustainability, it is important to gather information on the role played by the soil microbes in nutrient cycling as well on the interactions of the soil microbial

communities with other soil biotas in their various trophic levels in the soil environment. There exists a crucial information gap with respect to the nature of interactions and regulatory mechanisms in the belowground microbial communities in response to the plant defence chemicals in the context of biological invasion. Perhaps understanding the belowground process of allelochemical domination and responses of soil microbial communities may prove to be the key to controlling and reversing the unregulated range expansions of the invasive plants.

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Microbial Biodiversity in Agricultural Production Processes

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Abstract Microbial diversity is fundamental for the sustainable development of the production system, in the particular case of fish and agricultural production systems, as well as the use of native microorganisms for the development of probiotics positively impacts animal development and diminishes the environmental damage caused by the traditional management of the production system. An example of agricultural systems such as fish farming and grape production, in which animals within the aquatic system and plants in vineyards are exposed to aetiologies of viral, parasitic, fungal and bacterial origin that can cause economic losses to the producer associated with decreased yield and shelf life or mortality, causing the excessive use of antibiotics and other chemicals. For this reason, it is opportune to develop products with probiotic properties, which allow the control of pathogenic microorganisms and generate advantages to the host such as: strengthening of the immune system, better assimilation of food, gain in weight and height, besides preserving the physicochemical and microbiological quality of the environment. Regarding post-harvest and post-production management, microbial biodiversity is of special importance, mainly when it comes to conservation processes. Therefore, in many sectors there is great interest in those biomolecules with bioactive capacity to protect fresh foods that contribute to the development of products and their freshness for commercialization.

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1 Microbial Ecosystem in Fish Production Systems

The method of fish production has gradually changed and although intensive aquaculture has brought great economic benefits, the environmental impact associated with the production process has also increased. From a microbiological point of view a pond can be considered as a complex ecosystem that includes the microorganisms in the water, those contained in the fish and those concentrated in the sediments; this microbial community present in the pond plays an important role in fish health and water quality, however, it can be affected by different factors such as ambient temperature, predation pressure, disease pressure and the intensity of human intervention, therefore, the structure of the bacterial community in the pond ecosystem reflects the health status of the aquaculture ecosystem and provides a basis for the regulation of the ecological balance (Liu et al. 2020). It is worth mentioning that the microorganisms present in fish farms are a mixture of infectious agents and beneficial microorganisms that compete for space and nutrients and according to the dominant populations contribute to the recycling of nutrients and to the maintenance of water quality; however, information about the structure and functionality of the microbial community in fish farm ponds is scarce (Del'Duca et al. 2015).

An important factor to consider in an aquatic ecosystem is the proportional increase of microbial infections that cause economic losses and require the use of chemical or biological compounds that act as prophylactic or therapeutic agents. It is known that the aquaculture industry uses hundreds of tons of antimicrobials annually to prevent and treat infections. However, the disproportionate use of antimicrobial compounds in the aquatic environment increases pollution, affects the microbial structure of water and sediments, which can have consequences for fish health, and is the gateway for the emergence of resistant and mutant bacteria that transfer resistance genes to other microbial populations through genetic mechanisms such as transformation or conjugation (Park et al. 2016; Araújo et al. 2015; Cabello et al. 2016; de Azevedo et al. 2015; Liu et al. 2020). Therefore, the current interest of producers and academics is to develop methods to counteract pathologies, thus promoting the use of prebiotics, probiotics and plant extracts as additives to water or included in diets (Carbone and Faggio 2016; Etyemez and Balcazar 2016; de Azevedo et al. 2015; Bentzon-Tilia et al. 2016).

1.1 Study of the Diversity of Bacteria in Fish Farming Environments

In general terms the microbial community of an environment can be divided into cultivable and non-cultivable microorganisms. Cultivable microorganisms are studied by culture-dependent techniques where synthetic culture media are used and by basic microbiology techniques a pure culture of the bacteria present in the collected

sample is obtained. From this pure culture, different developments can be advanced using these microorganisms or their metabolites as an active principle of products that improve the productive system. However, through the use of culture-dependent techniques it is only possible to know 1% of the microbial diversity present in an ecosystem, therefore, other techniques must be used to obtain more information about the microbial diversity of these environments and in particular of non-cultivable microorganisms, among these techniques is the high performance sequencing. As more information is obtained about the diversity of bacteria present in a fish production system, the possibility of generating new biotechnological developments for the same system increases. Studies on bacteria associated with tilapia production systems using culture-dependent methods show that the dominant microorganisms in gills, tilapia gut and sediment are *Aeromonas hydrophila, Bacillus* spp., *Plesiomonas shigelloides, Shewanella putrefaciens, Pseudomonas fluorescens, Staphylococcus* spp. and *Vibrio cholerae* (Grande Burgos et al. 2018).

On the other hand, studies using high-throughput sequencing technologies reveal a broader view of the microbial diversity associated with fish production systems and the dominant phyla Proteobacterias, Cianobacterias, Bacteroidetes, Actinobacteria, Planctomycetes, Chlorobi, Firmicutes, Fusobacteria, Nitrospirae and Planktomycetes. Regarding the bacteria observed in sediments, they can be found Dechloromonas, Anaeromyxobacter, Geobacter, Proteobacterias, Actinobacterias, Bacteroidetes and Verrucomicrobia; many of these microorganisms are mainly involved in bioremediation processes and their prevalence is associated with the highly stratified characteristics and chemical gradient of the sediment. It should be noted that the microbial diversity of a fish production system can be affected by the type of fish, its state of development and management strategies (Zhou et al. 2018; Grande Burgos et al. 2018; Liu et al. 2020). On the other, hand, the use of probiotics in fish ponds has been found to modify the intestinal microbiota, for example, studies in tilapia have found the phylum Firmicutes to be dominant and relatively lower readings of Proteobacteria, Cianobacteria, Actinobacteria, Bacteroidetes, Fusobacteria, Nitrospirae and Spirochaetes (Grande Burgos et al. 2018). In front of this, several authors sustain that the microbiota of the intestinal tract of healthy fish can be involved in the epithelial proliferation, immune response and the metabolism of nutrients, therefore, it is the main source for the development of alternative products that fortify the health status of the host, the previous considering that the wild microorganisms are better adapted to the physiological conditions of the host and its environment in general (Etyemez and Balcazar 2016; Park et al. 2016). In this context, studying the microbial communities of fish ponds contributes to obtaining probiotics with native strains of fish or their environment and thus avoids the introduction of exotic microbial species since most commercial probiotics are obtained from terrestrial animals (Del'Duca et al. 2015).

1.2 Probiotics in Fish Production Systems

Probiotics have been known since 1965 where reference was made to substances produced by microorganisms that prolong the logarithmic growth phase in other species, thus having an opposite function to antibiotics. Later, in 1989, probiotics were understood to be a live microbial supplement that benefits the host and maintains the balance of microbial populations in the body. These can be supplied as monocultures or mixtures of microorganism cultures improving the properties of the native microbiota. Ten years later it is proposed that probiotics are microbial cells that administered in a certain way reach the gastrointestinal tract and remain alive with the objective of improving health and microbial balance in the host; in the same year other authors add that a probiotic can be made up of live or dead microorganisms or their cellular fractions with beneficial effects on the host (Martínez Cruz et al. 2012; Ibrahem 2015).

The above definitions do not conform 100% to the nature of aquatic species, one reason being that in aquatic environments there is no line of demarcation between the microbial community within and outside the host. Therefore, it is assumed that bacteria present in the environment influence the composition of the gut microbiota and vice versa. In this context, probiotics for aquatic systems were considered as living microorganisms, metabolites or cellular elements that once administered via food or water, modify the microbial community associated to the host and confer an advantage associated to characteristics such as: increase of resistance to diseases, greater capacity of food assimilation, improvement of the response to stress, increase of weight and height, cause a positive effect on the immune system, the activity of enzymes such as lysozyme and superoxide dismutase and in general it contributes to maintain the physicochemical and microbiological quality of the environment (Carbone and Faggio 2016; Ibrahem 2015; Araújo et al. 2015). It should be noted that when probiotic microorganisms are administered in the food, they must resist the conditions of the digestive tract, that is, be tolerant to acids and bile salts (Martínez Cruz et al. 2012).

Davis (2014), propose to extend the definition of viable probiotic bacteria to all microbes in the population that are metabolically active and/or have their membrane intact. These strains still possess the capacity to exhibit their beneficial effects when re-acclimated in the host environment; this, under the argument that there are microorganisms that can enter in a state of viable but not cultivable when they are subjected to stress either by the formulation process of the probiotic product or by the conditions of the host's digestive tract. However, when the microorganism finds the optimal conditions for its growth it develops and can fulfill its probiotic functions, an example of this is the sporulated bacteria of the genus Bacillus spp. The functions of a probiotic microorganism are clear, however, before seeking functional efficacy it is necessary to measure safety aspects of the strains evaluated. In this order of ideas, a probiotic microorganism must not harbor or transfer antibiotic resistance genes, nor must it synthesize enzymes or metabolites that can cause damage to the host such as biogenic amines (Bujnakova et al. 2014; Muñoz-Atienza et al. 2014;

Guo et al. 2016). Microorganisms that do not exert a positive effect within the host or its environment cannot be considered probiotics (Ibrahem 2015). However, as probiotics for aquatic animals, Gram-positive bacteria of the genera *Bacillus (B. amyloliquefaciens, B. subtilis, B. pumilus), Carnobacterium, Enterococcus* (*E. faecium*), *Lactobacillus* (*L. acidophilus, L. brevis, L. plantarum*), *Lactococcus, Bifidobacterium* (*B. bifidum*), *Leuconostoc, Clostridium, Micrococcus* (*M. luteus*), *Pediococcus* (*P. acidilactici*), *Streptococcus* (*S. salivarius* subsp. *Thermophilus*) as well as Gram-negative bacteria such as *Vibrio, Pseudomonas* (*P. fluorescens*), *Shewanella, Aeromonas, Enterobacter* and finally there are some mushrooms (*Aspergillus oryzae*), yeast (*Saccharomyces cerevisiae*) and microalgae like Spirulina (Hai 2015; Muñoz-Atienza et al. 2014; Martínez Cruz et al. 2012).

It is important to mention that a probiotic microorganism for some species can act as a pathogen for others, therefore, probiotics can be specific to appropriate fish species, *Citrobacter freundii* and *P. fluorescens*, for example, have been associated with fish diseases, but are potential probiotics for Tilapia (Hai 2015). In this context, it is considered that the host and its environment are a potential source for the isolation of probiotic microorganisms since it is presumed that they would have an adaptive advantage over other microorganisms from different environments.

1.3 Mechanisms of Action of Probiotic Microorganisms

Probiotic microorganisms present different mechanisms of action, most of these mechanisms have been observed during *in vitro* experiments, however, the response of a microorganism to these conditions can change significantly when administered to the host in its natural environment where host microbiota, pathogenic microorganisms, probiotic strains and environmental factors are integrated. Some of the mechanisms of action of probiotics are outlined in Table 1.

1.4 Selection of Probiotic Microorganisms for Fish

Among the important characteristics to consider a probiotic microorganism are that it is fast growing, remains in the intestinal tract and completes the adhesion to the tissue, if this does not happen, the probiotics can be washed out of the intestine during evacuation, therefore, a study to select probiotic microorganisms should combine in vitro assays and tests *in vivo* (Akhter et al. 2015). Entre las pruebas *in vitro* para seleccionar un microorganismo probiótico se destaca el crecimiento a diferentes pH, la resistencia a sales biliares, la sobrevivencia a condiciones de tracto digestivo simulado y actividad antibacteriana.

The pH is an important factor to consider since the digestive tract of fish can reach pH 1.8–2.0 in the stomach and 6.8 in the intestine, therefore, a probiotic microorganism must have the ability to survive these conditions. The growth of

Table 1 Mechanisms of action of probiotic microorganisms. Adapted from: Ibrahem (2015), Hai (2015), Martínez Cruz et al. (2012)

Mechanism of action	Concept
Competitive exclusion	It is carried out in response to a natural mechanism of interaction between microorganisms that generates an antagonism between populations to maintain the balance
Competition for adhesion and colonization sites	Probiotic bacteria compete with pathogenic microorganisms for adhesion sites in the intestine or other organs of the digestive tract and thus prevent the completion of the disease cycle. A successful probiotic bacterium attaches to the intestinal tract and stimulates its repair. The colonization process requires host-specific conditions such as body temperature, redox potential levels, enzymes and genetic resistance. It also requires the microorganism to secrete substances with an antagonistic effect, present enzymatic activity associated with proteases, peroxidase, lysozyme, and produce organic acids to modify the pH
Competition for nutrients and energy sources	It is presumed that this mechanism plays an important role in the microbial composition of the intestinal tract or environment, since when the available substrate is consumed by the probiotic microorganism, the other microbial populations among which pathogens can be found, are displaced
Improve host digestion	Probiotic microorganisms can participate in the digestive processes of the host through the production of extracellular enzymes such as proteases, lipases and/or growth factors such as fatty acids and vitamins
Competition for iron	Iron is an essential element for most organisms as it acts as a cofactor for several enzymes. The siderophores take part in the processes of capture and transport of iron
Adherence to intestinal mucus	The adherence of bacteria to intestinal mucus serves as a first barrier of defense against microbial attack; it is also believed to stimulate the host's immune system. On the other hand, adherence of pathogenic bacteria has been associated with the first step of bacterial infection and development of virulence. It is worth mentioning that the ability to adhere depends on the bacterial strain
Production of antagonistic or inhibitory compounds	It is a natural mechanism of microbial populations related to environmental conditions and nutrient availability. The aim is to produce substances such as antibiotics, antivirals, enzymes and organic substances, which displace other microbial populations and allow them to survive in a given space
Increased immune response	The supply of probiotics increases the levels of red blood cells, white blood cells, as well as respiratory activity, phagocytic activity, and enzymes such as lysozyme

microorganisms is directly affected by the pH of the medium. One way to know the tolerance of a microorganism to pH is to prepare a culture medium adjusted to different pH and then inoculate the microorganism of interest, leave it in incubation for a certain time and temperature (optimal for the growth of the microorganism) and evaluate the concentration of the microorganism at the beginning and end of the

experiment by plate count. Those microorganisms that achieve a higher count at pH 1.8–2.0 are of interest for use as probiotics (Pieniz et al. 2014; Solieri et al. 2014). Authors such as Garcia Marengoni and Menezes Albuquerque (2015) performed the inclusion of *B. subtilis* C-3102 and *B. cereus* as probiotics in red tilapia feed (*O. niloticus*), found that specific growth rate and feed conversion were not affected by the inclusion of probiotics. However, *B. subtilis* C-3102 and *B. cereus* were observed to influence intestinal colonization and survival rate. They conclude that the bacterial species *B. subtilis* and *B. cereus* are potential candidates for use as probiotics in aquaculture because they survive at high temperatures after the palletization process, can be stored at room temperature, and are resistant to low pH.

Las sales biliares hacen parte de los jugos gástricos que se liberan en el proceso digestivo cuya función principal es la degradación de lípidos, sin embargo, afectan directamente la viabilidad de las células bacterianas ya que pueden degradar algunas estructuras como la membrana celular y por consiguiente lisar las células. A nivel de laboratorio se puede determinar la resistencia de un microorganismo esta condición mediante la preparación de un medio de cultivo suplementado con sales biliares a concentraciones desde 0,1% a 20%. En cada medio de cultivo se inocula una concentración conocida del microorganismo de interés y se deja en incubación por tiempos que van desde 2 hasta 24 o 48 horas a la temperatura optima de crecimiento del microorganismo. Finalmente se cuantifica la concentración final del microorganismo mediante recuento en placa (Speranza et al. 2017; Solieri et al. 2014). Un ejemplo de la incidencia de esta condición es el trabajo realizado por Mukherjee et al. (2016) quienes aislaron 208 bacterias autóctonas desde intestino de *Cirrhinus mrigala,* de los cuales finalmente seleccionaron cuatro por su actividad antagónica contra al menos dos patógenos de pescado. Las cepas seleccionadas producen diversas enzimas extracelulares, crecen mejor en el mucus intestinal que en el de la piel y resisten a jugos biliares en una concentración de 2 a 20%. Estas cepas se identificaron como *Bacillus stratosphericus* (KM277362), *Bacillus aerophilus* (KM277363), *Bacillus licheniformis* (KM277364) y *Solibacillus silvestris* (KM277365).

Survival to simulated digestive tract conditions refers to a combined procedure where adverse conditions related to the presence of digestive enzymes, gastric juices and pH are replicated under laboratory conditions that the probiotic microorganism eventually faces when ingested by the fish. At laboratory level, a known concentration of bacteria is inoculated into a gastric solution adjusted to pH 2.0, it is left in incubation for 2 h at 37 °C, then the biomass is transferred to an intestinal solution characterized by being adjusted to a pH of 6.8, it is kept in incubation for 4 h at 37 °C. Finally, a plate count is made to determine the amount of microbial cells that managed to survive the treatment (da Silva et al. 2013).

The antibacterial activity allows to measure the capacity of a microorganism to inhibit the growth of another one, taking into account that one of the main mechanisms of action of a probiotic microorganism is the production of antagonistic or inhibitory compounds. It is necessary to measure this activity at laboratory level, for this, a Petri dish confrontation is made between the pathogenic microorganism and the potentially probiotic (Mahmoudi et al., 2016; Etyemez and Balcazar 2016). The

antibacterial activity of microorganisms is quite diverse, authors such as Etyemez and Balcazar (2016) isolated 120 bacteria from the intestinal mucus of Tilapia (*Oreochromis niloticus*) aide which 5 showed antagonistic activity with inhibition halos between 12 and 16 mm against *Streptococcus iniae* and *Edwardsiella piscicida*. Analysis of 16S rRNA gene sequences showed that the antagonistic isolates are closely related to *B. endophyticus, B. flexus, B. mojavensis, B. sonorensis,* and *B. subtilis.* Araújo et al. (2015) found heterogeneous results regarding the diversity of microorganisms in the different stages of development of rainbow trout (*Oncorhynchus mykiss*), obtaining up to 1620 isolates, of which 71.5% showed antimicrobial activity with at least one of the pathogens evaluated (*Lactococcus garvieae* JIP29-99, *L. garvieae* CECT5807, *L. garvieae* CF01144, *L. garvieae* CF00021 and *Carnobacterium maltaromaticum* LMG14716, *Yersinia ruckeri* LMG3279, *Aeromonas salmonicida* LMG3776 and *Vibrio campbellii* LMG21363).

The in vitro evaluation for the selection of probiotic microorganisms is an important activity, however, the main objective is to achieve that the probiotic microorganism generates a positive response when it faces the fish in its natural environment, that is, it is required to validate the probiotic effect through in vivo tests. There are many probiotic products on the market such as Lycogen^{tm}, Biogen$^{®}$, Organic Green^{tm}, PROB (AquaStar$^{®}$) and Levabon$^{®}$ which contain probiotic microorganisms that generate different effects in the host related to an increase in the intestinal villi and a decrease in the incidence of pathogenic microorganisms such as *Vibrio* sp. However, there are products that are inefficient in reducing the pathogenicity of aquatic species and on the contrary affect their health causing greater economic losses, so it is recommended that a probiotic is obtained from the natural environment where the host is (Hai 2015; Ibrahem 2015; Batista et al. 2016).

It is noteworthy that the effect of the probiotic on fish is very diverse, for example Ozório et al. (2016) used a probiotic composed of *Bacillus* sp., *Pediococcus* sp., *Enterococcus* sp. and *Lactobacillus* sp., supplemented in the diet of *Oncorhynchus mykiss*. After 9 weeks of experimentation, growth was not affected by the diet with probiotics supplied, however, changes were observed in the biochemical indicators and in the intestinal microbiota with respect to the control diet. While other works such as that carried out by Dawood et al. (2016) where the influence of four concentrations of *Lactobacillus rhamnosus* in the food of *Pargus major,* It was observed that the parameters final weight, weight gain, specific growth rate, protease activity, protein digestibility, superoxide dismutase activity and intestinal count of *Lactobacillus* sp., they were significantly higher in all groups fed probiotics compared to the control. On the other hand, Reda and Selim (2015) using *B. amyloliquefaciens* supplemented in red tilapia fingerling diets (*O. niloticus*) found that the diet with the highest concentration of probiotic showed significant differences in weight, specific growth rate and feed conversion compared to the control. In general it has been found that indigenous bacteria have greater potential and that the mixture of bacteria improves growth, immunity and resistance to diseases (Ridha and Azad 2016). Without a doubt, microorganisms play an important role in the life cycle of all organisms and the microbial diversity present in the

different fish production systems still holds many secrets that little by little will be discovered with the application of new microbiological and molecular techniques, this knowledge will be an opportunity for the biotechnological development of the fish farming sector and for the implementation of sustainable production systems that improve the quality of life of the families dedicated to this activity.

2 Microbial Ecosystem in Grape Cultivation

The grape crop is affected by different pathogens: fungi and viruses. Among the most economically important fungi is: Mildeo velloso *Plasmopara viticola* (Bert. and Curt.), Berl. and, Oidio o Mildeo powdery *Uncinula necator* (Schw.) and the Gray Rot *Botrytis cinerea* Pers. (Table 2), which can cause losses of up to 80%, when control methods are not established in a timely manner. In Latin American wine-growing regions, the presence of viruses has been reported affecting varieties of *Vitis vinifera* and *Vitis labrusca* L, such as *Grapevine corky bark virus* (GCBaV) and *Grapevine stem pitting* (GSP), in varieties of Queen and Italy (Chavez and Varón de Agudelo 1994); *Grapevine leaf roll virus* (GLRaV) in variety Isabella (Muñoz-Atienza et al. 2014). In these studies it has been suggested that as the transmission of the detected viruses is mechanical, it is probable that the majority of vine crops in the department could be infected with these parasites, due to the fact that the planting material is not certified (cuttings), and pruning practice is periodic.

Diseases of viral etiology cause a reduction in the productive yield per plant (t/ha), due to physiological damage caused by the presence of viruses in the plant system (deformations in the stem affecting xylem and phloem tissues, deformations in the leaf blade affecting photosynthesis and chemical transformation, reducing their growth between 79 and 89% for varieties of *V. vinifera*), decreasing the organoleptic characteristics of the fruit, changes in color, size, number of grapes per bunch and reduction in maturity indices, °Brix, increased acidity, and decrease in productive life (approximately 30–15 years).

2.1 Grape Species Grown in Latin America

Wild and domesticated grapes belong to the *Vitaceae* family, which includes 14 living genera and 2 fossils, more than a thousand species and approximately 8000 varieties. The plants are herbaceous or woody, with tendrils opposite the leaves, their inflorescences are generally located at the site of the tendril but are almost never axillary or pseudo-axillary. The plants can be perfect or unisexual, male, or female. The genus Vitis is divided into two sections is *Vitis* (*Euvitis*) and *Muscadinia*. The section *Vitis* (*Euvitis*) consists of the real grapes, to these belong most of the varieties grown worldwide. The shoots have an extra-ambiental bark (which includes the pericyclic fibers, primary phloem and secondary non-functional

Table 2 Viruses present in grapes and their taxonomic position[a]

Family	Gender	Species
Virus belonging to a genus with an assigned family		
BROMOVIRIDAE	*Alfamovirus*	*Alfalfa mosaic* (AMV)
	Cucumovirus	*Cucumber mosaic* (CMV)
	Ilarvirus	*Grapevine line pattern* (GLPV)
BUNNYAVIRIDAE	*Tospovirus*	*Tomato spotted wilt* (TSWV)[b]
COMOVIRIDAE	*Fabavirus*	*Broadbean wilt* (BBWV)
	Nepovirus	*Artichoke italian latent* (AILV)
		Arabis mosaic (ArMV)
		Blueberry leaf mottle (BBLMV)
		Grapevine bulgarian latent (GBLV)
		Grapevine chrome mosaic (GCMV)
		Grapevine fanleaf (GFLV)
		Grapevine tunisian ringspot (GTRV)
		Peach rosette mosaic (PRMV)
		Tobacco ringspot (TRSV)
		Tomato ringspot (ToRSV)
		Tomato black ring (TBRV)
		Strawberry latent ringspot (SLRSV)
TOMBUSVIRIDAE	*Tombusvirus*	*Petunia asteroid mosaic* (PAMV)
		Grapevine algerian latent (GALV)
	Carmovirus	*Carnation mottle* (CarMV)
TOMBUSVIRIDAE	*Tombusvirus*	*Petunia asteroid mosaic* (PAMV)
		Grapevine algerian latent (GALV)
	Carmovirus	*Carnation mottle* (CarMV)
CLOSTEROVIRIDAE	*Closterovirus*	*Grapevine leafroll-associated 1* (GLRa1)
		Grapevine leafroll-associated 2 (GLRa2)
		Grapevine leafroll-associated 3 (GLRa3)
		Grapevine leafroll-associated 4 (GLRa4)
		Grapevine leafroll-associated 5 (GLRa5)
		Grapevine corky bark (GCBaV)
Virus belonging to a genus without assigned family		
	Sobemovirus	*Soybean mosaic* (SoMV)
	Necrovirus	*Tobacco necrosis* (TNV)
	Potexvirus	*Potato X* (PVX)
	Tobamovirus	*Tobacco mosaic* (TMV)
		Tomato mosaic (ToMV)
	Trichovirus	Grapevine B (GVB)
		Grapevine C (GVC)[c]
		Grapevine D (GVD)
	Capillovirus[c]	*Grapevine berry internal necrosis* (GIBNV)[c]
		Virus sin asignación de nombre
	Furovirus[c]	*Grapevine labile rod-shape* (GLRSV)

(continued)

Table 2 (continued)

Family	Gender	Species
Virus belonging to a genus without assigned family		
	Vitivirus	*Grapevine A* (GVA)
		Grapevine B (GVB)
	Tentative new genre	*Grapevine fleck* (GFkV)
		Grapevine asteroid mosaic (GAMV)
Unclassified viruses		
		Grapevine ajinashika (GAV)
		Grapevine stunt (GSV)

[a] Modified by Martilli, G.P., 1994
[b] The correct identification of this virus is doubtful
[c] Tentative assignment

phloem). The berries ripen uniformly in the bunch. The seeds are generally peripheral. The basic chromosome number is n = 19 or 2n = 38. The Muscadinia section is comprised of grapes of lesser economic importance, because they have smaller size and fewer berries in the same bunch, some of which are studied for the genetic improvement of varieties.

***Vitis vinifera* L.** Civilization spread the Eurasian *V. vinifera* species from western Asia throughout Europe. Although it is very tolerant of limestone soils, it is extremely sensitive to all American pests and diseases, such as Phylloxera *Daktulosphaira vitifoliae* Fitch; Oidio *Uncinula necator* (Schw.) Burr; Mildiu *Plasmopara viticola*, (Bert. and Curt.) Berl. and Toni; and Pierce's disease, *Xylella fastitidiosa* Wells and Cols. It is also sensitive to grey rot *Botryotinia fuckeliana* (de Bary) Whetzel, *Botrytis cinerea* Pers. Anthracnose *Elsinoë ampelin* (de Bary) Shear, *Sphaceloma ampelinum* de Bary; *Gloesporium ampelophagum* (Pass.) Sacc., *Ramularia ampelophaga* Pass.; Angular leaf spot *Mycospharella angulata* Jenkins, Excoriosis *Phomopsis viticola* (Sacc.) Sacc. and crown gills *Agrobacterium tumefaciens* (Smith and Townsed) Conn. Due to the quality of the fruit in V. Vinifera varieties, it has been used in most of the interspecific hybrids used both for winemaking and for table grapes.

***Vitis labrusca* L.** Native to the wilds of the United States, especially east of the Mississippi River. This variety as some of its hybrids (*V. Labrusca* × *V. vinifera, Vitis* × *V. Labruscana*) and in the company of other American varieties *V. riparia, V. cinerea, V. rupestris, V. elvira, V. concord, V. catawba,* and *V. champini*, have been widely cultivated around the world, as they present rooting characteristics in most soils and allow grafting with other varieties, especially *V. vinifera*. Varieties such as Concord, Isabella, Noah, Otello and Campbell are grown in rainy regions due to their moderate resistance to fungal diseases (Oidio and Mildiu), although in certain soils they are sensitive to Black Rot *Guignardia bidwellii* (Ellis), Pierce's disease and Phylloxera. Some American species have played an important role in the genetic improvement of the grape in the world. American interspecific crosses have been used to obtain patterns adaptable to different soil types, diseases and climatic

conditions, also American species have been crossed with the European species V. vinifera to create patterns of cultivars.

2.2 Viruses, Causal Agents of Diseases in Grapes

There is a high number of viral agents that affect the cultivation of grapes, currently there are 44 different viruses belonging to 5 families and 16 genera (Table 1), identified worldwide. The first identification of grape viruses was in the early 1960s (Cadman et al. 1960), among the most prevalent viruses belonging to: *Nepovirus, Closterovirus* and gender *Trichovirus*, which are verifiable pathogens and disease agents that have undoubtedly caused a negative impact on the quantity and quality of production.

The trend of viral infections in *Vitis* species lies in: the susceptibility of the grape to viruses, the variety, the way of seed propagation, the climatic conditions for its growth and production. Most of the *Vitis* species are vegetatively propagated, thus the infectious agents have progressively accumulated in different places and times (cultivation cycles), perpetuating "clonally" the viruses. Along with the viruses, the grape is susceptible to other intracellular infectious agents. So far, six different viroids have been reported, three or more phytoplasmas, and *Xylella* fastidiosa limited to the xylem of the plant (Martelli 2014).

Compared to the high number of potential viral pathogens in grapes, the list is reduced to viruses of higher incidence, especially in species of industrial importance: *Vitis, Vitis berlandieri* × conder1613, *Vitis berlandieri* × *V. riparia, Vitis labrusca*, *Vitis rupestris*, *Vitis rupestris* var. *rupestris*, *Vitis thunbergii*, *Vitis vinifera*, which were reported in the January 16, 1997 version of ICTV (The International Committee on Taxonomy of Viruses) (Table 3).

There are two major groups of viral diseases affecting grape cultivation: those caused by *Closterovirus* and those caused by Nepovirus. As we consider of greater importance and incidence worldwide will be described in more detail the viruses: grapevine leafroll-associated viruses (GLRaV), grapevine corky bark-associated virus (GCBaV) and grapevine fan leaf virus (GFLV).

2.3 Grapevine Leafroll-Associated Viruses (GLRaV)

The *Grapevine leafroll-associated viruses* GLRaV (Namba et al. 1979) or leaf roll virus, produces the disease that bears its name, and is present in almost all grape production in the world. It is characterized by expressing symptoms on the leaf, weakening and coloration (yellow, reddish, purple) but the veins remain green. In the fruit, it produces alteration in the pigmentation, decrease in the indexes of maturity, °Brix, increase of acidity, reduction of sugar and reduction of the number of bunches per plant. To this kind of viral particle, other symptomatic variants have

Table 3 Viruses with the highest global incidence in species of industrial importance

Grape species	Susceptible to
Vitis	*Grapevine fanleaf nepovirus*
	Sowbane mosaic sobemovirus
	Tomato black ring nepovirus
Vitis berlandieri × *conder1613*	*Grapevine corky barck-associated closterovirus*
Vitis berlandieri × *V. riparia*	*Grapevine corky barck-associated closterovirus*
Vitis labrusca	*Grapevine bulgarian latent nepovirus*
	Grapevine fanleaf nepovirus
	Grapevine stem pitting associated closterovirus
	Peach rosette mosaic nepovirus
Vitis rupestris	*Grapevine bulgarian latent nepovirus*
	Grapevine corky bark-associated closterovirus
	Grapevine fanleaf nepovirus
	Grapevine fleck virus
	Grapevine stem pitting associated closterovirus
Vitis rupestris var. Rupestris	*Grapevine stem pitting associated closterovirus*
Vitis thunbergii	*Grapevine stunt virus*
Vitis vinifera	*Arabis mosaic nepovirus*
	Artichoke italian laten nepovirus
	Grapevine A vitivirus
	Grapevine ajinashika disease (?) luteovirus
	Grapevine algerian latent tombusvirus
	Grapevine B vitivirus
	Grapevine bulgarian latent nepovirus
	Grapevine corky barck-associated closterovirus
	Grapevine fanleaf nepovirus
	Grapevine fleck virus
	Grapevine leafroll associated closterovirus
	Grapevine line pattern (?) ilarvirus
	Grapevine stem pitting associated closterovirus
	Grapevine stunt virus
	Petunia asteroid mosaic tombusvirus
	Strawberry latent ringspot (?) nepovirus

Fuente: The International Committee on Taxonomy of Viruses ICTV, 1997

been associated such as *Potyvirus* (Tanne 1977), Isometric particles (Castellano et al. 1983), and *Closterovirus* (Namba et al. 1979; Tanne 1977; Zee et al. 1987), Most of these studies have used electron microscopy as their main methodology. Different results obtained support the hypothesis that grape leaf roll disease can be caused by one or more viral particles belonging to *Closterovirus* (Castellano et al. 1983; Namba et al. 1979; Zee et al. 1987), especially the suberosal bark virus (GCBaV), possibly representing a new group of viruses (Gugerli et al. 1984).

Different varieties of V. vinifera and Vitis americas, keep the expression of infections of this virus latent, giving them asymptomatic characteristics. Its symptoms are evident in indicator species such as Nicotiana glutinosa L. and N. Benthamiana, where results can be obtained between 1 and 2 years (Monette and James 1990; Wilcox et al. 1998). Grape leaf roll virus (GLRaV) has six associated strains: GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, and GLRaV-6, none of which have been fully characterized (Candresse and Martelli 1995). For this reason several laboratories have purified viral particles from these and have created monoclonal (Mabs) and polyclonal (Pab) antibodies for each of them (Monis 2000).

GLRaV Diagnostics The techniques of serology and molecular methods have been widely used in worldwide studies, in these techniques several laboratories have optimized their methodologies for the purification of grape viruses. Gugerli et al. (1984) were the first to purify filamentous Closterovirus viral particles from grape leaf roll disease and produced an antiserum for ELISA, successful in many crops. The second to obtain a successful method was Zee et al. (1987), purifying viral particles from *Closterovirus* of diseased plants with Leafroll of the variety "Pinot noir" in New York (Estados Unidos) and developed a highly specific antiserum for ELISA (NY-1). Since that year there have been modifications in the method of purification of Zee et al. (1987), as described Hu et al. (1990).

By ELISA technique, it has been determined the best time and tissue for the early identification of the virus, affirming that, the rate of advance of viral antigens in the plant appear according to the location of the virus in the growth of this one, the obtained results were: first, from the sprouting (bud break) to the inflorescence (cluster), the viral antigens are not detected in leaves. Second, from the inflorescence to the development of berries (25–75 days after the sprouting), the antigens are detected in leaves but do not reach the terminal leaves. Third, from berry to harvest, antigens are detected on all leaves including the terminal ones (Teliz et al. 1987).

There are differences between diagnostic methods (indicator plants, ELISA, Western Blotting) for viruses associated with Leafroll disease, such is the case of GLRaV-1 and GLRaV-3 viruses, which are detected by ELISA in a material that was apparently free of virus by the technique of grafting in indicator plant. In the same samples an identification study by Western Blotting Assay, detected viral polypeptides of different associated viruses of "Leafroll" and "Corky Bark" (Monis and Bestwick 1997). Recently, diagnoses are made with Mabs because they are monospecific and provide better immunological reaction for the detection of plant pathogens. These have been developed with specificity for GLRaV-1 and GLRaV-2 (Gugerli and Ramel 2003), GLRaV-3 (Hu et al. 1990), GLRaV-4, GLRaV-5 (Gugerli and Ramel 2003) and GLRaV-6 (Gugerli et al. 1984). The in vitro micropropagation is a technique to clonally multiply a cultivar ensuring its cleanliness from diseases, as a preventive measure to not propagate material with viruses, has been diagnosed through stress conditions (4% Sorbitol in the culture medium), which induced symptoms of stem malformation and reddish coloration in all leaves

(4 and 8 weeks) typical of the leaf roll disease in varieties "Petite Sirah", "Gamay" and "Mission" (Tanne et al. 1996).

GLRaV transmisión Currently, the transmission of two Closteroviruses associated with grape leaf roll disease (GLRaV-1 and GLRaV-3), have been transmitted from plant to plant by Pseudococcus longispinus and P. calceolariae (Homoptera: Pseudococcidae) (Petersen and Charles 1997).

2.4 Grapevine Corky Bark-Associated Virus (GCBaV)

The "*Grapevine corky bark-associated virus*" (GCBaV), belongs to the family Closteroviridae, of the genus Closterovirus, which has been reported in the United States (California), Mexico, Brazil, France, Spain, Germany, Italy, Yugoslavia, Bulgaria, South Africa and Japan (Namba et al. 1991; Hu et al. 1990). The symptoms of this virus are: in leaf, the delay of the opening of the bud, coloration and foliar rolling in some varieties of *V. vinifera*. In stem, growth reduction, weakening (brittle at the base and tendency to bend), corkiness, reduction in production due to deficiency in conductive tissues (Namba et al. 1991). As a main characteristic in the Closteroviridae family, the symptoms in this virus are the malformation in the phloem tissue, disorganization of mitochondria, chloroplasts, endoplasmatic reticulum and accumulation of viral vesicles, these observations are similar to those of the grapevine leaf roll-associated virus "GLRaV".

Diagnostic GCBaV A diagnostic method to identify the GCBaV, is the micrografting, the results are obtained in 8–12 weeks, observing symptoms like reduction of growth, malformation of the stem and reddish coloration in its leaves; its methodology is made with the micrografting of "indicator plant" LN-33 (Counderc 1613 × Thompson Seedless), the results are shorter as opposed to field or greenhouse grafting which lasts between 12 and 24 months (Tanne et al. 1996). A study with a polyclonal antibody for ELISA from purified extractions of GCBaV, determined that the grapevine corky bark-associated virus (GCBaV) is the causal agent of the disease that bears his name and not by sharing characteristics with other viruses (GLRaV-2, GLRaV-3 and grapevine Virus A "GVA") of the family *Closteroviridae* (Namba et al. 1991).

GCBaV transmission The form of transmission has varied since 30 years ago, a report in Czechoslovakia stated that it was transmitted by rickettsia producing necrotic infections (Ulrychova and Vanek 1975; Hewitt and Chiarappa 1977), and recently it was determined that it can be transmitted by grafting and by the insect *Psyllidae* and not by *Planococcus ficus* (Namba et al. 1991) (Anexo A).

2.5 *Grapevine Fanleaf Virus (GFLV)*

A species of the gender *Nepovirus*, is the GFLV (Hewitt et al. 1958), causing the fanleaf syndrome or infectious degeneration, in almost all winegrowing areas of the world (Table 4). It is a virus of polyhedral particles of 30 nanometers (nm) of diameter, with a bipartite genome constituted by two linear molecular filaments of ribonucleic acid (RNA) with molecular weights of 2.4×10^6 (ARN-1) and 1.4×10^6 (ARN-2), its total size is 11. One hundred sixteen kilo-base, it is in two parts, the first 7324 kb and the second 3774 kb (Quacquarelli and Gallitelli 1976; Pinck et al. 1988; Fuchs et al. 1991).

GFLV presents diversity of symptoms in different varieties, inducing the appearance of the fan shape in leaf and zig-zag inter-node growth, sometimes it is observed foliar lamina winding with yellowish in white fruit varieties (*V. vinifera*), reddish winding in interventional tissues leaving green bands along the main vein in red fruit varieties (*V. vinefera*) (Table 4). In stem presents: loss of vigor, short internodes, weakening and malformation (Namba et al. 1979; Quacquarelli and Gallitelli 1976). The virus is transmitted by vegetative propagation, less by pollen, nor by sexual seed (Cory and Hewitt 1968).

Diagnostic GFLV The control strategy for GFLV has been through evaluation tests of planting material by indexing techniques in herbaceous plants (*Chenopodium quinoa* and *C. amaranticolor*) and timber (Zee et al. 1987), serology "ELISA" with monoclonal antibodies (Huss et al. 1987), double-stranded ribonucleic acid analysis (cD-RNA), dexoribonucleic acid hybridization tests "cDNA" (Fuchs et al. 1991) and "PCR" Polymerase Chain Reaction technique (Rowhani et al. 1993). Cytopathology studies have detected the GFLV virus in root tissues in the mesophilic and vascular parenchyma; in the cytoplasm and nucleus, capsid accumulation and inclusions in the cellular and nuclear membranes are observed (Saric and Wrischer 1973). The best tissues for the diagnosis of GFLV by the ELISA technique are those with low, medium and bark leaves maintained at 6 °C, a few months after growth and roots (Walter and Etienne 1987).

The hybridization of DNA/RNA nucleic acids between two nucleotide sequences, is a technique that by its specificity, sensitivity and speed of molecular hybridization, allows to be more valuable than conventional immunological methods such as ELISA. For such a method the complementary DNA copies of the GFLV RNA are cloned into the Escherichia coli plasmid pUC9 and then used in the identification in GFLV purifiers (Fuchs et al. 1991). The PCR technique (Polymerase Chain Reaction) for GFLV, is used in viral diagnosis in seed cleaning programs and resistance research in different varieties (Table 4). The PCR methodology has been modified for RNA viruses, using oligonucleotides (Primers) for *Nepovirus* (Sánchez et al. 1991). However, there are problems when applying this PCR technique, because of the high levels of phenolic components, polysaccharides and other substances present in grape tissues, inhibiting the polymerase chain reaction (Flores et al. 1985; Newbury and Possingham 1977; Rezaian and Krake 1987).

Table 4 General information on viruses with the highest global incidence in grapes

Topic	VIRUS		
	Grapevine leafroll virus	Grapevine fanleaf virus	Grapevine corky bark virus
ACRONYM	GLRaV	GFLV	GCBaV
TYPES OR RACES	GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5	Fanleaf (Hewitt et al. 1958), Yellow Mosaic (Hewitt et al. 1958), Vein Tape (Zee et al. 1987)	None currently
	They are associated viruses, and none are characterized		
TAXONOMY	Virus RNA	Virus RNA	Virus RNA
	GENDER: *Closterovirus*	**GENDER:** *Nepovirus*	**GENDER:** *Closterovirus*
	FAMILY: *Closteroviridae*	**FAMILY:** *Comoviridae*	**FAMILY:** *Closteroviridae*
MORPHOLOGY	Nucleocapside filaments, flexible, 1800–2200 nm long	Isometric virion, not wrapped, diameter 30 nm, angular in profile, exterior conspicuous capsomer	Nucleocapside filamentosa, flexible, 1400–2000 nm long
HOSTESS NATURAL	*Vitis vinifera*	*Vitis vinifera*	*Vitis vinifera*
	Vitis american and hybrids	*Vitis labrusca*	*Vitis berlandiere* × *Conder 1613 (LN 33)*
	Variedades:	*Vitis rupestris*	*Vitis berlandiere* × *V. Riparia*
	– Pinot noir	Hybrids inter-specific	*Vitis rupestris*
	– Mission		
	– Gamay		
	– Cabernet		
SINTOMAS	**LEAVES:**	Systemic Yellow Mosaic Disease	**TREE STEM:**
	Balancing and discoloration (metallic yellow, reddish, purple, green veins) are maintained	**TREE STEM:**	Severe winding, delayed growth
	Asymptomatic in *American Vitis*	Weakening, delay, malformation	Weak, corky, brittle at base, tendency to curve, reduced vigor
	FRUIT or BAY:		
	Sugar reduction		**LEAF:**
	Delayed ripening	**LEAVES:**	Delay in the opening of the yolk
	Pigment alteration	Malformation and nodal segments	Leaf coloring completely

(continued)

Table 4 (continued)

Topic	VIRUS		
	Grapevine leafroll virus	Grapevine fanleaf virus	Grapevine corky bark virus
DIAGNOSTIC HOST	*Vitis vinifera*	*Vitis* ssp.	*Vitis berlandiere* × *Conder 1613 (LN 33)*
	Variedades:	*Chenopodium amaranticolor*	*Vitis berlandiere* × *V. riparia*
	– Piinot noir	*Chenopodium quinoa*	*Vitis rupestris*
	– Mission	*Cucumis sativus*	*Vitis vinifera* (LN33)
	– Gamay	*Phaseolus vulgaris cv*	
	– Cadernet	*Gomphrena globosa*	
		Nicotiana benthamiana	
		Nicotiana clevelandii	
TRANSMISSION	By grafting	By grafting	By grafting
	By *Pseudococcus longispinus*	By mechanical inoculation	By *Psyllidae*
	Planococcus ficus	By seed (abundant virus in the endosperm but not inside the embryo in *V. Vinifera*)	Not transmitted by:
		By nematodes: *Xiphinema index* (Hewitt et al. 1958)	– *Planococcus ficus*
		Xiphinema italie (Cohn et al. 1970)	
		Not by pollen (although the virus is found in Vitis pollen)	
		It is not transmitted by contact between plants	
		It does not require virus helper as a vector	
PURIFICATION AND/OR MAINTENANCE IN HOST	*Nicotiana glutinosa*	– *Chenopodium amaranticolor*	– *Vitis vinifera*
	Datura metel	– *Phaseolus vulgaris cv.*	– *Vitis repestris*
		Good purification resources:	– LN 33 (also as sp. indicator)
		– *Chenopodium quinoa*	
		– *Nicotiana benthamiana*	
		– *Nicotiana clevelandii*	
		– *Gomphrena globosa*	

(continued)

Table 4 (continued)

	VIRUS		
Topic	Grapevine leafroll virus	Grapevine fanleaf virus	Grapevine corky bark virus
GEOGRAPHICAL DISTRIBUTION	Possibly all over the world	Possibly all over the world, in areas of cultivation of *Vitis* è *American* hybrids	African regions, in areas where *Vitis* è hybrids are grown

GFLV Transmission One of the transmission vectors identified are the nematodes *Xiphinema index* (Hewit et al. 1958) and *Xiphinema italie* (Cohnet al. 1970). *Grapevine fanleaf virus* and probably other viruses transmitted by nematodes in grapes, are easily eliminated by thermotherapy treatment. A treatment of 30–40 days at 37–38 °C is sufficient, but for other viruses such as leafroll and fleck require more temperature and days, in most cases it can last 3 months (Gifford and Hewitt 1961; Nyland and Goheen 1969; Goheen and Luhn 1973; Stellmach 1973). It is likely that among the causal agents of these diseases with apparent viral symptoms, are the viruses with the highest incidence worldwide: two species of the family *Closteroviridae*, genus *Closterovirus*, grape leaf roll virus GLRaV (Namba et al. 1979), GCBaV bark virus (Frison and Ilkin 1991), and a species of the family *Comoviridae*, genus *Nepovirus*, Grape Leaf Fan Syndrome Virus GFLV (Fuchs et al. 1991).

3 Post-harvest and Post-production Alternatives for the Control of Microorganisms in Food

Studies recognize the importance of evaluating preformed matrices (PC), with the task of quantifying various parameters such as mechanical, optical and antimicrobial properties, thus determining its possible application as a new package, since it creates a modified atmosphere (AM), restricting the transfer of gases (O_2, CO_2) and also becoming a barrier for the transfer of aromatic compounds (Arcondéguy et al. 2008; Miller and Krochta 1997). Packaging plays a key role in conservation, distribution, and marketing. Some of its functions are to contain the food, protect it from physical, mechanical, chemical, and microbiological action. A CR or PC has the ability to work synergistically with other packaging materials, is the case of corn starch CR added with glycerol as a plasticizer and applied on brussels sprouts (Brassica oleracea L. var. Gemmifera); these were treated with such solution, stored in expanded polystyrene plates and covered with polyvinyl chloride (PVC) films; preserving quality parameters from different factors such as: commercial acceptability, weight loss, firmness, food surface color, and nutritional quality, since the content of ascorbic acid, total flavonoids and antioxidant activity remained constant during 42 days of storage at a temperature of 0 °C (Dea et al. 2011; Viña et al. 2007).

Research, its applicability in post-harvest management and social importance are strongly correlated, since they are a tool for generating progress in the face of the panorama that continues to be evident and a response to the generation of losses that amount to 40% (Pérez-Gago 2011). Yang et al. (2019), ensure that the post-harvest losses in Castille arrears can reach a percentage of 60–70% when harvesting and post-harvest tasks are carried out carelessly and inefficiently and depending on the packaging system, when using the wooden crate, losses of up to 90% can be reached (Tahir et al. 2018).

The use of a PC or CR in food applications and especially in highly perishable products, such as those belonging to the fruit and vegetable and meat chain, is based on certain characteristics such as cost, availability, functional attributes, mechanical properties (tension and flexibility), optical properties (brightness and opacity), its barrier effect against the flow of gases, structural resistance to water, to microorganisms and its sensory acceptability. These characteristics are influenced by parameters such as, the type of material implemented as structural matrix (conformation, molecular mass, load distribution), conditions under which the films are preformed (type of solvent, pH, concentration of components, temperature, etc.), the type and concentration of additives (plasticizers, crosslinking agents, antimicrobials, antioxidants, emulsifiers, etc.) (Galus and Kadzińska 2015; Rojas-Graü et al. 2009).

Bioactive films (PCs) and edible coatings (RCs) are a trend in emerging technologies applied to food preservation and biofortification (Baldwin et al. 2011). Through the use of hydrocolloids (sodium alginate, chitosan, starch, protein, lipids) emulsified mixtures can be designed (hydrocolloid + plasticizer + bioactive compound extracted from one of the previous sources), which allow the generation of formulations to form coatings (FFR) (Ben-Fadhel et al. 2017). These biopolymer matrices, the latter will be evaluated by quantifying their mechanical properties (tension, elongation), optical (transparency, color parameters), water solubility, PVA (water vapor permeability) and finally determine the ability of these matrices to inhibit the growth of bacteria and molds of relevance to public health and post-harvest management (E. coli, Staphylococcus aureus, Salmonella, Botrytis cinerea and Aspergillus niger) (Aitboulahsen et al. 2018; Vital et al. 2016a).

The diversity of fruit and vegetable products, both in Colombia and in other tropical fruit producing countries, the complexity of their management and post-harvest stabilization make endogenization, study and application of standardized technologies viable and necessary. In which emphasis is placed on benefits such as the content of bioactive compounds encapsulated in biodegradable matrices, which at the same time exert their bioactivity as antifungal, antibacterial and antioxidant compounds in products that present deterioration of their quality attributes as a result of low structural resistance, weight loss, susceptibility to attack by microorganisms and short half-life during post-harvest handling (Behbahani and Imani Fooladi 2018). The high activity of microorganisms in fruit and vegetable products, the loss of quality parameters, and the reduction of shelf life, have led to the constant use of artificially synthesized substances to extend the shelf life of fresh and minimally processed products (Vital et al. 2018). Therefore, the design and optimization of bioactive packaging regains vital importance since passive packaging only provides

a barrier against the outside, in addition the increased bacterial activity is reflected in the surface of the food and in many cases, compounds implemented in the control of such biota do not have the efficient activity as a result of the heterogeneity of the constituents in such products (Tharanathan 2003). This variant is attractive for the food industry due to the increased demand for minimally processed foods free of synthetic additives (Bergel et al. 2018).

Microorganisms have the capacity to generate resistance to products that have been implemented on a recurring basis and therefore could generate a greater risk to public health, for this reason it is essential to make use of bioactive compounds extracted from plant genetic resources found in the Colombian territory such as propolis, mangosteen (*Garcinia mangostana*), copoazú (Microorganisms have the capacity to generate resistance to products that have been implemented on a recurring basis and therefore could generate a greater risk to public health, for this reason it is essential to make use of bioactive compounds extracted from plant genetic resources found in the Colombian territory such as propolis, mangosteen (Garcinia mangostana), copoazú (Theobroma grandiflorum) and aloe (Aloe barbadensis Miller) and that in previous studies have shown to possess bioactive compounds) and aloe (*Aloe barbadensis Miller*) and that in previous studies have shown to possess bioactive compounds (Islam et al. 2019; Lee et al. 2003; Vital et al. 2016b), que podrían tener influencia en el crecimiento de flora microbiana. Pero su sinergia junto a matrices biopoliméricas no ha sido evaluada, no se conoce su incidencia en las propiedades mecánicas de películas comestibles, if the activity of its compounds decreases due to interactions with hydrocolloids which generate the polymeric structure, or if on the contrary they function synergistically maintaining the bioactivity of such compounds. The current problem of the fruit and vegetable chain has a special item such as the lack of generation of value through the application of innovations within the treatment of products without generating adverse effects such as: loss of colour (Homez et al. 2018), odor (Alsaggaf et al. 2017), taste (Rojas-Graü et al. 2007), vitamins (Mei et al. 2002), minerals (Tahir et al. 2019), nutraceuticals (Suhag et al. 2020), contamination with excess chemicals and proliferation of microorganisms. In addition, post-harvest losses continue to be reiterative in labile products, especially those that cannot withstand strong changes in their surrounding environment (Kumar and Neeraj 2019).

3.1 Biopolymer Matrices as Structural Matrices Based on Carbohydrates, Proteins, and Lipids

Different research initiatives have focused on the development of biocompatible, biodegradable and economically viable materials as a consequence of the increasing volume, inefficient handling and final disposal of synthetic food packaging materials (Lacroix and Vu 2013). The concern for maintaining the quality attributes of fresh products and prolonging their shelf life has brought about the design and application

of edible matrices that interact with the food called films and coatings (Falguera et al. 2011). These new packaging materials are designed from a wide variety of renewable compounds such as polysaccharides, proteins and lipids (Bosquez-Molina et al. 2003; Hernández-Muñoz et al. 2008; Kim and Hong 2018; Pavinatto et al. 2020; Ponce et al. 2016; Sánchez-Ortega et al. 2016; Vital et al. 2016a; Wu et al. 2016). However, the functionality of these depends on their chemical nature, as well as on the existing interactions, which has allowed us to obtain biologically active packaging (Das et al. 2013).

Chitin is the second most abundant polymer on the planet after cellulose. It is found in the form of crystalline microfibrils in the exoskeleton of arthropods, as well as in the cell walls of fungi and yeasts. The chitosan (Ch) derived from chitin is one of the most important polysaccharides and attention in the development of active packaging. The structure of this polymer is the result of the partial N-acetylation and depolymerization reactions of chitin (Rinaudo 2006). The molecular architecture of Ch consists of units of β-(1–4)-2-acetamido-2-deoxy-D-glucose and β-(1–4)-amino-2-deoxy-D-glucose. It is soluble in water under acidic conditions which promotes its polycathionic nature (Daza et al. 2018; Homez-Jara et al. 2018), resulting in a number of applications in the pharmaceutical, biomedical, chemical and agricultural industries (Benbettaïeb et al. 2019; Villafañe 2017). Its antimicrobial property is the most important aspect, since it inhibits the sporulation and germination of some fungi (Alsaggaf et al. 2017; Barzegar et al. 2020; Behbahani and Imani Fooladi 2018; Ferreira et al. 2020; Pavinatto et al. 2020; Vital et al. 2016a), as well as the growth of bacteria of food safety importance (Chung 2002; Erkan and Yıldırım 2017; Robledo et al. 2018).

Sodium Alginate (SA) is a binary, anionic, unbranched polysaccharide of high molecular weight, constituted by the union (1 → 4) of the acids β-D-mannuronic (M) and α-L-guluronic (Glc). The proportion of these units depends on the species of algae extracted (Fu et al. 2011; Grant et al. 1973; Ibrahim et al. 2019). One of the most important particularities of AS is its rapid and irreversible bonding reaction with multivalent cations, where it undergoes conformational changes, giving rise to the egg-box structure (Fu et al. 2011; Lawrie et al. 2007; Lin and Zhao 2007).

The ability to form polysaccharide and protein films and coatings has a disadvantage over moisture diffusion. Thus, hydrophobic compounds (neutral lipids, fatty acids, waxes and resins) are used to reduce this deficiency, especially those with high melting points, such as beeswax and carnauba wax (CW) which are more resistant to moisture transport than other lipid and non-lipid coatings (Benbettaïeb et al. 2019; Khan et al. 2013a, b; Shin et al. 2017).

Due to the electronegativity of SA and the polycathionic nature of Ch, there is a high interest in studying the formation of polyelectrolyte complexes (PEC) from these biopolymers. These complexes are the result of the interaction of SA carboxyl groups and Ch amino. Factors such as the fraction of acetylated monomers in the polymer chain of Ch (Lee and Mooney 2012; Won et al. 2018), the mixing ratio of both polymers, the molecular weight and the pH of the solutions, are determining variables in the formation of the CEP (Goff and Guo 2019; Silva et al. 2019), verifiable by atomic force microscopy or scanning electron microscopy, showing

structural conformations, including fibrils (Grosso et al. 2020; Tabassum and Khan 2020). PECs have been used to produce different biomaterials in the form of microcapsules, hydrogels, and films (Jaworek 2008; Yan et al. 2019), with medical and engineering applications thanks to its biocompatibility and muco-adhesiveness (Eom et al. 2018; Ruan et al. 2019; Xiong et al. 2020a, b).

PC and RC have been classified based on the structural material, this way we talk about films and coatings based on proteins, lipids, carbohydrates, or composites. A composite film consists of lipids and hydrocolloids combined to form a bilayer or a conglomerate (Cisneros-Zevallos and Krochta 2003; Dangaran and Krochta 2000; Miller and Krochta 1997; Sothornvit and Krochta 2005). In recent studies, edible and biodegradable film technologies contemplate the production of PCs through the combination of various polysaccharide proteins and lipids, with the task of taking advantage of the properties of each compound and the synergy between the implemented components, since the mechanical and barrier properties depend on the compounds that make up the polymeric matrix and their compatibility (Falguera et al. 2011; Scott and Penlidis n.d.).

Composite edible films and their optimization are in one of the most important study phases, since they are formulated according to the needs and characteristics of the fruits and vegetables to be treated (Rojas-Graü et al. 2009). Hence the importance of characterizing and testing various coating solutions on fresh and minimally processed products since each of them has different quality attributes that must be maintained and enhanced during storage (Oms-Oliu et al. 2008a, b).

Hydrocolloids are the most implemented biopolymers in CP and CR research, a set of these are: carboxymethylcellulose, casein (Ponce et al. 2016), gum tragacanth, guar gum, ethyl cellulose, mesquite rubber (Bosquez-Molina et al. 2003), gelatin added with glycerol, sorbitol and sucrose as plasticizers (Avena-Bustillos and McHugh 2011; Chiumarelli and Hubinger 2011, 2012, 2014; Hurley et al. 2013; McHugh and Avena-Bustillos 2011), PC gelatin-casein composites cross-linked with transglutaminase (Chambi and Grosso 2006; Ju et al. 2019), pectin (Bartolomeu et al. 2012; Conforti and Zinck 2002; Xiong et al. 2020b), cassava starch incorporated with natural antimicrobial compounds. Standard and pre-gelatinized corn starch (Basiak et al. 2019; Ferreira et al. 2020; Pagella et al. 2002), wheat gluten (Bertan et al. 2005; Chambi and Grosso 2006; Grosso et al. 2020) as well as mixtures of sodium alginate and pectin and the effect of the addition of $CaCl_2$ as a crosslinking material on the mechanical properties (Altenhofen da Silva et al. 2009; Xu et al. 2003), water solubility, moisture content, film thickness and the ability of the film to contain the Ca^{2+} ions (Altenhofen da Silva et al. 2009; Cathell and Schauer 2007; Zactiti and Kieckbusch 2006), Similarly, multi-component or composite films have been optimized in terms of their mechanical properties and transparency, since the aim is for them to be accepted by the consumer and to be able to withstand mechanical stress and handling during the transport of coated food (Hurley et al. 2013). For this reason, the response surface methodology has been implemented in the design of experiments, with the aim of determining the optimal mixtures of components that allow to take advantage of each of the functionalities of the added substances (Bernhardt et al. 2017; Walkenström et al. 2003; Xu et al. 2003).

Polysaccharides and proteins are good materials for the formation of PCs and RCs as they show excellent mechanical and structural properties, but on the other hand, they have a poor capacity as a barrier against humidity. This problem is not found in lipids due to their hydrophobic properties, especially those with high melting points such as beeswax and carnauba wax (Jiménez et al. 2003; Shellhammer and Krochta 1997; Velickova et al. 2013).

To overcome the poor mechanical strength of lipid compounds, they can be used in association with hydrophilic materials by forming an emulsion or by laminating the hydrocolloid film with a lipid layer. The efficiency of an edible film against moisture cannot be simply improved by adding hydrophobic materials to the formulation unless a homogeneous and continuous lipid layer is achieved in or on the hydrocolloid matrix (Debeaufort et al. 1998; Hambleton et al. 2008; Quezada Gallo et al. 2005). As other hydrocolloids, chitosan has been studied together with other biopolymers, in this case films composed of corn starch-chitosan plasticized with glycerin, show that the mixture of these two hydrocolloids improves their mechanical properties such as elongation at break and water vapor permeability, in contrast to membranes developed with only one of the structural components. This is a result of the interactions between the hydroxyl groups of starch and the amino groups of chitosan. In addition, their antibacterial activity was clear when inhibition zones were observed by diffusion of discs of the agar material containing *Escherichia coli* O157:H7 (Liu et al. 2015).

Oleoresins of rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), olive tree (*Olea europea*), chili (*Capsicum frutescens*), garlic (*Allium sativum*), bulb onion (*Allium cepa L.*) and common blueberry (*Vaccinium oxycoccus*) were supported in edible coatings based on sodium caseinate, carboxymethylcellulose and chitosan, applied on pieces of pumpkin or auyama (*Cucurbita moschata Duch*) with the aim of inhibiting and/or delaying the adverse effect of the reigning microflora in this type of fruit and evaluating its possible effect on *Listeria monocytogenes*. The combined effect of film-forming solutions added with oleoresins at a concentration of 1.0% w/v on native microflora and L. monocytogenes and evaluated through agar diffusion methodology showed that native microflora was sensitive to chitosan enriched with olive, rosemary and chili oleoresin, as well as for CMC solutions. *L. Monocytogenes* was sensitive to CMC and very sensitive to chitosan film forming solutions added with rosemary (Ponce et al. 2016). Similarly, to date there have been few in vivo applications of edible coatings carrying bioactive compounds in food products such as fresh fruits and vegetables (Palou et al. 2015). And the reports of studies carried out on fruits and vegetables with export potential in Colombia are reduced, since the great majority of investigations refer to the postharvest stabilization of deciduous plants; even more important, the effect of these edible coatings on the physicochemical, physiological and microbiological properties that can be affected in storage is not known (Hernández-Muñoz et al. 2004).

Meanwhile, meat due to its high humidity, high protein content and high lipid oxidation (saturated and polyunsaturated fatty acids) is very perishable and susceptible to microbial spoilage (Rasouli et al. 2019). Therefore, there is a great interest in the development of conservation strategies focused on maintaining quality and

freshness attributes in meat as a function of time, process variation factors associated with the product, atmosphere control, packaging technology (Adams et al. 2015) and storage temperature (Ayres 1955; de Almeida et al. 2015). These adverse conditions of deterioration are associated with intrinsic and extrinsic characteristics of meat, especially its composition (proteins, fats and carbohydrates) (Bazargani-Gilani et al. 2015; Khan et al. 2013a, b), which makes it highly susceptible to the proliferation of microorganisms associated with foodborne diseases (FBDs) such as *Enterobacteriaceae*, lactic acid bacteria (LAB), *Pseudomonas* spp. and *Brochothrix thermosphacta* (Nowak et al. 2012; Sánchez-Ortega et al. 2016), the main ones being *Salmonella* spp. (Shigematsu et al. 2018), *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella Typhimurium* (Kim et al. 2015) and *Staphylococcus aureus* (Latifasari et al. 2019). Also, the pH of the meat (5.4 6.4) and the water activity (aw = 0.99) create favorable conditions for its development, and therefore, reduce its useful life (Tahir et al. 2018). Fundamentally, microbial action on protein, lipid and carbohydrate substrates generate low molecular weight compounds such as histamines, tyramines, putrescines, tryptamines, aldehydes, alcohols, and ketones that affect attributes such as freshness, color, and texture. These are important parameters in the evaluation of physicochemical, biochemical, and microbiological quality of meat (Adams et al. 2015; Jenko et al. 2018).

Consequently, the scientific community and industry have developed techniques for microbial control and inhibition, modifying the extrinsic environmental conditions that affect the growth and survival of unwanted microbial flora. Among these techniques, there is the control of temperature during storage, either under refrigeration conditions, bacterial growth due to the high concentration of substrate and reduction of the useful life of the meat by lipid oxidation (Zhao et al. 2007; Zhao 2012). On the other hand, storage at freezing temperatures proves to be viable since the metabolic processes associated with deterioration are inactivated. However, under these conditions the meat cannot be called fresh when it is taken to the consumer (Vital et al. 2018). Something similar happens when implementing curing processes that seek to reduce the aw in meat, since in addition to changing the characteristics of fresh produce, nitrosamines, mutagenic and carcinogenic compounds can be formed in humans (Talalay and Fahey 2001). In addition, the use of chlorine solutions, electrolyzed water and organic acids has been investigated to reduce the initial microbial load on the product and extend its shelf life (Shin et al. 2017). On the other hand, the effect of the use of ozone treatments on microbial contamination of meat has been evaluated with apparently favorable results (Rasouli et al. 2019), have evaluated the effect of irradiation to decrease the initial microbial load. However, this treatment accelerates lipid oxidation producing unpleasant odors under aerobic conditions (Kang et al. 2005).

In addition to these treatments, different packaging techniques have been tested, since they promote barrier and inhibition mechanisms against microbial development, extend the shelf life of the product, improve its presentation and reduce the use of artificial preservatives (Atarés and Chiralt 2016; di Pierro et al. 2018). These alternatives include active packaging techniques and modified atmospheres (MAP) (Chen et al. 2020; Cooksey 2013; Vergara et al. 2003). APMs are widely used in the

industry to prolong the shelf life of fresh meat (Moreira et al. 2019; Zhai et al. 2018), by changing the concentration of gases such as CO_2 in the headspace of the package, which destabilizes the development of microorganisms in the fresh product (Kim et al. 2011; Tabassum and Khan 2020). Also, active packaging has become important by integrating natural antioxidants and/or antimicrobial agents into the packaging material. Materials such as glass and metal have also been tested, however, the use of multilayer plastic films in fresh meat packaging has been highlighted, given their oxygen barrier and moisture permeability properties. Some authors have evaluated the synergistic effect between different conservation technologies to minimize the alteration of quality attributes in the product, therefore, it has been evaluated the application of treatments such as gamma irradiation prior to packaging (Adams et al. 2015; Hambleton et al. 2008; Miller and Krochta 1997) and the effect of oxygen-absorbing compounds together with citrus extracts (Bonilla et al. 2012).

Nowadays, the continuous changes in the consumer's demands and the trends of the market have caused the innovation in processes of conservation of the meat products (Zhai et al. 2018), reason why PC and RC have been developed (Kai et al. 2013) that improve the overall quality of the food (Galus and Kadzińska 2015), since they have greater interaction with the product, constituting an additional layer of protection and creating an effect equivalent to the modified atmosphere (Won et al. 2018). Thus, PC and RC reduce oxygen and water vapor permeability, flavor emission, antimicrobial activity, alteration of sensory attributes such as color and texture, while allowing the incorporation of additives for antimicrobial control, such as chitosan (Martínez-Abad et al. 2014; Rojas-Graü et al. 2007) and protein isolates (Kai et al. 2013), as well as functional and bioactive ingredients to meat (Antoniewski et al. 2007; Bazargani-Gilani et al. 2015; Cardoso et al. 2016; Kanatt et al. 2013), creating a healthy food approach (Atarés and Chiralt 2016; di Pierro et al. 2018; Henriques et al. 2016). This type of packaging can be easily developed in inedible coatings, by designing low density polyethylene (LDPE) films, where the inner cover is impregnated with bioactive compounds that will be in contact with the product (Li et al. 2013; Wu et al. 2016). Of course, there is a better performance of functional and bioactive compounds in edible coatings. In this order of ideas, peptides, especially of animal origin (Zhang et al. 2010), have been considered in the design of functional meat products due to their antioxidant and antihypertensive activity (Barzegar et al. 2020; Hamedi et al. 2017; Islam et al. 2019; Robledo et al. 2018).

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Coastal Sediments of La Paz Bay BCS: Bacteria Reserve with Biotechnological Potential

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Abstract Coastal marine sediments are ecosystems rich in nutrients and microorganisms, these environments are considered to be highly competitive, so these microorganisms have developed strategies that allow them to regulate or inhibit the growth of other microbial populations. In sediments obtained from different locations in the La Paz Bay, in Baja California Sur México, different microorganisms have been identified that, according to their morphological, biochemical and molecular characteristics, belong to a group of Gram-positive bacteria known as actinobacteria. These bacteria have been shown to have the ability to grow in extreme conditions of low nutrient concentrations, and have the capacity of generating micro-ecosystems based on the production of secondary metabolites that allow them to develop properly in that ecosystem. Among the genera present in these ecosystems, the genus *Streptomyces* stands out. Cultures of this bacterium, extracellular extracts rich in peptides and other low molecular weight bioactive compounds have been obtained, which have been shown to have antimicrobial effect against different pathogenic strains. Thanks to the identification and characterization of the strains present in the microbial communities through massive sequencing techniques, it has been possible to infer the mechanisms by which these strains can carry out the synthesis of antimicrobial compounds. Knowing the genes involved in the synthesis of these metabolites, it is possible to design the appropriate mechanisms to carry out production at the laboratory level and to evaluate their activity

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using *in silico* and *in vitro* tools. The objective of this proposal is to describe the species of actinomycetes that have been identified in the bay, that present antimicrobial activity and that are potentially useful for their subsequent use at the semi-industrial level.

1 Marine Sediments and Bacteria Present

Microorganisms are the most abundant life forms in the ocean, they constituted the basis of marine trophic webs, and are considered to be responsible for approximately 50% of primary production and are therefore considered to play a fundamental role in the biogeochemical cycles in the marine environment (Arrigo, 2005; Middelburg 2018). Even though they participate in the remineralization of organic matter and they are involved in the processes of energy transfer in marine ecosystems, relatively little is known about microbial diversity and its function in these habitats. It is necessary to carry out a hard work to identify and describe the patterns of microbial biodiversity that will allow us to understand the biological foundations of ecosystem function (Das et al. 2006).

Understanding the structure and function of ecosystems requires more than recognition of the interrelationships between microbial populations; quantitative information is needed about the number of organisms, biomass, activity rates, growth and death rates, and energy transfer cycles and rates within ecosystems. Numbers, biomass and activity represent different ecological parameters, and although they normally correlate with each other, these parameters should not be used interchangeably (Atlas and Bartha 1998).

The detection and characterization of microbial populations in marine environments presents serious problems. Several techniques have been used to enumerate and identify bacteria in samples of different origins, but all of them have certain limitations. Culture techniques that use selective media have their own limitations and the results may be underestimating in situ microbial populations, because most microorganisms in the environment are not cultured in the laboratory. To overcome these difficulties, methods involving the deoxyribonucleic acid (DNA) analyses (Amann et al. 1995; Head et al. 1998) have been of great value in understanding bacterial phylogeny and taxonomy. The use of molecular tools has made it possible to reveal the presence of non-cultivable bacteria, which has which has allowed to recognize groups of organisms that are known only by their molecular sequences, and which are quantitatively significant in many environments. Advanced molecular technologies such as genomics, transcriptomics, proteomics, etc. have now been used to explore the microbial diversity and function in the ocean (Giovannoni and Stingl 2007; Duncan et al., 2014).

Marine sediments harbor diverse bacterial communities, which are influenced by geophysical parameters (Böer et al. 2009; Jorgensen et al. 2012; Middelburg 2018). Previous studies show bacterial counts ranged between $1.5 \pm 0.2 \times 10^8$ and

$53.1 \pm 16.0 \times 10^8$ cells g^{-1} in sandy and muddy coastal sediments respectively (Luna et al. 2002).

Coastal zone sediments are characterized by a complex and diverse microbial community both taxonomically and physiologically. They are characterized by spatial and temporal variability at different scales of time and space, and the water-sediment interface is a complex system characterized by physicochemical gradients that change with depth (Brady et al. 2013). The principal phyla in these communities are Firmicutes, Verrucomicrobia, Bacteroidetes, Proteobacteria (Delta y Gammaproteobacteria), Planctomycetes, Actinobacteria y Cianobacteria (Musat et al. 2006; Gobet et al. 2012; Patin et al. 2017).

Marine sediment harbors numerous microbial populations, in this environment microorganisms are subject to strong inter.- and intra-specific interactions and have therefore developed, as a chemical defense mechanism, the capacity to synthesize various metabolites that they use as allelopathic substances. These substances allow them to inhibit the growth of some microorganisms with which it competes for nutrients and/or space (Patin et al. 2015).

Sediments in marine environments are currently attractive for bioprospecting programs, being an abundant source of actinobacteria, which have a high potential to produce new compounds with biotechnological applications. The research carried out with these bacteria shows their great capacity to produce secondary metabolites, so they are considered the richest sources of these metabolites (Murphy et al. 2012).

Actinobacteria are responsible for the production of almost 90% of the antibiotics used in the pharmaceutical industry, which places them as one of the most important sources in the natural production of these compounds. Within this group, the genus *Streptomyces* is one of the most studied and is attributed to the production of commercial antibiotics such as streptomycin, daptomycin, erythromycin, neomycin and tetracycline, to name a few (Arul Jose et al. 2013).

Many metabolites of interest produced by these bacteria are peptide in nature, highlighting the antimicrobial peptides (AMPs) for their structural diversity, the mechanism of action of AMPs is usually through the formation of pores thus destroying the integrity of the cytoplasmic membrane of the pathogen (Shai 2002). Other AMPs act by inhibiting enzymatic activities or interrupting processes such as DNA replication or transcription (Brogden 2005). AMPs are susceptible to proteolytic degradation and an important property of many AMPs is their low toxicity to eukaryotic animal cells (Guina et al. 2000).

Considering that the marine environment has different conditions than the terrestrial environment, it is to be expected that marine actinobacteria have different characteristics than their terrestrial counterparts, and therefore may produce totally different bioactive compounds. During their evolution these bacteria have adapted to live in extreme conditions, and it is likely that this is reflected in the genetic and metabolic diversity of these microorganisms (Manivasagan et al. 2013).

Understanding the patterns of bacterial biodiversity is of importance, because it will allow a better understanding of the diversity and function of these bacteria in marine environments.

2 Peptides and Antimicrobials from Extracellular Extracts

Since the discovery of penicillin by Fleming in 1928, the battle against diseases caused by pathogenic bacteria was thought to have been won (Tan and Tatsumura 2015), however, two years after its introduction in 1940 for infection treatments for *Staphylococcus aureus*, the first isolate of strains resistant to this antibiotic was recorded (Lowy 2003). Letting see that the war was far from being won. In our days the phenomenon of microbial resistance has put the development of new antibiotics in crisis, this because the abuse in their use and self-medication cause resistant strains to appear in a short time, which is why some pharmaceutical companies do not see profitability. Economic and have neglected the line of development and search for antibiotics almost entirely in the hands of the academy (Ventola 2015).

In this search for alternatives to combat diseases caused by pathogenic microorganisms, a number of sources have been explored: microorganisms, plants and animals, both terrestrial and marine, in addition, various strategies for their production have been explored, such as the extraction of natural sources, chemical synthesis and semisynthesis (transform from a previous one), however, natural sources remain one of the best alternatives to find new chemical entities useful for fighting microorganisms (Paul et al. 2019).

2.1 Peptides

The term refers to those compounds formed by more than two amino acids, according to their size, they can be classified into oligopeptides (from 2 to 20 amino acids) and various oligopeptides polypeptides (mainly proteins), according to their biosynthetic origin, the orders are classified as ribosomal synthesis such as bacteriocins that are small low molecular weight proteins (<100,000 KD) and with between 30 and 60 amino acid residues (Yang et al. 2014) and non-ribosomal (secondary metabolites), the latter are small-sized peptides synthesized by most microorganisms and have a wide range of biological activities such as toxins, siderophores, antibiotics, cytostatics, anticancer drugs, among others (Klapper et al. 2018; Martínez-Núñez and López 2016).

Among the most promising chemical entities to combat pathogenic microorganisms, are antimicrobial peptides (AMP's), which are produced by all organisms from bacteria to superior animals, the latter being the first line of defense against bacteria, fungi, viruses and in some cases cancer cells (Zhang and Gallo 2016) as part of the response of their innate immune system (Faye et al. 2002; Pushpanathan et al. 2013). To date, 3228 AMP's have been reported in the antimicrobial peptides database (APD Antimicrobial Peptides Database) (http://aps.unmc.edu/AP/main.html), of which 357 are bacteriocins and antibiotic peptides of bacterial origin. Table 1 shows some peptides obtained from marine microorganisms in the last two decades.

Table 1 Antimicrobial peptides from marine microorganism

Microorganism source	Compound name	Activity against	Reference
Streptomyces. sp	Mohangamides A and B	*Candida albicans*	Semreen et al. (2018)
Streptomyces champavatii	Champacicline	*Erwinia amylovora*	Pesic et al. (2013)
Streptomyces scopuliridis	Desotamide B	*S. aureus; S. pneumoniae*	Song et al. (2014)
Streptomyces drozdowiczii	Marfomycins A, B, E	*M. luteus*	Zhou et al. (2014)
Marinactinospora thermotolerans	Mathermycin	*Bacillus* spp.	Erquan et al. (2017)
Streptomyces atratus	Ilamycins	Mycobacterium tuberculosis	Ma et al. (2017)
Bacillus subtilis	Gageotetrins	G+ and G-	Tareq et al. (2014)
Saccharomonospora sp	Taromycin A	G+	Yamanaka et al. (2014)
Brevibacillus laterosporus	Tauramamide	Enterococcus sp	Desjardine et al. (2007)
Brevibacillus laterosporus	Bogorols B-E	MRSA, E. coli	Barsby et al. (2006)
Bacilus sp.	Bogorol A		Barsby et al. (2001)

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Currently, there is a great interest in compounds of marine origin due to their wide variety of biological activities such as antibacterial, cytotoxic, antioxidant, antiviral, anti-inflammatory, just to name a few, in some cases, with novel mechanisms of action that can be the answer to the phenomenon of microbial resistance (Semreen et al. 2018; (Lei et al. 2019).

Although most of the known antimicrobial compounds have been obtained from terrestrial organisms, the marine environment offers a wide variety of environmental conditions that favor the production of unique compounds with properties and structures different from those found in terrestrial organisms, which gives them the potential for the development of new antimicrobial drugs (Aneiros and Garateix 2004). In this sense, marine bacteria have been cataloged as a promising source of new antibacterials, especially actinobacteria, example of this, until 2010 around 13,700 bioactive compounds had been reported, 10,400 of them obtained only from the genus Streptomyces, both from terrestrial strains as marine (Lei et al.

2019; Solanki et al. 2008). Year by year the number of bioactive compounds obtained from microorganisms is increasing, with Streptomyces being one of the main producers with 167 new compounds reported for 2018 (Carroll et al. 2020).

In the marine environment, the formation of bacterial biofilms is a very complex process that is related to both the environmental conditions and the physicochemical nature of the substrate, various microorganisms are capable of colonizing a wide range of substrates, whether living or inert, other microorganisms only colonize or a type of substrate is developed where they find the necessary conditions for its establishment and development (Caruso, 2020; Dang and Lovell, 2016). Given the wide range of biotic and abiotic interactions in the marine environment, microorganisms are considered a factory of secondary metabolites that they use as a mechanism to adapt, defend or communicate within such a complex environment, some of these metabolites are useful in various industrial branches, mainly pharmacological for the development of new antibacterial drugs (Carroll et al. 2019; Gerwick and Fenner 2013; Javed et al. 2011).

3 Isolated Bacteria from Marine Sediments and their Identified Compounds

Marine sediments are known to have the capacity to host an abundant bacterial diversity (Petro et al. 2017). Therefore, they are considered as highly competitive environments, where it is essential that the microorganisms present develop strategies that allow them to contend for the nutrients in their environment (Challis and Hopwood 2003). One of these strategies is the production of antagonist substances, which can inhibit or regulate the growth of certain bacterial communities (Laport and Gandelman 2016). The ability of bacteria to produce these secondary metabolites offers them a more efficient response to different stress factors and a competitive advantage (Das et al. 2006; Torres-Beltrán et al., 2012).

Marine sediments are one of the most studied environments for isolation of bioactive Actinobacteria (Claverías et al. 2015). Actinobacteria are currently recognized as a source of molecules, with great biotechnological and pharmaceutical importance (Soria-Mercado et al., 2012). To date of the 500,000 natural compounds reported around the world; obtained from biological sources, approximately 70,000 are derived from microbes (both bacteria and fungi), of which 29% were isolated from actinomycetes (Subramani and Sipkema 2019).

Among the Actinobacteria, Streptomyces genus has been widely studied in the last sixty years due to its ability to produce a wide variety of chemical compounds with medical importance and high commercial value for the pharmaceutical industry (Chater 2016). It is currently the source of more than 7600 compounds, including antitumor, anti- and pro-inflammatory drugs, antibiotics, among others (Olano et al. 2009).

On the other hand, Salinispora genus has three species: *S. arenícola*, *S. pacifica* and *S. tropica* (Millán-Aguiñaga et al. 2017). These species produce different compounds, recently identified with cytotoxic activity against different cancer cell lines. One of these compounds is Salinosporamide A, isolated from *S. tropica*, which are in clinical trials (Gulder and Moore 2010). Other Actinobacteria include the genus Marinispora (Kwon et al. 2006), which produces chemical compounds such as Marinomycin A and D, with potent antibacterial activity and against human melanoma cell lines LOX-IMVI, M14, SK-MEL-2, SK-MEL-5, UACC-257 and UACC-62. While Marinomycin B and C also show significant activity against cancer cells with LC 50 values in the μM range, with a specific mechanism of action (Kwon et al. 2006). These new compounds have a mixed polyketide/terpenoid origin structurally related to A80915C antibiotic (Becerril-Espinosa et al. 2012). Likewise, the antibiotic Abisomycin C, obtained from Verrucosispora strains, has also been isolated (Kwon et al. 2006; Keller et al. 2007). Although the marine Actinobacteria; particularly the genus *Streptomyces*, have been widely recognized for their importance as a pharmaceutical resource, little is known about their diversity and ecological role in the ocean (Becerril-Espinosa et al. 2012).

It is noteworthy that recently there have been few findings of new compounds, which diverted attention to Actinomycetes 'non-Streptomyces'. These have been called 'rare Actinomycetes' and are defined as the strains of Actinomycetes isolated with less frequency, compared to those of the genus Streptomyces. It is important to note that this definition does not argue that they are rare in the environment (Subramani and Sipkema 2019).

Since 2013 to 2017, 167 different new bioactive compounds have been documented, from 58 strains of these 'rare Actinomycetes' belonging to 24 genera. Among them, genera such as Nocardiopsis (40 new compounds), Micromonospora (37 new compounds), Salinispora (21 new compounds) and Pseudonocardia (14 new compounds) are the ones that stand out the most with respect to the number of new secondary metabolites obtained (Subramani and Sipkema 2019).

Additionally seven different chemical classes of natural products from Nocardiopsis spp. have been found, where α-pyrones predominated (18 out of 40 compounds). These molecules have a wide range of biological activities, such as anti-inflammatory, anti-bacterial and cytotoxic activity. Furthermore, the genera Streptomonospora and Saccharomonospora also produce a substantial number of α-pyrones. Furthermore, the nocarimidazoles of Nocardiopsis sp., possess a 4-aminoimidazole ring that is rarely found in secondary microbial metabolites (Leutou et al. 2015) and uncommon prolinyl-macrolactam polyketides in Nocardiopsis sp. (Raju et al. 2013; Subramani and Sipkema 2019).

Although enough information has been generated about abundance, diversity and application of microorganisms isolated from marine sediments, few studies on bacteria in this environment have been generated in Mexico compared to those carried out in other parts of the world (Soria-Mercado et al., 2012). The foregoing, despite the fact that Mexico has a maritime territory of 1,378,620 km^2 of coastal and oceanic zones, which are 1.5 times the area of the land territory (Becerril-Espinosa

et al. 2012). This is why Mexican marine ecosystems offer a great study opportunity in this important and prolific field of research (Becerril-Espinosa et al. 2012).

3.1 Bioactive Compounds from Microorganism Isolated of the California Gulf

The Gulf of California (Sea of Cortez) in Mexico, is one of the seas with the greatest biodiversity, is considered by many to be the world's aquarium, where its waters host an immense variety of organisms (Brusca and Hendrickx 2010). This great wealth of organisms, many of which are sessile or with slow movements, use to defend themselves against their predators and against epibiotic secondary metabolites (chemical defenses) that make them a potential source of bioactive compounds for the development of new drugs (Puglisi et al. 2014). Undoubtedly, this same richness serves as a substrate for an infinity of microorganisms with which they develop various biotic interactions (mutualism, commensalism, parasitism, symbiosis, etc.) and which, for the most part, are responsible for the production of bioactive compounds. (Thomas et al. 2010).

In the works related to evaluating the marine microflora of the coasts of the Baja California Peninsula as a source of new antimicrobials, most of the microorganisms obtained are associated with substrates such as macroalgae, mangroves, sponges and mainly marine sediments, where Most of the isolates are actinobacteria (Maldonado et al. 2009). Most of the information related to these works is found as gray literature in theses and project reports (Hernández-Guerrero et al. 2018), however, the few studies that have reached Being published shows the great potential of bacteria in the peninsular seas.

In 2009 the isolation of around 300 strains of actinobacteria from marine sediments of the Gulf of California was reported, the most prominent genera are*: Actinomadura, Dietzia, Gordonia, Micromonospora, Nonomuraea, Rhodococcus, Saccharomonospora, Saccharopolyspora, Salinispora, Streptomyces*. It is widely documented that actinobacteria are the main producers of antibiotics, mainly the genus Streptomyces (Manivasagan et al. 2014; de Lima Procópio et al. 2012), only in 2017, 242 new compounds were reported, of which 137 (more than 50%) were from *Streptomyces* and the number increased to 167 by 2018 (Carroll et al. 2019, 2020), which makes this genus an important source for the production of antimicrobials compounds. Analyzing organic and aqueous extracts obtained from marine actinobacteria from the Gulf of California, they found 17 extracts with cytotoxic activity against cancer cells and three that inhibited the growth of methicillin-resistant Staphylococcus aureus (Torres-Beltrán et al. 2012). Through the analysis of the Polyketide synthetase (16sRNA) gene, the potential of *Salinispora* species to produce bioactive metabolites has been shown, once again showing the great potential of the extracts obtained from marine bacteria (Becerril-Espinosa et al. 2012).

In a more recent bioprospecting study, 125 marine bacterial strains were evaluated for their potential to inhibit biofilm-producing microorganisms as a result of this work. 16 strains showed antagonistic and inhibitory activity against the bacteria evaluated, 10 of the crude extracts evaluated showed activity against *Bacillus. altitudinis*, *B. pumilis* and *B. subtilis* all of them biofilm-forming (Sánchez-Rodríguez et al. 2018). Despite the documented richness and diversity of marine microorganisms in the Gulf of California, studies aimed at evaluating their metabolic richness are scarce. Even so, the few studies available show the great potential they have for the extraction and purification of bioactive metabolites useful in the development of new antimicrobial drugs.

4 Characterization and Identification of Bacterial Communities Using Molecular Techniques

Marine actinobacteria are often difficult to grow compared to terrestrial species, mainly due to their special growth requirements (Zotchev 2012) or unknown growing conditions. It is currently known that only <2% of bacterial cells can form colonies by conventional culture conditions. A large number of them belong to strains known as "viable but not cultivable" (VBNC) (Bernard et al. 2000). A frequently used strategy for their isolation is to mimic the natural environment in terms of pH, oxygen gradient, nutritional compositions, etc. With these variations, some species previously considered VBNC can now be cultivated (Kaeberlein, et al. 2002).

On the other hand, recent advances in genomics, proteomics and bioinformatics have emerged for the identification and characterization of microbial diversity in a broad way (Ngara and Zhang 2018). Genomic analysis by genetic fingerprinting (Muyzer, 1999), DNA-DNA hybridization techniques (Pinhassi et al. 1997) and the construction of metagenomic libraries and sequencing (Kisand et al. 2012), are have been used to identify and characterize bacterial diversity within marine samples. The development of next generation sequencing (NGS) (Webster et al. 2010) and nanopore sequencing (Deamer et al. 2016), has allowed the process to be more in-depth and agile.

Likewise, the analysis of the expression and regulation of RNA transcripts using metatranscriptomics (Ogura et al. 2011) or the determination of protein profile by metaproteomics (Slattery et al. 2012), can be directly linked to large banks of genomes available. To mention one example, coupled metagenomic and metatranscriptomic analyzes have been used successfully to determine bacterial communities in the deep waters of the North Pacific Ocean (Wu et al. 2013). Therefore, the combination of culture-dependent (growth and isolation) and culture-independent (nucleic acid and protein analysis) approaches has revolutionized the characterization and isolation of various marine organisms (Hirayama et al. 2007; Dhakal et al. 2017).

Similarly, the rapid development of data mining methods; including the identification of secondary metabolite gene clusters, has led to the discovery of the genetic machinery that encodes novel actinomycete natural products that have yet to be chemically identified (Ziemert et al. 2016). Most of these gene groups code for polyketides (PK), non-ribosomal peptides (NRP), post-translationally modified peptides (RiPP) and aminoglycosides (Ziemert et al. 2016). Bioinformatic analysis of genomes has also revealed silent clusters of secondary metabolite genes, which are not expressed under standard laboratory conditions (Hug et al. 2018).

So far more than 23,000 PKs and NRPs have been identified, many of them found in actinomycetes, which are being extensively tested for pharmaceutical applications (Wei et al. 2018). This approach has also been used for the identification of new antibiotics from actinomycetes from marine sediments (Schwager et al. 2015). Recently, Schorn and colleagues (Schorn et al. 2016) have shown that the genomes derived from these actinomycetes possess a high degree of diversity, with *Corynebacterium*, *Gordonia*, *Nocardiopsis*, *Saccharomonospora* and *Pseudonocardia* as genera representing the greatest diversity of biosynthetics gene groups. A total of 13 new bioactive compounds have been derived from rare marine actinomycetes, such as *Saccharomonospora* sp., *Salinispora* spp., *Micromonospora* spp. and *Streptosporangium* sp. using metagenome-based approaches (Subramani and Sipkema 2019).

Specifically, regarding the analyzes of microbial communities in marine sediments collected in the Gulf of California and the Gulf of Mexico, Maldonado et al. (2009) used seventeen different culture media to promote the growth and isolation of Actinobacteria. The recovery of this microbial group was almost 300 genera. 16S rRNA gene sequencing revealed genera such as *Actinomadura*, *Dietzia*, *Gordonia*, *Micromonospora*, *Nonomuraea*, *Rhodococcus*, *Saccharomonospora*, *Saccharopolyspora*, *Salinispora*, *Streptomyces*, *Solwaraspora*, and *Verrucosispora*. This study provided further proof that the actinobacteria present in marine habitats are not limited to the Micromonospora, Rhodococcus and Streptomyces grouping, thus showing the abundant diversity of Actinobacteria that can be found in marine sediments collected in Mexico and probably, worldwide (Maldonado et al. 2009).

5 *In Vitro* and *In Silico* Evaluation of Antimicrobial Peptides (AMPs)

Usually the evaluation of the antimicrobial activity of different compounds including peptides is carried out *in vitro*, starting from the search for inhibition against some of the most common pathogens. Among the most used strains are *Escherichia coli*, *Sthaphylococcus epidermidis*, *Candida albicans*, *Klepsiella pneumoneae* among others (Khan et al. 2018; Chávez-Jacobo 2020), all of them preferably belonging to one of the standardized culture collections such as the American Tissue Culture Collection (ATCC) or the European Collection of Authenticated Cell Culture

(ECACC), this because being strains already characterized, their phenotypes are perfectly known and it is also possible to have the sequencing of these strains by more specialized techniques (Zhu et al. 2020). These *in vitro* tests can be divided into two methods, dilution and diffusion methods, where those of diffusion in agar, acrodiscs, plate counts and others, are carried out to evaluate how effective the chemical compound is against any of the model strains, this by inhibiting growth, forming the so-called inhibition zone (Balouiri et al. 2016). This allows to obtain in a first scrutiny which are the compounds that can potentially provide an antimicrobial or antifungal effect. Additionally, *in vitro* tests can also be carried out by other methodologies validated by institutions such as The Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Some of these methods are agar diffusion methods, where the compound of interest or extract is evaluated in a Petri dish which was previously inoculated with the microorganism of interest, there are other methods such as well diffusion, cross streak plug diffusion and poisoned food method, these methods have already been standardized and allow the identification of compounds with relevant activity, as well as allowing estimating the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBI) with which it can be determined if the effect is bactericidal or bacteriostatic (Parvekar et al. 2020). Additionally, an interesting methodology is the antimicrobial gradient method in which a strip loaded with different concentrations of compound of interest is placed on agar surface previously inoculated with the microorganism of interest, with this the MIC can be determined without the need to perform the serial dilution method (Benkova et al. 2020). It is possible to determine by this method the effectiveness of the synergistic effect of two antimicrobial compounds, placing two strips with a concentration gradient and calculating the fractional minimum inhibitory concentration with which it can be estimated whether the effect is synergistic, antagonistic, additive or indifferent (Gupta et al. 2015).

Other diffusion methods consist of autobiography using thin layer chromatography (TLC) plates, where the compounds are resuspended in a suitable solvent and separated by capillary action on the TLC plate, then the plate is placed on the agar previously inoculated with the strain of interest to cause diffusion of the compounds. (Nuthan et al. 2020). With this, interesting results can be obtained, especially when looking for the effect of compounds that are still in complex mixtures and that in crude oil may not have an effect, but that being with a chromatographic separation the effect can be revealed. In order to corroborate the existence of microorganisms that may be alive, the use of tetrazolium salts is used, with which it is possible to identify if the cells are alive or have any damage to their replication mechanism (Panphut et al. 2020).

A good antimicrobial effect is highlighted by dilution in growth broth and dilution in growth agar, additionally, the time at which the fungicidal, bactericidal or bacteriostatic effect is had can be calculated by estimating a lethality curve versus growth time, in which evaluates the concentration 0.25 and 1 times the MIC value, compared with a reference value at intervals of 0, 4, 6, 8, 12 or 24 h. With these data, the percentage of mortality is calculated from the CFU/mL that grow in the control

plate (Jönsson et al. 2018); This method can be used to determine the synergistic or antagonistic effect between two or more compounds in different combinations.

Other tests involve the quantification of the bioluminescence produced by living cells based on their production of ATP and the calculation of the reaction between this compound and luciferase (oxyluciferin) with which the growth of organisms can be estimated by measuring the amount of light emitted since there is a directly proportional relationship between cell viability and the luminescence emitted by those cells.

Another *in vitro* method can be flow cytometry, however, it is a costly process for the equipment although testing times are reduced (O'Brien-Simpson et al. 2016). When the microbiological activity of the compounds of interest is already known, one of the strategies to follow may be bio-directed isolation, with which it is possible to know which is the compound or mixture of compounds responsible for the antimicrobial activity. In the case of antimicrobial peptides, isolation and purification must be performed with care to avoid precipitation or degradation of the peptides, separation must be done by molecular exclusion chromatography, ion exchange or other validated technique and characterization is done by magnetic resonance, FTIR and other techniques (De La Cruz-Sánchez et al. 2019). Characterization using these techniques allows to elucidate its structure and even its three-dimensional arrangement by means of crystallography. Additionally, knowing the sequence of amino acids that make up this peptide chain, it is possible to perform a *in silico* analysis using different protocols, with which it is possible to know specifically how the peptides are interacting with the target molecules, performing a rapid screaning (Basith et al. 2020).

There are currently databases where the crystallographic structures of peptides of different structural arrangement (alpha elicoidal, beta plate, extended and coiled or in the form of a loop) such as the Protein Data Bank, or the PepBank (Wen et al. 2019) are already defined or the antimicrobial peptide database (Waghu and Idicula-Thomas 2020), which can serve as ligands to evaluate antimicrobial activity *in silico*. Once it is known which are the peptides that present a better molecular coupling in the target molecules of interest and that the aspects of hydrophobicity, neutrality and structural arrangement are considered, what proceeds is to evaluate these peptides in the *in vivo* tests to determine if the modification of some amino acid residues are those that could give a better activity or modify it in such a way as to reduce its adverse effects (Amaral et al. 2012; Li et al. 2012; Sarveswari et al. 2015).

Antimicrobial peptides, most of which are approximately 20–30 amino acid residues in length, have certain chemical properties such as the small number of amino acid residues, cationicity and amphipaticity, with which they may have the characteristic of being able to interact in different ways but in reality they act in three main mechanisms, one of them is the barrel-stave form in which the peptide acts on the bacterial membrane modifying the membrane and penetrating it, the other mechanism is the formation of a toroid in which they are reached the interiors of the membrane producing a large hole that damages the cell membrane and the third mechanism is defined as the mat or carpet mechanism in which the accumulation of peptides outside the membrane which modifies the density of the membrane

dissolving it and generating channels inside the membrane, which causes cell death; however, the fact that peptides have this effect does not mean that other compounds cannot cause structural damage (Henderson et al. 2016; Falanga et al. 2018; Bueno 2020).

It is difficult to develop a general method to predict the nature and antimicrobial activity of peptides due to the low sequence homology as may occur. One strategy used to predict the bioactivity of antimicrobial peptides is by comparing the models using comparative methods based on the sequence similarities that the peptides have and the other is through in silico modeling in which the identification of the structures and the selection of the templates is carried out so that in a second step the alignment of the target sequences with the model structure is performed and a third step is the generation of the models for the target structure based on the information of the obtained structures and finally the validation of the generated models is carried out (Tavares et al. 2013; Sampaio de Oliveira et al. 2020).

In the evaluation of the antimicrobial activity *in silico*, it is necessary to perform a molecular dynamics of the membrane of interest to be able to elucidate the effect either by membrane disruption, disruption or interaction with transporters. (Maccari et al. 2015; Maróti et al. 2011).

6 Potential Industrial Application of Antimicrobial Peptides

When the sequence of amino acids with potential antimicrobial activity is already known, what proceeds for application at an industrial level is to know if these amino acid sequences are possible to replicate in a model microorganism whose genome is perfectly known to carry out biotechnological engineering and achieve the expression of these peptides with the help of the cellular machinery of the selected microorganism. Among the strains that are most used for industrial generation are *Sacharomyces cereviseae* and *Escherichia coli* (Bommarius et al. 2010; Sampaio de Oliveira et al. 2020).

Undoubtedly, antimicrobial peptides (AMPs) have a wide application in various areas of the industry, Fig. 1 shows the areas in which antimicrobial peptides can be used, in which those fields in which infections derived from the group stand out. of human infections caused by the microorganisms ESKAPE, which is an acronym for *Enterococcus faecium* (E), *Staphylococcus aureus* multiresistant (S), *Klepsiella pneumoneae* and *Escherichia Coli* (K), *Acetinobacter baumanni* (A), *Pseudomonas aeuroginosa* (P) and Enterobacter (E) species. These microorganisms are responsible for multiresistant antimicrobial infections (Chávez-Jacobo 2020).

These industrial applications are aimed at the following areas: biocides and others, veterinary applications, infections by both great positive bacteria and great negative bacteria, antifungals, crop protection, post-harvest treatments of both fruits

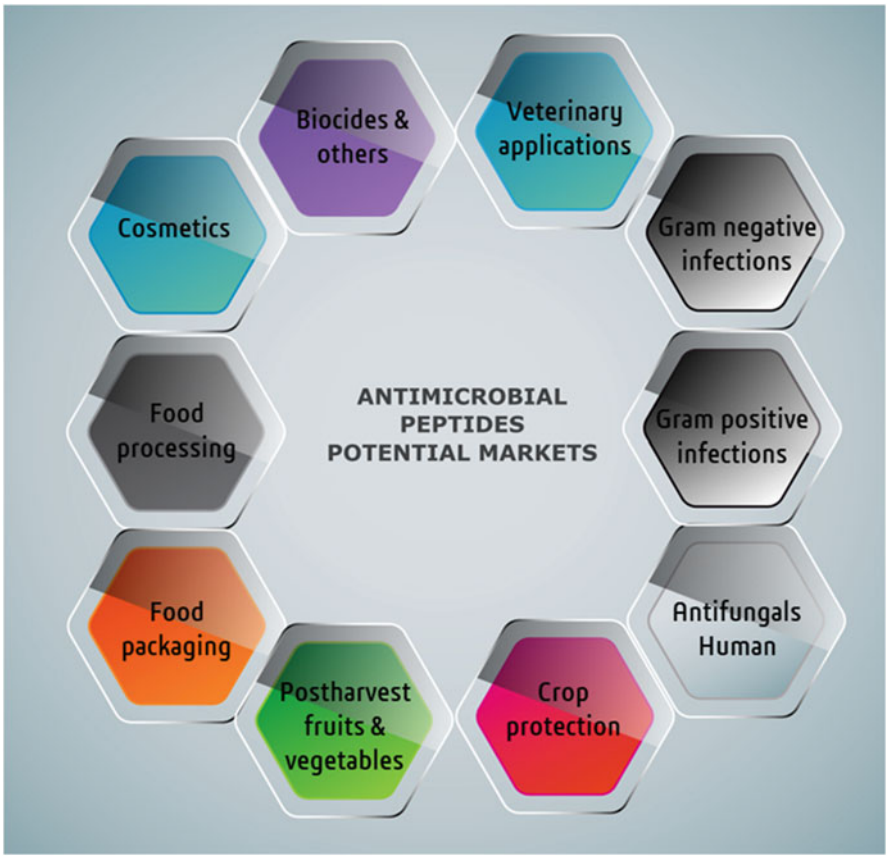

Fig. 1 Areas of application of antimicrobial peptides. Taken and modified from Bardají (2011)

and vegetables, food packaging and food processing in addition to the development of cosmetics (Nakatsuji and Gallo 2012; Juturu and Wu 2018; Ghosh et al. 2019).

The different applications take into consideration that previously an exhaustive investigation had to have been carried out, which usually involves the work of research groups from different institutions of higher education or research at an international level. However, when these AMPs are in the development process, the translation of the research part to scaling at an industrial level is where some discrepancies arise, since many times the strategies to demonstrate their effectiveness require technologies that are not fully validated or they are not integrated into processes that are already established, which significantly affects the efficiency of the MPA of interest.

Usually the development process of new MPAs follows this scheme (Fig. 2):

The basic research part (Step 1) involves identifying the possible peptides formed and that they present the biological activity that is considered (antimicrobial, antioxidant, anti-inflammatory), where their activity is evaluated following in vitro and

Fig. 2 Stages for the application of AMPs at an industrial level. Taken and modified from Bardají (2011)

in silica methodologies Once the activity is known, the optimal extraction, isolation and purification conditions are determined following consolidated methodologies or new methodologies. Once the compound has been purified, it is necessary to evaluate its stability over time and test it in the final product or in its field of application (Step 2). En esta etapa donde se obtienen los resultados de la invención y que se realiza la protección de la tecnología ante las instancias relacionadas con la propiedad intelectual, lo que sigue es realizar la prueba del prototipo, el modelo inicial o el mínimo producto viable (Step 3). In this stage where the results of the invention are obtained and the protection of the technology is carried out before the instances related to intellectual property, the next step is to carry out the test of the prototype, the initial model or the minimum viable product (Step 4). This is where many products take a long time to overcome these stages, since the prototype does not meet the expectations of the industry or its mechanisms by which it is obtained are so new that the necessary technology is still developed at the laboratory level or failing that. to pilot plant.

During the purification processes of antimicrobial peptides, a structural modification may occur, which may compromise their bioactivity, for which reason adequate strategies must be in place to avoid these conformational modifications (Dullius et al. 2018).

However, when these peptides are produced by microorganisms (bacteriocins), by acting as a defense mechanism for the bacteria that produce them, in order to adapt to the environment or to regulate the growth of other microorganisms in the culture system or medium, this makes their production not undergo structural modifications (Yi et al. 2015). As they are more conformationally stable, many of these bacteriocins are already in the final stages of clinical testing to be commercialized (Ghosh et al. 2019).

Among the main problems that arise when using AMPs is low stability, low solubility, toxicity, and high production costs, which can be reduced considering among these strategies the use of carriers or encapsulation, emulsification or

functionalization with which the effectiveness of these compounds can be maintained or even increased (Agyei and Danquah 2011; Zaccaria et al. 2018). Additionally, it is possible to generate solid-state cultures, using the microorganism that produces it and thus, from a stock of the strain, batch produce the bacteriocin with which the harvest, purification and obtaining is subsequently carried out (Mulyani et al. 2019).

The most relevant aspect for the industry is that the production and procurement costs must be low or that they do not require investment in emerging technologies such as electroporation, electrodeposition or foam drying (Alajlani et al. 2016) with which the application is oriented towards the areas application where its application is most economically viable.

7 Bacteria Isolated from La Paz Bay of and Other Associated Organisms

Marine ecosystems are considered a potential source of novel drugs, as they provide a wide variety of natural products obtained mainly from invertebrates such as sponges, jellyfish, anemones, corals, bryozoans, molluscs, echinoderms, tunicates and crustaceans, as well as bacteria and cyanobacteria (Donia & Hamann, 2003; Bhakuni & Rawat, 2006). The organisms have developed a series of metabolic and physiological strategies, which allow them to survive in this environment, which may mean the synthesis of secondary bioactive metabolites with novel chemical structures (Jensen & Fenical, 1994; Faulkner, 2001; Bhakuni & Rawat, 2006).

Within invertebrates, sponges are a rich source of natural compounds with unique structures, which exhibit a wide range of biological activities (De la Rosa & Gamboa, 2004; Anand et al., 2006; Hernández-Guerrero et al. 2007; Blunt et al., 2010; Gándara-Zamudio 2011). However, it has been considered that many of these metabolites may be of microbial origin because of the remarkable similarity with metabolites produced by bacteria, (Unson et al. 1994; Santos et al. 2010). It has been reported that sponges show a constant relationship with associated microorganisms, which can represent up to 50–60% of the sponge biomass (Lee et al. 2001; Donia & Hamann, 2003; Taylor et al., 2007).

In marine environments associations between microorganisms and invertebrates are common, bacteria associated with marine invertebrates are a clear example of interactions that demonstrate a variety of colonization and growth strategies used by some bacteria, perhaps inhibiting competing microorganisms through the production of antimicrobials or to protect against pathogens or opportunistic organisms (Engel et al., 2006; Hentschel et al. 2001).

In the Gulf of California, in the rocky reef of Punta Arena de la Ventana, B. C. S., (24° 02' LN and 109° 49'LW), two species of the genus *Aplysina* are found. Previous studies report that bacteria belonging to the classes Actinobacteria, Bacilli and Proteobacteria were isolated from *A. gerardogreeni* (α and

γ-proteobacteria) (León-Deniz., 2003; Montes-Plascencia et al., 2010). Biological activity assays showed that the strains had antimicrobial activity against *Staphylococcus*. *aureus* and *Escherichia coli*, (Valencia-Agami 2010; Águila-Ramírez 2012). For *A*. *clathrata* the isolated bacteria belonged mostly to the phyla Actinobacteria and Firmicutes, the Actinobacteria were represented by five families of the order actinomycetes, while for Firmicutes the bacteria belonged only to the family Bacilliaceae. The isolated strains also showed antimicrobial activity against *S. aureus* and *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Montes-Plascencia 2013). In another work carried out in the area of Pichilingue within the Bay of La Paz, B. C. S., with the sponge *Mycale* sp. it is observed that the bacteria associated with this sponge correspond to the phyla Proteobacteria, Firmicutes and Actinobacteria, with a predominance of the classes Bacilli and α-proteobacteria (Parera-Valadez 2012). It is important to note that for both the genus *Aplysina* and in *Mycale* most of the strains isolated from the sponge tissue are Gram-positive bacteria, with a predominance of Firmicutes and Actinobacteria. It is widely recognized that the genus Bacillus is a producer of bioactive compounds, among which are non-ribosomal polyketide synthases and peptide synthases (Donadio et al. 2007); also the production of metabolites with antibacterial properties, anti-adherents, algicides and several substances of interest in pharmacology has been reported (Ivanova et al. 1999; Jeong et al. 2013; Pabel et al. 2003; Zhang et al. 2004), so they have a high potential in the search for new antimicrobial substances.

Another group of bacteria that form part of the microbial communities in the marine environment are the actinobacteria, which are filamentous Gram-positive bacteria and are characterized by the production of biologically active compounds (Fenical and Jensen 2006). The search for biologically active compounds is currently focusing on the isolation of these bacteria in marine sediments, since they offer the possibility of finding native strains that produce novel compounds (Prieto-Davo et al. 2008).

8 Conclusions

Considering the potential that sediments offer for the isolation, characterization and search for biologically active compounds, two projects are currently being developed in La Paz Bay, one of them related to the search for peptides with antimicrobial activity from actinobacteria isolated from coastal and mangrove sediments. In this study, 75 strains of actinobacteria isolated from marine sediment were used to evaluate the potential for producing antimicrobial peptides and the inhibitory spectrum against clinical pathogens. Fifty strains have been identified by sequencing the 16S gene of the rRNA. The most represented genus is Streptomyces sp., with the identified strains the process of obtaining the extracellular products for the search of antimicrobial peptides is being carried out. The second one is related to the evaluation of the production of extracellular enzymes of biotechnological interest. For this purpose, strains preserved at −80 ° C are being, that are part of the collection of

actinobacterial strains of Microbiology and Molecular Biology Laboratory of CICIMAR-IPN.

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Microorganisms Bioindicators of Water Quality

Margarita del Rosario Salazar-Sánchez, Arnol Arias-Hoyos, Diana Carolina Rodríguez-Alegría, and Sandra Morales-Velazco

Abstract Lotic and lenitic ecosystems tend to the stability of physicochemical parameters in time, and sometimes they can present extreme conditions or in conditions of natural environmental stress for different organisms that are habiting them. Some of the impacts that affect the microorganisms are the permanent contribution of nutrients by the particulate matter and energy favouring the growth of producing organisms, which conform dense communities, with diverse populations of algae, distributed both in the water column (ticoplankton) and in peripheral growths, formed these communities an important refuge and source of food other organisms, including various species of fish, aquatic macroinvertebrates in its adult and larval phases, and zooplankton, which is why it is of interest to present some species are used as bioindicators of environmental quality given its ecological importance, with a view to preserving the environment, making decisions for mitigation or restoration.

Keywords Phytoplankton · Bioindicator

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1 Introduction

In the different types of water sources that exist on our planet earth it is very common to find a great variety of organisms interacting with each other and with their abiotic environment. The water presents ideal conditions for the development of many organisms, including bacteria, algae, protozoa, viruses, fungi, animals and plants, which exchange matter and energy from biogeochemical cycles that may occur in different aquatic ecosystems, but in turn can also affect the physical-chemical, bacteriological and organoleptic properties in water. Some of the mentioned micro-organisms can be non-pathogenic, and others on the contrary, can be pathogenic (causing diseases) which are transported and reach other organisms and especially humans through the water; according to the World Health Organization – WHO, one of the 10 causes of death related to the environment worldwide is caused by diarrheal diseases (World Health Organization [WHO], 2020), which are affecting approximately 1. 700 million children each year, of which 525,000 die in the under-five age range, due to difficult, access to safe water or good basic sanitation systems (WHO, 2020). Micro-organisms that can reach water by ways such as soil, animal and human feces, decomposing organisms, air, or discharges from some anthropogenic activities.

1.1 Bioindicators in the Water

Bioindicators can be considered as a complement to physicochemical and microbiological analyses to determine water quality (Guillén et al. 2012); moreover, at a given moment they can determine the ecological or trophic quality of the water ecosystem or its quality according to its intended use. Some of the main bioindicators are presented in Table 1, considering that among the indices usually used to identify their relationship as bioindicators of water quality is given by the functional redundancy of the co-occurring species in the communities (Xu et al. 2018).

1.2 Phytoplant Communities as Bioindicators of Nutrient Concentration in Lentic Ecosystems

Phytoplankton communities represent the main component in primary productivity because this community transforms light energy and essentially inorganic materials such as carbon dioxide, phosphorus, nitrogen into other energy forms, with the consequent release of oxygen, thus contributing to the trophic richness of a lake and therefore, the biological richness of these ecosystems and their organisms are used as bioindicators of water quality (Roldán and Ramírez 1992; Wijeyaratne and

Table 1 Bioindicator organisms are used to determine water quality

Group	Individual/Bio-indication
Phytoplankton	Chlorophytes/Eutrophy Cyanophysics/Eutrophy Pyrophytes/Oligotrophy Euglenophytes/Meso to oligotrophy
Zooplankton	Cylates/Water with high organic matter and low oxygen concentration. Copepods/Oligotrophy Cladocera/Eutrophic waters
Macroinvertebrates	Tubificidae/Water contaminated – the presence of feces. Ephemeridae/Water well-oxygenated – oligotrophic Plecopteros/Clean water
Macrophytes	*Eichornia crassipes*/Contaminated water *Stratiotes*/Water pollution *Juncus effuses*/Absorbsorbs heavy metals
Periphyton	*Cladophora*/Organic matter contamination *Hyemic diatom* / Oxygenated water – oligotrophic environment *Navicula gracilis*/Alkaline and oxygenated environment

Nanayakkara 2020). Also from the ecological perspective, the characteristics of water and nutritional requirements of phytoplankton are directly related to the adaptation to the environment, productivity, and population dynamics (Reynolds 1995, Glibert 2016). These relationships allow determining the fluctuations, organization, evolution, and functioning of phytoplankton populations and aquatic ecosystems.

The communities of primary producing organisms are distributed in lakes and other inland water masses basically in two ways: forming extensive blooms attached to sediments, rocks or vegetation, thus forming the peripheral community, or presenting themselves freely and suspended in the water mass, forming the community known as phytoplankton (Roldán and Ramírez 1992; David et al. 2020). Peripheral and phytoplankton communities are made up of a great diversity of algae of variable size, which can be present from a few microns to a few millimeters or even form dense growths several meters long; for a great variety of forms and for possessing broad metabolic capacities, which turn aquatic ecosystems into areas of high trophic productivity (Margalef 1984, Markert et al. 2003). Therefore, biological communities are supported by the photosynthetic production of organic matter.

The freshwater phytoplankton communities are located in the two major domains: the prokaryotic and the eukaryotic. In the first one, there is the Cyanophyta division and the photosynthetic bacteria (Lewin 2002); in the second one, the following divisions are included: Chlorophyta, Chrysophyta, Euglenophyta, Pyrrophyta, and Cryptophyta (Roldán and Ramírez 1992). Margalef (1983), includes as primary producers the Rhodospiral bacteria within the Cyanophyta (Vachard 2020). Table 2 indicates the algae divisions and their main characteristics, based on the system proposed by C (Hoek et al. 1995; Queiroz et al. 2020).

Table 2 Main characteristics of the algae

Division	Pigment	Food reserve	Cell wall	Flagellum	Environment
Cyanophyta Unicellular, filamentous, and colonial. Prokaryotic organisms.	Chlorophyll a, phycocyanin c, phycoerythrin, beta carotene, and xanthophyll	Cyanophicine granules (arginine and aspartic acid), glycogen type poly glucose	Murein or peptidoglican. Diamimo-pimelic acid, glucosamine and alanine. Mucilages are present.	Absent. Movement is observed due to mucilage effects	All environments, even in wetlands. Produce highly toxic toxins. Indicate alteration of aquatic ecosystems
Prochlorophyta Non-branched cellular or filamentous	Chlorophyll a and b, carotenes, zeaxanthin arranged in tilacoids	Starch and cyanophine granules	Peptidoglycan	Absent	Fundamentally marine
Glaucophyta. Unicellular	Chlorophyll a in chloroplasts. No other types of chlorophylls have been detected. Chloroplasts have a thin layer of peptidoglycan. There are phycocyanin and allophocyanin	Starch is located outside the chloroplast.	Peptidoglycan	Present with two flagella of different sizes formed by two rows of fine filaments.	Aquatics
Haptophyta **(Prymnesiophyta)**	Chlorophyll a and c. Never chlorophyll b. Fucoxanthin, Beta carotenoids, diatoxanthin, and diadinoxanthin.	Chrysolamines. Some accumulate paramilon.	Cellulose, with coatings of organic materials and calcium plates with characteristic radial patterns	Present. Two equal or unequal scourges.	Aquatic
Euglenophyta. Flagellated unicellulars. The cell has an invagination called an ampulla.	Chlorophyll a and b; beta carotenes and xanthophylls. 2–6 tilacoids sometimes grouped	Paramilon (beta 1,3 glucopyranoside) Oil in some.	Absent	1–3 and sometimes up to 7. Apical y subapical.	All habitats. Widely distributed. Some feed heterotrophically. They indicate the presence of organic matter.

Phaeophyta Currently part of the Heterokontphyta.	Chlorophyll a and c; beta carotene, fucoxanthin, and xanthophylls. 2–6 Tilacoids.	Laminaran (beta 1,3 glucopyranoside with predominance of mannitol.	Cellulose microfibre with fractions of calcium alginate and sulphated mucopolysaccharides (fucoidan)	2, uneven and in a lateral position	Essentially marine and estuarine; few freshwater species. Generally filamentous. They have industrial applications.
Chrysophyta Currently being studied as a class of the Heterokontophyta Division. Unicellular, filamentous, and colonial.	Chlorophyll a and c; alpha, beta and gamma carotenes, xanthophylls, and fucoxanthin. 3 tilacoids grouped	Chrysolaminaran (beta 1,3 glucopyranoside) oils.	Cellulose, with silica coatings, calcium carbonate, some with mucilaginous substances and even chitin in some	1–2 unequal or equal apicals	All habitats, with wide distribution. Some can be feeding heterotrophically.
Xanthophyceae Unicellular, colonial, and thalloid.	Chlorophylls a and c. Beta carotenoids, vaucheriaxanthin, diatoxanthin	Chrysolaminaran	Cellulose, with silica prints forming caps or shells.	Present	Aquatic (essentially freshwater, although a few marines) and terrestrial.
Bacillariophyceae	Chlorophyll a and c. no chlorophyll b found	Chrysolaminaran. Some accumulate lipids in the form of oils.	Formed mortgage and epiteca of polymerized silicic acid amorphous (not crystalline) called frustula.	Absent. Flagellated sex cells	Aquatic. Due to their abundance and the presence of frustules, when they die they are deposited in aquatic sediments and give rise to diatomaceous earth, of industrial application.
Dictyochophyceae	Chlorophylls a and c. Contains fucoxanthin, diadinoxanthin, diatoxanthin, lutein, and beta carotene.	Chrysolaminaran	It has a kind of siliceous skeleton like a basket where the cell is located.	Present	Marine

(continued)

Table 2 (continued)

Division	Pigment	Food reserve	Cell wall	Flagellum	Environment
Dinophyta.	Chlorophyll a and c; Beta carotene and xanthophylls. 3 tilacoids grouped	Starch, alpha 1.4 glucan. Oil in some species	Celulosa o ausente.	2; one rolled up and the other free	Aquatic, they produce highly toxic toxins. They form red tides. Some with industrial application
Crypsophyta	Chlorophyll a and c; alpha, beta and gamma carotenes, very typical xanthophylls (alloxanthine, crocoxanthine, and monadoxanthine). Phycobylins. 2 grouped tilacoids	Starch	Ausente	2: Unequal and apical	All environments.
Rhodophyta	Chlorophyll a; in some d. Chlorophyll b and c absent. Phycocyanins and Phycoerythrin.	Floridic starch (amylopectin)	Celulosa, xilanos, polisacáridos sulfatados (galactanos). Calcificación en algunas. Alginatos en algas coralinas. Ausente en otras	Absent	The majority are marine or estuarine. Freshwater filaments. With industrial applications due to the composition of the cell wall and the accumulation of iodine.

The evaluation of the quality of the environment, in particular of aquatic communities, has traditionally been developed based on methods supported by measurements and determinations of the physical and chemical characteristics of water, but the use of biological indicators complements this type of method, quantifying and qualifying the impacts generated by human activities.

Particularly in lakes, another secular component of the succession is observed, the system changing slowly, through annual cycles, as a result of the interaction between the lake and the surrounding terrestrial ecosystems and intensified by the action of man. This is the phenomenon known as eutrophication, which consists of the enrichment of the water with nutrients at such a rate that it cannot be compensated for by its definitive elimination through total mineralization so that the decomposition of the excess organic matter causes an enormous decrease in the concentration of oxygen (Margalef 1983; Zhang et al. 2019).

These processes are generated naturally, most notably in lentic-type systems (lakes, lagoons, and wetlands), where they have accelerated due to different anthropogenic activities, which have increased the rate of sedimentation (through the destruction of surrounding vegetation and increased runoff), nutrient colmatation (leaching of agrochemicals and dumping of domestic and/or industrial wastewater) causing changes in the physicochemistry of water and thus limiting the species associated with these ecosystems (Roldán and Ramírez 1992).

On the other hand, a deterioration of the quality of the ecosystem is generated, since the release of toxins can favour colonisation by emerging macrophytes, which will reduce the effective area of the aquatic system, with negative effects on local biodiversity and aquatic food webs, a unique situation that occurs when certain biological, anthropogenic and environmental (physicochemical) factors interact in the environment (Margalef 1983; Dalu et al. 2020).

Limnologically, water bodies can be classified in a simplified way into three main types:

1. Oligotrophic with low nutrient content and minimum vegetal production.
2. Mesotrophic with intermediate characteristics between oligotrophic and eutrophic.
3. Eutrophic with high nutrient content and excessive vegetal production.

Some of the most important biological factors are the presence of a phytoplankton colonising population, as an anthropogenic factor the dumping of organic loads, which abnormally increases the amount of nutrients such as nitrogen and phosphorus. And amongst the environmental factors, we consider the contribution of nutrients from the atmosphere, runoff, and leaching (Herrera-Silveira and Ojeda-Alayón 1999). Biolo and Rodrigues (2011), found that the high concentrations of phosphates and organic load present in do Alto Rio Paraná (Brazil), determining the composition and biocenocis of the diatomaceous community, presenting differentiated groups according to the trophic state of the stations where the sampling was done.

The structural properties of the periphyton have considerable importance about nutritional requirements, adaptive behavior to the environment, productivity, and population dynamics (Reynolds 1984). For this reason, not only the concentration of

a nutrient or element is important, but the essential material available in quantities that are more similar to the critical minimum necessary to fulfill the functions of the organisms is a limiting factor (Odum and Barrett 1971). On the contrary, the elements can constitute a device that increases its reproductive rate and the ranges in which it can survive are known as the tolerance range (Shelford 1931). For the aforementioned reason, the recognition of species in different concentrations of nutrients allows the determination of bioindicator species that contribute to glimpse impacts and conservation strategies of lentic ecosystems.

The main methods for monitoring water quality are based mainly on physico-chemical analyses, which are relatively accurate and easy to perform. However, from a biological perspective, it is possible to make estimates, which, although they require a greater effort, have sufficient advantages to justify its use (Prosperi 2004).

In this respect, bio-indication is important for the valuation of ecosystems, and therefore the following should be taken into account when using a species as an ecological indicator (Odum 1959; Stevenson and Smol 2015).

1. Species must be generally stenotic, rather than euronic, since by developing in more limited ranges they allow a better reflection of the characteristics that are to be evaluated.
2. Before certain species are relied upon as indicators, there should be ample field testing and, if possible, experimental evidence that one or more factors are limiting.
3. Knowing the adaptive capacity, if there are pronounced ecotypes with greater adaptive capacity
4. Numerical relationships between species, populations, and whole communities often provide more reliable indicators than single species, since the whole reflects a better integration of conditions than the part.

1.2.1 Algae Bioindicators of Nutrient Concentration

Research performed in three lakes (oligotrophic, mesotrophic, and eutrophic) with different concentrations of nutrients (Morales Velasco and Peña 2014), classify the peripheral microalgae as bioindicators under the following classification.

1.2.1.1 Stenoic Indicators of Pollution-Free Waters

According to the associations between the physical and chemical characteristics of water, three exclusive species were registered for the oligotrofic ecosystem (Fig. 1).

Bambusina brebissonii, indicator of oligotrophic lakes with a tendency to acidity (Duque and Donato 1992), a condition recorded in the species' habitat (pH 4.0–6.5); susceptible to changes in the concentrations of any of the nutrients and/or reactions during the production-breathing processes, altering the water conditions, causing the disappearance of the species (Esteves 1988).

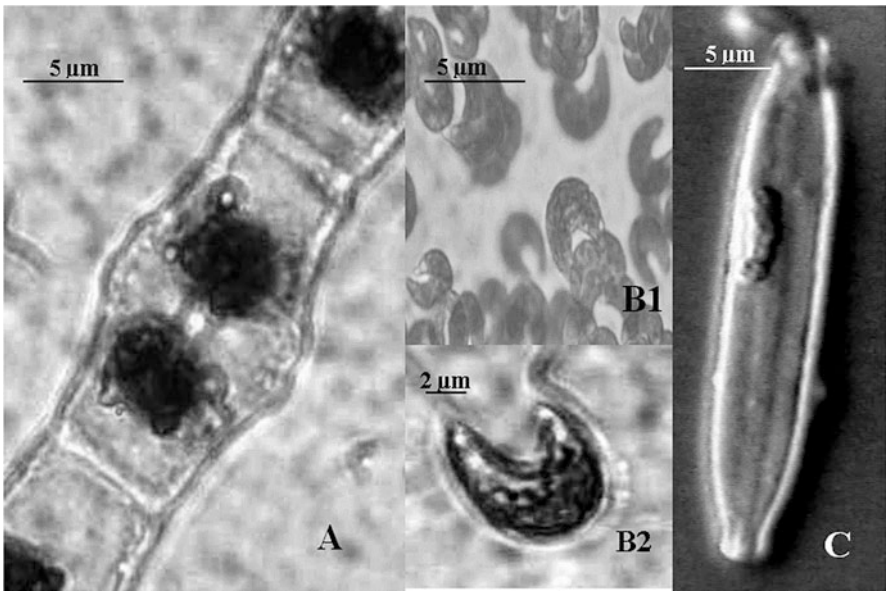

Fig. 1 Some stenoic indicators of pollution-free waters algae. (**a**): *Bambusina brebissonii*, (**b1, b2**) *Borzia sp*, (**c**) *Synedra ulna var ulna*

Synedra ulna var ulna, presents a preferential habitat towards oligotrophic and mesotrophic systems, a presentation given to the stability of the water column, maintaining the availability of the chemical components *(*Agbeti et al. 1997).

The presence of *Borzia sp.* (Cyanophyta), is explained by its capacity to fix atmospheric nitrogen that enables it to survive in waters of varied trophic state and maintain its growth (Biolo and Rodrigues 2011; Cutrim et al. 2019).

1.2.1.2 Species Indicating Low Phosphorus Concentrations

Phosphorus related species (0.01–0.4 mg/l) tolerant to low nutrient concentration (Fig. 2). The species are associated with relatively clean water with a moderate enrichment of organic material and are sensitive to seasonal changes where the nutrient content becomes more evident (Palmer 1969; Steinberg and Schiefele 1988).

Rhopalodia gibba is sensitive to changes in habitat, which are reflected in decreasing numbers of individuals as nutrient concentration increases (Bolgovics et al. 2017). Pinnularia graciloides and Pinnularia gibba, capable of accumulating and storing soluble and particulate phosphorus (detritus) in their cells, to be used when its concentration in the water is lower (American Public Health Association 2005), a similarity shared with the supply of combined nitrogen, which they fix from

Fig. 2 Some species Indicating low phosphorus concentrations. (**a**) *Ceratium hirundinella*, (**b**) *Cocconeis sp*, (**c**) *Pinnularia mesolepta*, (**d**) *Rhopalodia gibba*, (**e**) *Pinnularia graciloides*, (**f**) *Surirella sp*, (**g**): *Eunotia maior*, (**h**): *Pinnularia gibba*

atmospheric nitrogen and thus do not depend on it (Arroyo et al. 2012; Vázquez et al. 2002; Momeu and Péterfi 2009).

1.2.1.3 Species Tolerant to Low Nutrient Concentrations

Direct relation with total phosphorus (0.05 mg/l) and nitrates (0.1–1.2 mg/l), where the preferential habitat is the low temperature lenticular ecosystems (10 °C) (Fig. 3). The species indicate successional processes in the different trophic states, with strong relationships with nitrates and ammonium, which condition the processes of eutrophication of water (Donato et al. 1987; Fontúrbel Rada 2005; Nwankwegu et al. 2020).

Another conditioning factor is the dependence on iron and aluminium, ions that directly influence the pH of the water (pH: 4.6–5.5), which determined the presence of *Pediastrum piramidale*, *Pleurotenium* sp., *Synedra ulna,* and *Xanthidium* sp., species (Cordero et al. 2005; Roldán-Pérez 2009; Neustupa et al. 2011 & Duque and Donato 1992; Sarmaja-Korjonen et al. 2006, Cabral 2010).

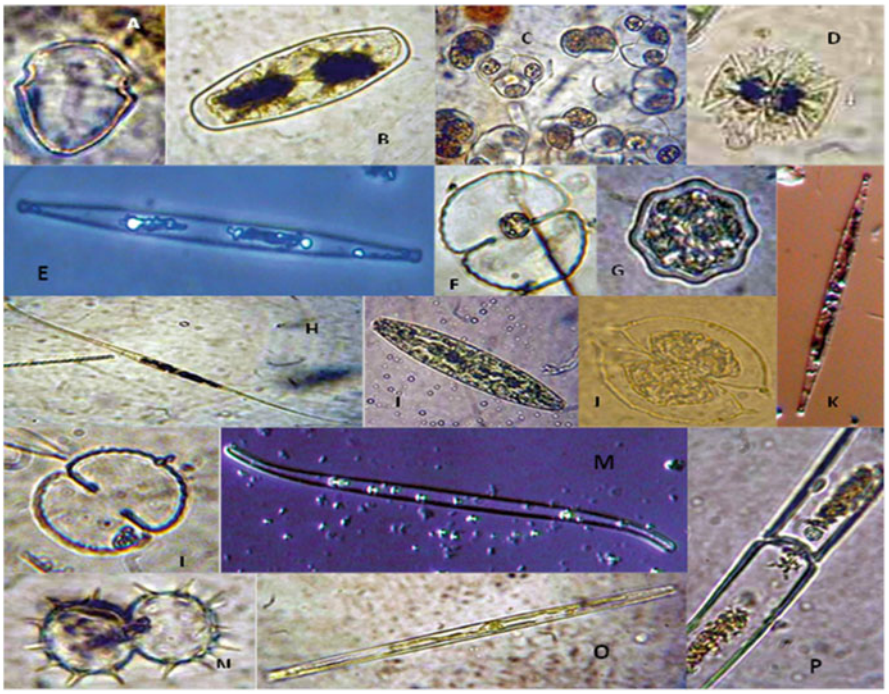

Fig. 3 Some tolerant species to low nutrient concentrations. (**a**): *Gymnodinium sp*, (**b**) *Netrium digitus*, (**c**) *Dityosphaerium pulchellum*, (**d**) *Mycrasterias denticulata*, (**e**) *Nitzchia linearis*, (**f**) *Cosmarium sp2,* (**g**) *Pediastrum piramidale*, (**h**) *Closterium setaceum*, (**i**) *Pleurotenium sp*, (**j**) *Cosmarium sp4*, (**k**) *Nitzchia intermedia*, (**l**) *Cosmarium reniforme*, (**m**) *Stenpterobia curvula*, (**n**): *Xanthidium sp*, (**o**): *Synedra ulna*, (**p**) *Zygnema sp*.

1.2.1.4 Indicators of Average Concentrations

Incidence of Other Variables: pH

The pH is proposed as one of the most important physical variables in the development of the species present in this group (Fig. 4).

The pH favours the growth of *Cymbella naviculiformis*, *Clamydomona sp*., *Encyonema mesianum,* and *Stauroneis sp*., *Eunotia zygodon*, *Eunotia camelus*, *Eunotia serra* (Díaz-Quirós and Rivera-Rondón 2004; Ramírez and Plata-Díaz 2008) which increased in number as the pH values increased, along with the concentrations of nutrients (pH: 5.5–6.1) indicating its capacity to tolerate acidic waters, classifying them as acidophilic, which develop at pH $<$ 7–5.5 (Moresco and Rodrigues 2014; Sabater and Admiraal 2005). Lane et al. (2007) reveal that acid deposition has had significant effects on aquatic communities in many lakes. Taxa numbers are decreasing, but some acid-tolerant taxa may increase significantly, specifically in the analysis of diatoms and other biological groups (e.g., Chrysophytas, etc.). Silva-Benavides (1996) & Duque and Donato (1992) have

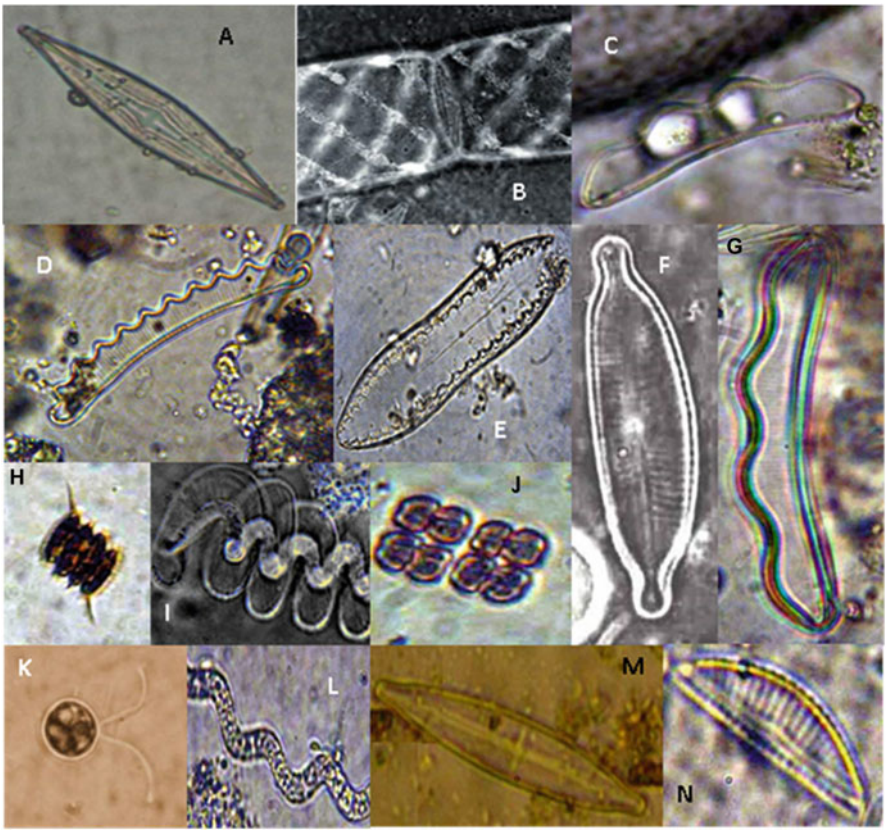

Fig. 4 Some species affected by pH. (**a**) *Anomoeoneis vitrea,* (**b**) *Spirogyra comunis,* (**c**) *Eunotia camelus,* (**d**) *Eunotia serra,* (**e**) *Surirella dydima,* (**f**) *Cymbella naviculiformis* (**g**) *Eunotia zygodon,* (**h**) *Scenodesmus subspicatus,* (**i**) *Oscillatoria* sp., (**j**) *Merismopedia convulata,* (**k**) *Clamidomona* sp., (**l**) *Spirogyra tabiques,* (**m**) *Stauroneis* sp., (**n**) *Encyonema mesianum*

recorded them in environments with enriched nutrients, mainly from agricultural activities (Rodrigues et al. 2005) that correspond to the strong correlation with dissolved oxygen, nitrites, nitrates, aluminium, pH and turbidity.

1.2.1.5 Stenotypes Indicating High Concentrations

Specialized in water with high organic matter content, from the discharge of effluents containing faeces and blood from the slaughtering of pigs (Fig. 5).

Species *Coccinodiscus perforatus*, *Gomphonema gracile*, *Gomphonema parvalum*, *Gomphonema pseudoaugur*, *Gomphonema angustum*; tolerant to pollination and eutrophication, but without losing its stenotic quality, caused by the

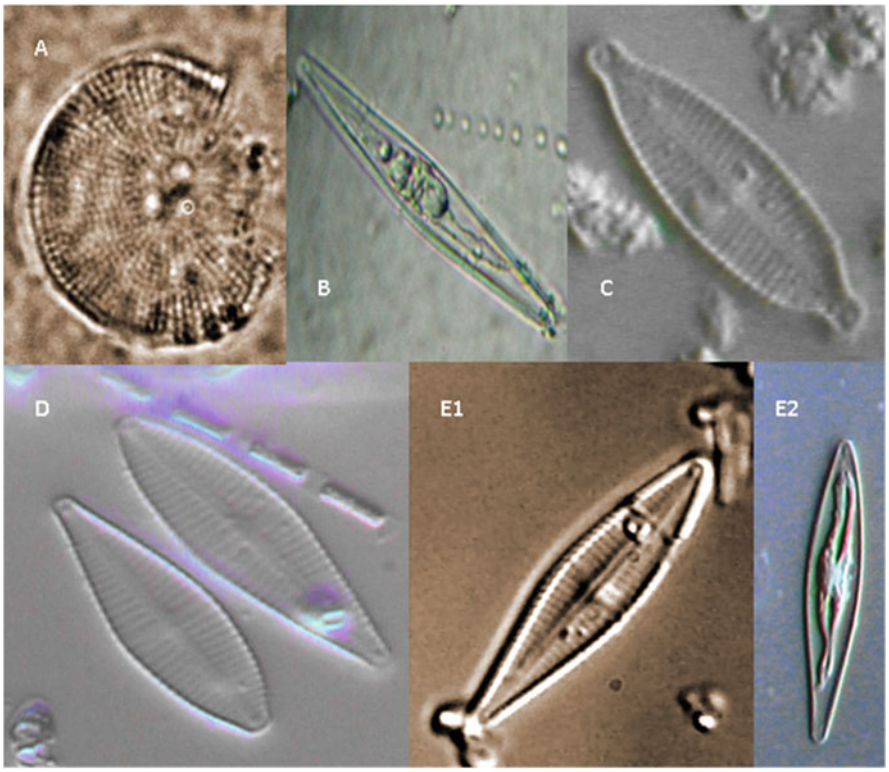

Fig. 5 Some species Stenotypes indicating high concentrations. (**a**) *Coccinodiscus perforatus*, (**b**) *Gomphonema gracile*, (**c**) *Gomphonema parvalum*, (**d**) *Gomphonema pseudoaugur*, (**e1**–**e2**) *Gomphonema angustum*

nitrophilic characteristic (Lobo et al. 2003; Duque and Donato 1992, Ramirez 1992; Silva-Benavides 1996; Oliva-Martínez et al. 2005).

1.2.1.6 Eurioids Indicating Average Concentrations

Incidence of Other Variables: Iron and Chlorides

Blanco et al. (2008) mentioned that iron mineralization can be determined by chloride levels (1–4.6 mg/l) (Fig. 6), a condition that decreases iron mobility, reflected in the high concentrations of iron in the water (0.72–2.4 mg/l) affecting the processes of iron redissolution-precipitation, thus limiting the growth of the species *Eunotia sudetica*, *E. pectinalis* and *E. flexulosa*, *Staurodesmus* sp., *Cosmarium portianum*, *Scenodesmus* sp., *Closterium calosporum*, *Closterium macilentum*, *Cosmarium subcostatum*, *Fragilaria ulna*, *Cymbella lunata*, which for this study can be catalogued with iron indicator species (Jara Londoño and

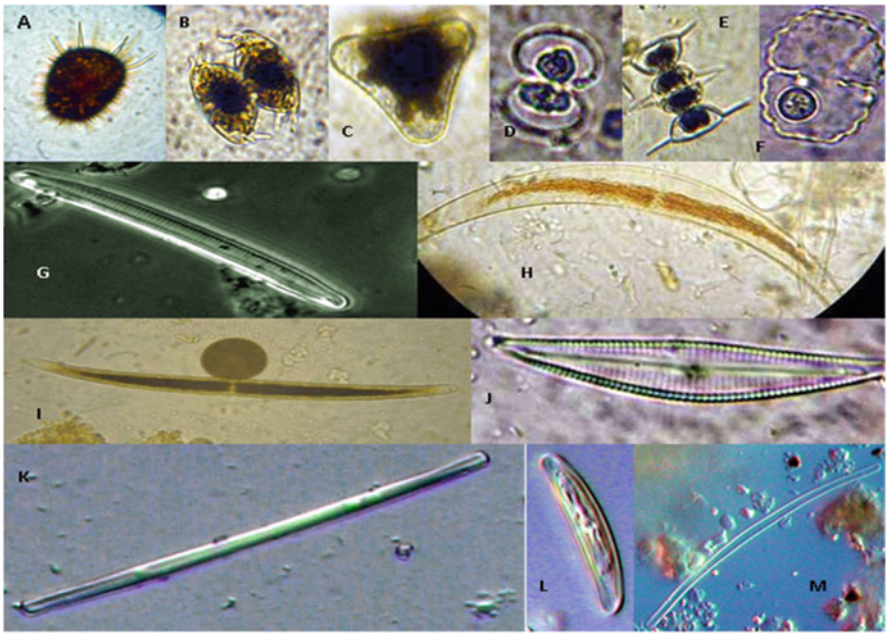

Fig. 6 Some species with incidence of Iron and Chlorides. (**a**) *Trachelomona armata*, (**b**) *Staurodesmus sp1*, (**c**) *Staurastrum sp*, (**d**) *Cosmarium portianum*, (**e**) *Scenodesmus sp2*, (**f**) *Cosmarium subcostatum*, (**g**) *Eunotia sudetica*, (**h**) *Closterium calosporum*, (**i**) *Closterium macilentum*, (**j**) *Cymbella lunata*, (**k**) *Fragilaria ulna*, (**l**) *Eunotia pectinalis*, (**m**) *Eunotia flexulosa*

Pinilla Olarte 2014). *Trachelomona armata, Staurastrum sp,* bioindicators of organic matter concentrations, since they have a rigid cover, or loriga that is frequently impregnated with iron salts (Attoungbre et al. 2019).

1.2.1.7 Tolerant Eurioids

To this group belongs 26 species of generalist type, which presents great ecological amplitude, without following a structure it does not follow any seasonal pattern, but it responds to the hydrological irregularity (Fig. 7) (Rodríguez Fuelantala and Miño Arias 2018).

The most abundant taxa were considered cosmopolitan and tolerant of fluctuations in salinity, temperature, and nutrient concentration (Vilches et al. 2016; Giorgio et al. 1991; Palmer 1969). Many of these have also been described as facultative heterotrophs (Cholnoky 1968; Hellebust and Lewin 1977; Chelf 1990), thus allowing them to reproduce in extreme ecosystems, which in many cases present very marked morphological variability, making a taxonomic determination very difficult (Trobajo et al. 2004).

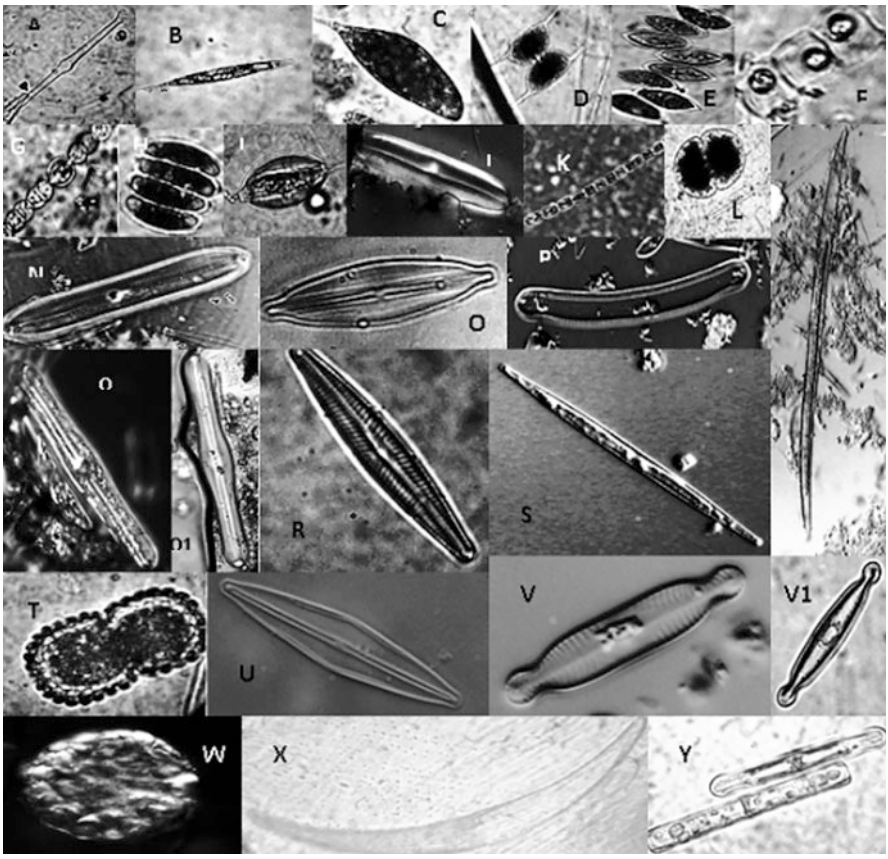

Fig. 7 Some species tolerant eurioids. (**a**): *Tabellaria fenestrata*, (**b**): *Lepocinclis acus*, (**c**) *Euglena gracile,* (**d**): *Staurodesmus sp2*, (**e**) *Scenodesmus alternos*, (**f**) *Schlorella sp*, (**g**) *Nostoc sp*, (**h**) *Scenodesmus sp1*, (**i**) *Cryptomona sp*, (**j**) *Pinnularia divergens*, (**k**) *Anabaena circinalis*, (**l**) *Cosmarium sp3*, (**m**) *Closterium gracile*, (**n**) *Pinnularia viridis,* (**o**) *Navicula gregaria,* (**p**) *Eunotia binularis,* (**q**) *Pinnularia sp*, (**r**) *Navicula cryptocephala*, (**s**) *Nitzchia filiformes*, (**t**) *Cosmarium sp1*, (**u**) *Frustulia rhomboides*, (**v**) *Pinnularia braunaii*, (**w**): *Oocistes sp*, (**x**): *Closterium archerianum*, (**y**) *Pinnularia abaujensis*

Cyanophytes, *Cryptomonas sp*., *Lepocinclis acus*, *Euglena gracil*e have been associated with eutrophication conditions and organic contamination, and in particular with increased phosphorus (Nalewajko and Lean 1980; Frizzo et al. 2004; Conforti and Pérez 2000; Bauer et al. 2012), but the presence of *Nostoc sp.,* species, *Anabaena circinalis*, is constantly and abundantly present during sampling, which can cause "Blooms" and therefore cause problems at the level of the food chain, because those species are little consumed by zooplankton and fish, due to the production of toxins (Kotak et al. 1995; Leigh et al. 2020).

The resistance of *Oocystis sp*. to high concentrations of pesticides (Salazar and Díez de Arango 1987; Zhao et al. 2020), may explain the frequency of this species in

the different study sites. Also, the different species appear when water situations occur that do not only affect nitrogen and phosphorus, but also other substances that the water carries in solution, allowing them to be tolerant of water changes (Quintana i Pou et al. 2006) as in the case of *Tabellaria fenestrata*, *Pinnularia viridis*, *Closterium gracile* and *Closterium archerianum*, which prefer clean or slightly alkaline waters (Duque and Donato 1992; Marella et al. 2020) where the pH with a neutral tendency in some places contributes to its presence.

The presence of cosmopolitan species (*Staurodesmus sp.*, *Cosmarium sp.*, *Scenedesmus sp.*) is favoured by the contributions of allochthonous organic matter, which in this case comes from runoff and the partial degradation of organic matter adjacent to the ponds; coinciding in their eurisustrial and pioneering character, as well as their nitrophilic preferences, that can be identified with a wide ecological tolerance (Jones 2000; Reynolds 2009).

1.3 Bacteria Communities as Bioindicators in Water Environmental

One of the groups that represent the greatest quantity and diversity in the water are bacteria, a large number of which are pathogenic. These bacteria are generally associated with the intestinal tract of humans and some animals and are subsequently eliminated in the faeces of the water, and are therefore the main indicators of water contamination of faecal origin and their presence indicates that the sample was exposed to conditions that could determine the arrival of dangerous microorganisms, allowing the proliferation of pathogenic species (Silva et al. 2004). These include: *Clostridium perfringes*, *Streptococcus*, *Escherichia coli*, *Enterobacter*, *Salmonella*, *Klebsiella pneumoniae*, *Helicobacter pilory*, *Shigella dysenteriae* among others (Aguilar-Maldonado et al. 2018).

1.3.1 Coliforms

Are non-sporulated, rod-shaped, Gram-negative, aerobic and facultative anaerobic bacteria, fermenting lactose with gas production at a temperature of between 35 and 37 °C. Their presence indicates fecal contamination (Madigan et al. 2003). Some coliforms do not inhabit the intestinal tract of mammals, but are found in the soil living as independent saprophytes (González 2012), which is why within this group they can be called fecal coliforms (those that inhabit the digestive tract) and total coliforms (those that do not inhabit the digestive tract). The following organisms are considered to belong to this group:

Escherichia coli, is the most common, is commonly found in human and animal faeces. It belongs to the Enterobacteriaceae family, with a size of about 0.5–2 microns. Most species are harmless, in fact, in the intestinal tract they do not

cause any problem, however, currently, six groups of strains have been described as pathogenic for humans causing urinary tract infections, gastrointestinal infections and meningitis These are: *enterohaemorrhagic E. coli*, *enterotoxigenic E. coli*, *enteropathogenic E. coli*, *enteroinvasive E. coli*, *enteroaggregative E. coli* and *diffuse adherence E. coli* (Ríos Tobón et al. 2017).

Enterobacter aerogenes, is a microorganism that is not associated with the intestine, is opportunistic and affects especially the urinary and respiratory tract. It can be found in soil, water, and the digestive system (González 2012; Khalifa 2020).

Klebsiella pneumoniae, this less common microorganism of fecal origin, is characterized by the formation of biofilms in storage tanks and distribution networks as long as it finds nutrients, appropriate temperature, and little disinfectant (González 2012).

The determination of this group of organisms in surface water, water for irrigation, and water purification systems are very important since they allow the evaluation of the efficiency of a treatment system or deficiencies in the distribution network. It is important to take into account that an indirect method for these organisms to reach people is through food that has been irrigated with untreated water in its production process and that may contain an excessive number of coliforms.

Pseudomonas, are bacillus-shaped, gram-negative, and aerobic organisms. It is characterized by its high resistance to residual chlorine and its capacity to inhibit coliform growth (Ríos Tobón et al. 2017). Therefore, the absence of any organism from the coliform group in a sample of treated water is not synonymous with its suitability for human consumption. Some of the species belonging to this group have been detected in storage tanks, distillers, water distribution networks, therefore, they can be used as indicators of the efficiency of a disinfection process. Within this group are the species *Pseudonoma aeruginosa* used as an indicator for wastewater discharges into other water bodies (González 2012), *Proteus vulgaris,* and *Proteus morganii* which cause strong diarrhoea and infections.

Clostridium, are gram-positive, anaerobic, gas producing organisms, used as indicators of fecal contamination (Hernández-Trujillo et al. 2007) and spore formers which makes them resistant to extreme conditions of drying, pH, temperature, lack of nutrients and disinfection among others (González 2012). One of the representative species of this genus is *Clostridium perfringes*, which is usually associated in water with the microorganisms *Giardia* and *Cryptosporidium*, and is therefore also considered an indicator of the presence of *protozoan cysts* (González 2012). Therefore, it is a good indicator of efficiency in water purification processes, but not for wastewater treatment processes due to its high resistance.

Faecal streptococci, a group of organisms found more in animals than faecal coliforms, are used as indicators of contamination and make it possible to determine whether recent faecal contamination is caused by humans or an animal. Within this group are the *Enterococcus* and Intestinal *Streptococcus*, which are characterized by their high resistance to very basic pH (around 9.6 units), high salt concentrations, and temperatures between 10 and 45 °C. Therefore, because of their resistance and longer survival time compared to coliforms, they are used as an indicator of long-

term fecal contamination in swimming pools, recreational waters, and marine waters (González 2012).

Salmonella and *Shigella,* are Pathogenic microorganisms, anaerobic optional, gram-negative in the form of a bacillus, do not form spores. They cause diseases such as typhoid fever and gastroenteritis (Madigan et al. 2003). The most representative species that are transmitted by fecally contaminated water to humans are *Salmonella tiphy* and *Shigella dysenteriae*. They indicate failures in the treatment process of drinking water, especially in the chlorination process, the mixing of wastewater with drinking water or recreational water, or the lack of a treatment system.

Heterotrophic bacteria are a group of aerobic bacteria of different species that join together to form biofilms, which is why they are more difficult to remove compared to free-living bacteria. Although they are not associated with the cause of any disease, this group of microorganisms indicates deterioration in the quality of water, allowing the efficiency of a treatment system to be evaluated, especially concerning chlorination and the state of the water that is conveyed through distribution networks.

Aeromonas, are bacteria in the form of *bacilli*, Gram-negative, and facultative anaerobes. They can develop at temperatures between 2 and 42 °C and a pH between 5.2 and 9.8 (American Water Works Association [AWWA], 2017). They are found in inland surface water, marine, soil, sewage, sludge, and some foods such as meat and milk. They are pathogenic to humans and some aquatic animals such as fish; the *A. hydrophilic* and *A. sober* species generate extraintestinal and gastrointestinal diseases (González 2012).

These microorganisms can form biofilms, be found in chlorinated or non-chlorinated waters with high organic matter content. There is no relationship between these and the presence of coliforms, and they are used as indicators of the trophic state of a freshwater ecosystem.

1.4 Protozoos Communities as Bioindicators in Water Environmental

Some species belonging to these taxa are parasitic, cysts, or oocysts that are found in contaminated waters and cause acute diarrhoeal diseases that can lead to death, especially in children and the elderly. The presence of these organisms in water for human consumption indicates deficiencies in the processes of filtration, coagulation, and disinfection (oocysts, for example, are resistant to chlorination) in the treatment system, although they are also found in contaminated groundwater or recreational water. The most common pathogenic protozoa are *Giardia intestinalis*, *Cryptosporidium spp*, *Entamoeba histolyca*, *Toxoplasma gondii*, *Encephalitozoon intestinalis*, among others; these can reach humans through a faecal-oral route by ingestion of contaminated water or food and in the case of cysts also through the air.

1.5 Virus Communities as Bioindicators in Water Environmental

This group of organisms are not part of the flora of the intestinal tract of humans and animals but are excreted in the faeces of individuals who have already been affected. At present, viruses or specifically the so-called enteroviruses are the cause of a large number of waterborne diseases. Around 140 viruses have been identified (González 2012) that cause gastrointestinal diseases, a worrying situation from a public health point of view since these pathogenic organisms have considerably increased the number of cases and deaths worldwide. Among the most important are *Poliovirus*, *Rotavirus Adenovirus* (Ríos Tobón et al. 2017), and *Coronavirus* (Tiwari et al. 2020). Within this group, *Polioviruses* and *Phages* (bacteriophages) are used as indicator organisms, the latter being the most accepted because of their easy and quick detection, as well as their correlation with enteric viruses. A specific bacteriophage that indicates good results is the coliphagus, which eliminates the microorganisms of the coliform group and shows the presence of *Salmonell*a, being also more resistant to chlorination (González 2012), which may finally indicate that good water disinfection processes have been performed.

1.6 Conclusion

The use of microorganisms as bio-indicators of water quality has received special interest from the academic community and from the agro-industry for obtaining alternative sources of protein through the use of microalgal, bacterial or animal biomass or their derived metabolites (protein, amino acids, peptides, enzymes, proteases) has become an innovative approach for the development of healthier food products without contaminants and providing a solution to the problems of leaching or dumping. In addition, to use individuals to give a vision of the behaviour and use of environments by man from marine and continental ecosystems, with a view to preservation, regeneration and restoration, obtaining effects on the improvement of the quality of water currents with different uses such as drinking water for domestic use, decontamination of environments that were previously pristine, recovery and generation of spaces for the conservation of species being no longer a perspective of microbiologists but of different disciplines of science.

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Modelling the Migration of Pathogens in Agricultural Settings: From Surface Land to Groundwater Reservoirs

Edgar Marín-Angel, Martín Rivera-Toledo, and Iván R. Quevedo

1 Introduction

Groundwater is an essential natural resource for the preservation of the natural ecosystems, as well as, for human life and health as for many communities it is their sole source to supply all their daily water needs (Oki and Kanae 2006). In brief, groundwater aquifers are formed when freshwater stores in underground saturated geological formations refilled by alluvial recharge. As water begins to seep into the ground, it enters to the unsaturated zone or vadose zone (a mixture of soil, water and air) that ideally acts as a natural filter retaining pollutants suspended before reaching the saturated zone (Stefanakis et al. 2015). It is estimated that water for human consumption comprises of up to underground water (95–96%) and consequently when water scarcity occurs, groundwater exploitation appears the easiest way to fulfill the growing water demands (Oki and Kanae 2006; Giordano 2009). Thus, the increasing reports on contamination of groundwater resources has a direct impact in all living organisms which depend directly or indirectly on the hydrologic cycle of the reservoir (Fig. 1).

Quality degradation in groundwater might occur as result by pollutants from anthropogenic or natural sources (Tufenkji 2007; Bradford et al. 2013). Activities such as aquifer over-abstraction, livestock farming and indiscriminate use of fertilizers in agriculture represent important sources of groundwater contaminants (Stefanakis et al. 2015). Wastewater, livestock farms, agricultural activities and landfill sites are a common source of biological contamination and the transport from the surface to the aquifer as well as their survival capacity of pathogens are key to predict the risk of groundwater pollution (Tufenkji 2007). Anticipating the

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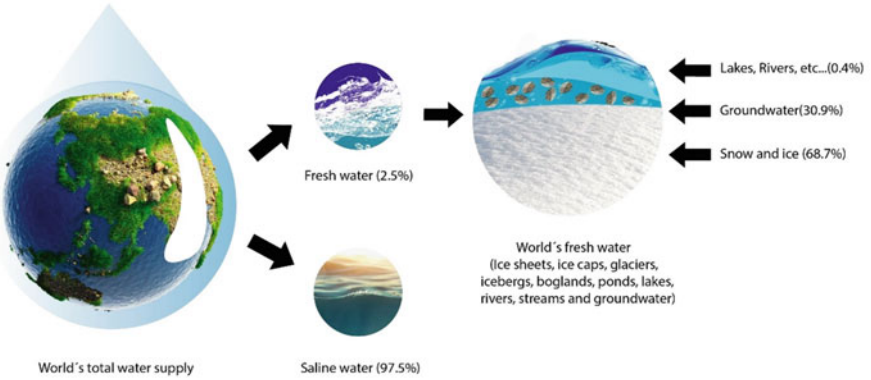

Fig. 1 World's fresh water supply (adapted from Shiklomanov 1993. Chapter "World freshwater resources" from Water in Crisis: A Guide to the World's Fresh Water Resources). *This figure/table is reprinted with permission from source Oxford University Press*

transport and fate of waterborne pathogens (i.e., protozoa, bacteria and viruses) coming from the topsoil are of significant interest in the protection of groundwater supplies (Tufenkji 2007; Bradford et al. 2013; Mawdsley et al. 1995; Smith and Perdek 2004).

Widespread groundwater contamination to drinking water supplies has been assessed using mathematical models of microbial migration in granular porous media and the models have been improved conducting experiments under controlled conditions. In brief, the transport of waterborne pathogens has been assessed experimentally on field-scale investigations or in the laboratory using columns packed with natural granular materials encountered in the soil matrix (Harvey and Harms 2001). Results of both: field-scale studies and laboratory experiments, have been useful to propose predictive models of biocolloid transport using simplified forms of the advection-dispersion equation where the biocolloid removal is controlled by physicochemical filtration (Tufenkji 2007; Bradford et al. 2013).

In this chapter, the most common waterborne pathogens encountered in groundwater reservoirs are presented, as well as, the classical models and governing equations to predict the transport of microbial pathogens in agricultural settings. Moreover, the gains than be obtained by increasing the knowledge in waterborne disease transmission and the importance of linking this fundamental science to the development of appropriate public policy and land use strategy.

2 Waterborne Pathogens: Sources and Impact on Water Quality

Pathogens are infectious microorganisms that cause a disease in the host they invade. Only in the United States it is estimated that groundwater contamination by pathogens cause between 750,000 and 5,000,000 illnesses per year (Macler and Merkle

2000). The pathogens of interest in this section have been restricted to biocolloids (i.e., viruses, bacteria and protozoal cysts) of zoonotic origin and in agricultural settings where improperly treated wastewater or waste leakage is the primary source of contamination.

2.1 *Pathogen Pollution Sources*

The Unites States Environmental Protection Agency ranks agriculture activities (i.e., zoogenic and anthropogenic) as the most probable source of pathogens to water bodies (U.S. Environmental Protection Agency 2010). Pathogens originate from point and nonpoint sources (Fig. 2); point sources are well defined and include wastewater treatment plants effluents, wastewater lagoons, or leaking septic or sewer systems, whereas nonpoint sources (i.e., surface runoff, and soil leaching) are difficult to predict and identify due to the variability and spatial translocation in the environment external to a host (Bowman 2010).

The use of manure and biosolids and their application to agricultural land is also a common strategy to enhance the nutrient levels in the soil (Lapworth et al. 2012), but it might also result in biological contamination of groundwater through surface runoff (Stefanakis et al. 2014).

Same applies in developing countries where wastewater is used to irrigate crops coming from sources with poor quality. The lack of adequate wastewater treatment

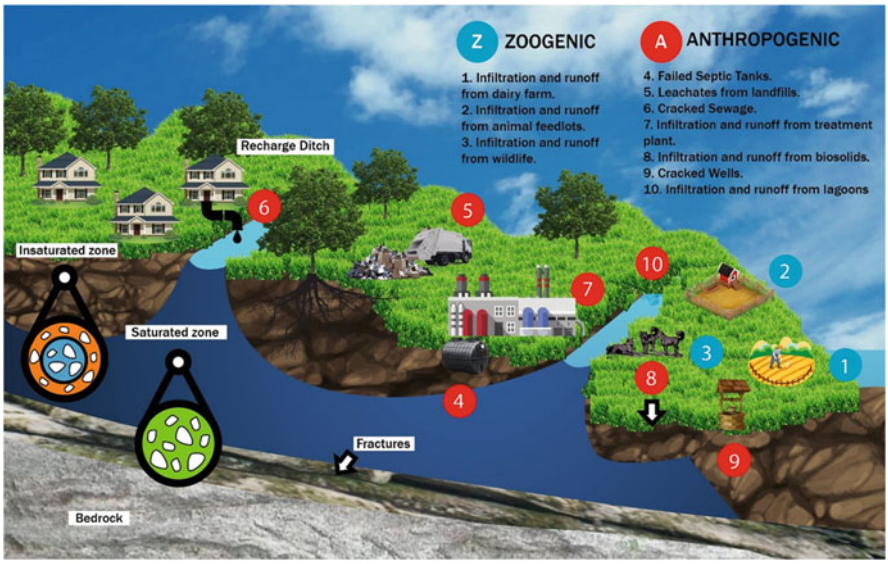

Fig. 2 Sources of waterborne pathogens (adapted from Tufenkji and Emelko 2011). *This figure/table is reprinted with permission from source Elsevier*

processes in the water used for irrigation of crops and replenishment of groundwaters has resulted in outbreaks of gastrointestinal diseases (Chávez et al. 2011). Thus, people involved in the process of aquifer exploitation need to recognize the essential biological aspects of the pathogens to plan and carry out corrective measures, or to foresee actions that can facilitate the mistaken migration of pathogens into the groundwater aquifer.

2.2 Waterborne Pathogens of Concern

Although most of the protozoa, bacterial, and viral pathogens have a common origin in human and animal feces. Only certain pathogens are responsible for most waterborne disease outbreaks recorded (Rosen et al. 2000). In Table 1, the most common waterborne pathogens causing gastrointestinal illness are summarized. These pathogens share the following characteristics:

- Organisms released in large numbers or potentially contagious to humans or animals in small doses.
- Organisms which may remain infectious (active) for long periods of time or are resistant to conventional water treatment processes.
- Certain species of pathogenic bacteria which can multiply outside the host under the appropriate environmental conditions.

However, proper identification of the pathogens present in drinking water is important as not all the disinfection process might be adequate to eliminate or inactivate the pathogens, particularly protozoa in groundwater (Jarrol et al. 1984).

2.2.1 Protozoan Parasites

Protozoa are microscopic, single-celled organisms that belong to the kingdom Protista (Rosen et al. 2000). From the environmental point of view, *Cryptosporidium parvum* and *Giardia* species (abbreviated as sp. in Table 1), are some of the most significant protozoa pathogens from zoonotic origin present in groundwaters (Tufenkji et al. 2004; Tyrrel and Quinton 2003). Both species have notable characteristics: Neither of the parasites can reproduce outside the host, do not effectively inactivate by conventional water treatment methods (i.e., chlorination) and have been identified as responsible of major waterborne disease outbreaks worldwide (Eisenberg et al. 2005). Infections caused by protozoa cause mild to severe diarrhea and severely shorten the life of immunocompromised hosts (Bradford et al. 2013). Human infections can be caused by low concentrations (ingestion of 10 cysts or less for both species) and their elimination from drinking water could be particularly difficult as it requires improved filtration methods (Rosen et al. 2000). Protozoa are a major concern due to their prevalence in the environment associated to their lifecycle (survive in the environment, enter a susceptible host, and develop in the newly

Table 1 Causes of waterborne disease outbreaks causing gastroenteritis 1989–2014 (Rosen et al. 2000; Centers for Disease Control and Prevention 2014)

Organism	Pathogen	Number of outbreaks	Outbreaks associated with	
			Surface water	Ground water
Protozoan parasites	*Giardia sp.*	68	22	
	Cryptosporidium parvum	55	4	21
	Cryptosporidium sp.	114	2	4
	Naegleria flowri	11	1	5
	Avian schistosomes	5	1	
Bacteria with potential for infecting multiple species	*Escherichia coli* O157:H7	49	5	13
	Escherichia coli sp.	4	2	
	Campylobacter jejuni	19	6	10
	Campylobacter sp.	18	2	11
	Salmonella typhimurium	4		4
	Salmonella java	1		
	Salmonella sp.	4		
	Leptospira grippotyphosa	4	1	2
Bacterial infections associated with humans	*Shigella sonnei*	37	2	9
	Shigella flexneri	5		1
	Shigella sp.	3	1	
Human viruses	*Hepatitis A*	5		2
	Norwalk virus	33	2	15
	Norwalk like virus	1		
	Small round structured virus	2	1	1
Acute gastroenteritis	Unidentified cause-many consistent with viral epidemiology	179	19	74

infected host). Protozoan lifecycle relates to development stages where the organism encysts by secreting an outer covering (cyst wall), which is durable. The encysted stage remains until environmental conditions allow the hatching of the cyst (Fig. 3).

Protozoa could be grouped into four main classes according to their motor skills (Table 2) (Rosen et al. 2000):

2.2.2 Bacteria

Bacteria are a group of micro-organisms that lack membrane-bound organelles. Most are unicellular and may exhibit a wide range of geometries (i.e., spherical, rod-shaped, comma-shaped, corkscrew-shaped, spiral-shaped). Their size ranges from 0.5 to 5.0 micrometers, and some of them are motile species with at least one fine hair (flagella) arising from their surface (Fig. 4). Despite most bacteria are beneficial for decomposing dead material and releasing nutrients back into the environment for sustenance of the ecosystem. Certain species (i.e., *Escherichia*

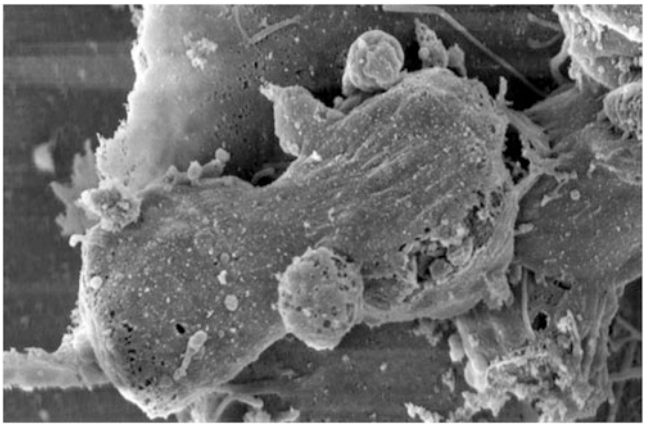

Fig. 3 Morphological characterization of *Cryptosporidium parvum* life-cycle stages in an in vitro model system (Borowski et al. 2009). *This figure/table is reprinted with permission from source Cambridge University Press*

Table 2 Properties of protozoan groups (Rosen et al. 2000)

Class	Description
Mastigophora	Flagellated cells for motility; major pathogens are *Trypanosoma, Leishmania, Trichomonas, Giardia.*
Sarcodina	Amoeba-life motility, Major pathogen is *Entamoeba hystolytica,* the cause of amoebic dysentery.
Sporozoa	All are nonmotile, animal parasites with a complex lifecycle that may require a different host for asexual and sexual reproduction. Do not engulf particular matter. Major pathogens are *Plasmodium* (malaria), *Toxoplasma* (toxoplasmosis), and *Cryptosporidium.*
Ciliophora	Cells with short, hair-like projections called cilia. Major pathogen is *Balantidium coli,* which causes dysentery in humans

coli O157:H7, *Salmonella spp.*, *Campylobacter spp*., *Listeria monocytogenes*) also present in manure are of high concern due to their characteristics (Table 3) (Bowman and Bowman 2009). *E. coli O157:H7* is a potentially deadly bacteria *that* has been isolated from animal feces (e.g., cattle, white-tailed deer, sheep, dogs, horses, birds) and might cause bloody diarrhea and dehydration in humans (Rosen et al. 2000). *Campylobacter* and *Salmonella* are some of the most common causes of bacterial gastroenteritis (Cotruvo et al. 2004). For instance, *Campylobacter jejuni* is a human pathogen commonly encountered in surface water, stream sediments, sewage effluents, and agricultural settings (Bradford et al. 2013). Whereas animals used for food production (e.g. poultry, meat, dairy products, eggs, seafood) are common carriers of *Salmonella* known to cause between 800,000 and four million human infections each year only in the U.S. (Rosen et al. 2000).

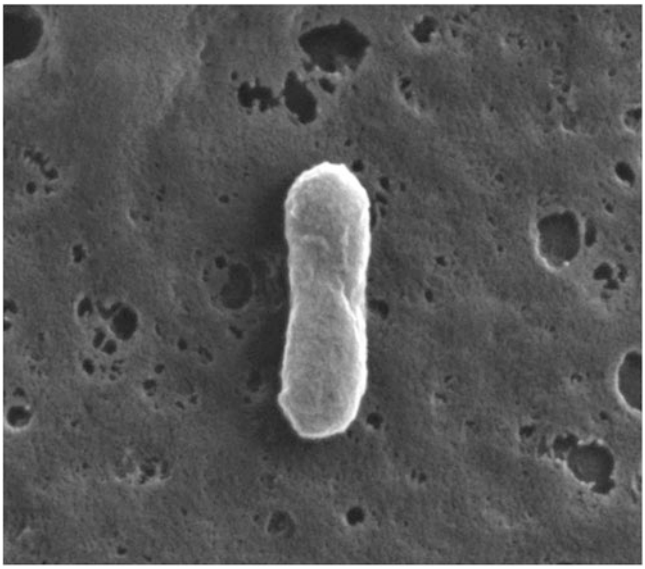

Fig. 4 SEM image of *E. coli K-12* treated by g-C_3N_4/TiO_2 hybrid photocatalysts under visible light irradiation at 1 hour (Li et al. 2015). *This figure/table is reprinted with permission from source Oxford Pergamon Press*

Table 3 General characteristics of pathogenic bacteria (Bowman and Bowman 2009)

Characteristic	Description
Persistence	*Traditional.* Responsible for persistent waterborne outbreaks. *Emerging.* Relatively recently identified as pathogenic species
Virulence	*General.* Particularly virulent and responsible for catastrophic loses of life. *Opportunistic.* Not pose a risk to healthy adult humans, but individuals with weakened immune system are susceptible
Morphology	*Size:* Range of μm; *Form*: Bacillus, coccus, spirillum, spirochete, etc.; *Breathing:* Aerobic/anaerobic*; Staining:* Gram (+) or Gram (−), *Motility:* Own ways of moving; *Fermentative and Sporulative*
Related diseases	Parts of body (tissues, cells, organs) where can cause insufficiencies or dangerous complications in wounds or illness. Deadly toxins capable of producing.
Reservoirs	Areas where are found or parts of body (animal and human) where can grow and reproduce indiscriminately.
Transmission methods	Forms of reach human and animal entrails. From exposure to consumption of contaminated water, sludge, food, or contact with contaminated feces or untreated wastewater.

2.2.3 Viruses

Viruses are the smallest known agents that might infect a great variety of hosts (i.e., plants, animals, bacteria). A source of pathogenic viruses for groundwater bodies

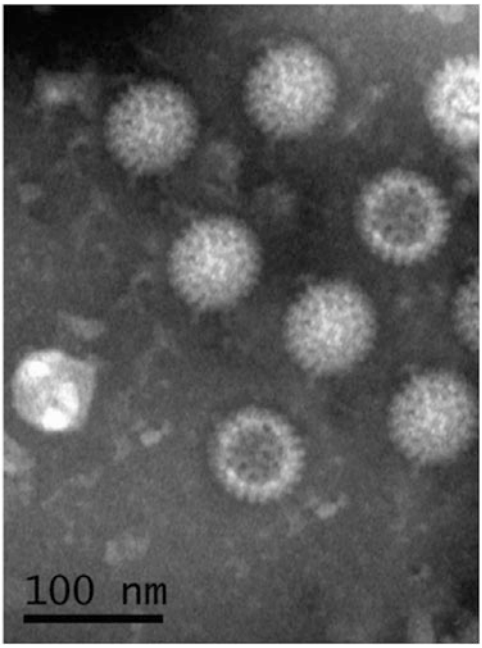

Fig. 5 Cryo-EM reconstructions of double-layered rotavirus virus-like particles. (Li et al. 2014). *This figure/table is reprinted with permission from source Elsevier*

may be runoff from manure applied on the surface or septic tanks. When viruses are outside host cells, they exist as DNA or RNA surrounded by a protein coat or capsid ranging from 20 to 300 nanometers (Fig. 5). Their classification depends on their arrangement and the type of genetic material: double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RNA, and retroviruses, a unique type of single-stranded RNA virus (Table 4). A wide variety of viruses are potentially zoonotic such as, *hepatitis E* virus (Meng et al., 2002), *Norovirus sp.* (Norwalk-like) the predominant cause of viral foodborne gastroenteritis (Mattison et al. 2007). Moreover, recent investigations have encountered that of infectious viral particles of *Coronavirus* (SARS-CoV, MERS-CoV, and SARS-CoV2) are present in human feces raising the alarm of potential infection for people exposed to untreated sewage/wastewater (Amoah et al. 2020).

2.3 Control of Groundwater Pollution

Agriculture, sewage and septic systems have been identified as the main source of biological contamination to groundwater aquifers (U.S. Environmental Protection Agency 1996). Figure 6 illustrates the source and potential entrance routes for

Table 4 Partial list of types of human viruses associated with animal feces (Adapted from Rosen et al. 2000; Amoah et al. 2020)

Group	Type[a]	Water relationship
Adenovirus	DS DNA	Isolated from sewage, rivers, lakes, groundwater, drinking water and recreational bathing water (swimming pools are a major source)
Astrovirus	SS RNA	Feces, therefore, inferred in sewage
Caliciviruses	SS RNA	Feces, therefore, inferred in sewage
Hepatitis	SS RNA	Sewage and polluted rivers
Norwalk-like	SS RNA	Municipal drinking water contaminated with sewage, recreational bathing
Rotavirus	DS RNA	Sewage, rivers and lakes, estuarine and marine water
Coronavirus	SS RNA	Feces, therefore, inferred in sewage

[a]*DS* double-strained, *SS* single-strained

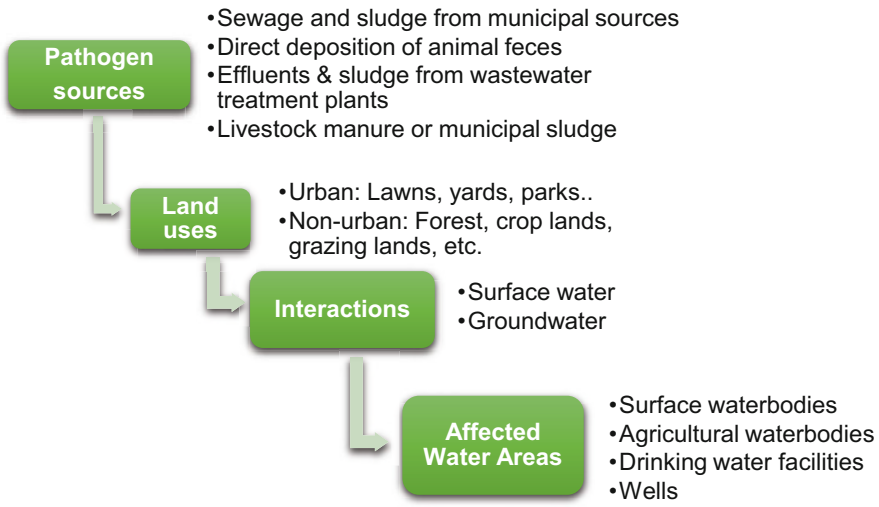

Fig. 6 Potential routes of exposure for waterborne pathogens in aquatic environments

waterborne pathogens and as inferred water inlets play an important role on the transport of biocolloids through the soil matrix. Rainfall entrances wash microorganisms from feces or vegetation surfaces and directs them into the soil or along the land surface. Surface water and groundwater bodies contaminated by pathogens are often a health risk to consumers as it must receive extensive treatment for drinking use. Being the goal to minimize the number of pathogens to a level lower than that needed to cause infections.

Table 5 Survival times of pathogens in agricultural media (Rosen et al. 2000)

Pathogen	Survival Times			
	Animal Feces	Soil	Water	Plants
Protozoan cysts (general maxima)			>180 days	>5 days (dry conditions)
Salmonella sp.	200 days	>150 days	~16 days	
E. coli	200 days	>200 days	~35 days	
Viruses (general maxima)		>1 year		~60 days

Nonetheless the quantitative determination of pathogen concentrations in complex environmental samples (manure, soil, waterbodies or sediment) is challenging and costly due to the need of specialized equipment and personnel. Specialized analytical techniques are required to determine specific pathogen concentrations and the results are most of the times semiquantitative due to method-specific limitations. Thus, environmental laws are regularly based on specific microorganisms such as total or fecal coliform (FC), Enterococcus, and E. coli. (Bradford et al. 2013).

Nonetheless, certain generalities can be made about survival of pathogens that may be useful in the implementation of control practices. Table 5 shows an estimate of survival times of pathogens in freshwater, sewage, on crops, and in soils.

Pathogen viability outside a host is a function of the environmental conditions (temperature, humidity and soil type). For instance, pathogens contained in manure have longer survival times, particularly in anaerobic conditions. Certain bacteria (*Salmonella typhimurium, E. coli, Pseudomonas aeruginosa, Staphylococcus aureus)* in cattle manure, may remain viable for up to 15 days in aerated liquid excrement, but up to 39 days under non-aerated conditions (Strauch 1987).

Agricultural soil rich in natural organic matter and clay content may effectively retain biocolloids (i.e., viruses, bacteria, or small protozoa) due to electrostatic interactions. Yet larger pathogens (i.e., large bacteria, protozoa or large macroparasites known as helminths) might filter through the narrower pores contained in the soil matrix (straining). For the specific case of viruses, the mobility might be enhanced through permeable soils or with a high content of coarse elements (Mawdsley et al. 1995).

The transport of bacteria and viruses is enhanced in the saturated area and the filtered water provides the mechanism for downward movement as it will be discussed in detail in the following section.

3 Biocolloid Mobilization and Fate through the Soil Matrix

Soil is the natural matrix that covers the land which bulk consists of four main dispersed components: minerals, organic matter, water and air. Biocolloid transport can be seen as a heterogeneous transport process which involves more than one

phase. Natural waters serve as habitat for different biocolloids (i.e., bacteria, viruses and protozoa), a portion of which might come from fecal material and are transported from the surface to aqueous interfaces (e.g., groundwater reservoirs).

Certain mechanisms may act independently or simultaneously to drive the migration and fate of biocolloids, these processes are classified as: (a) transport in liquid phase, (b) transfer between the liquid phase and the solid phase because the attachment or detachment may be carried out, and (c) inactivation, grazing or death (Molnar et al. 2015). Some biocolloids could also exhibit self-propelled transport and these have been classified as pushers or pullers if whether the dipole is extensile or contractile, respectively. For example, *Escherichia coli* or *Bacillus subtilis* are pushers, whereas *Chlamydomonas reinhardtii* is considered a puller. From the hydrodynamic point of view, the microorganisms can modify the fluid properties, for instance, the pushers decrease the viscosity while the puller increases it. This behavior creates a non-equilibrium system then the equilibrium adsorption mechanism is considered inappropriate for predicting the microbiological transport (Tufenkji 2007).

Mathematical modelling is a way to represent a system to investigate the response of the system under certain conditions, as well to predict the behavior of the system in the future. It is a powerful tool used in different studies to predict pathogen transport and retention in groundwater systems (Bradford and Harvey 2017), the deposition dynamics of rod-shaped colloids during transport in porous media under favorable conditions (Li and Ma 2019), the modeling effect of cover condition and soil type on rotavirus transport detected in agricultural runoff (Bhattarai et al. 2017). Decision makers may use models to predict the behavior of any system prior to implement a project or a remediation scheme. It is easy to see that is a simple and economic solution compared to project establishment and management (Fig. 7).

3.1 Mechanisms and Governing Equations in Colloidal Filtration Theory

For all scientists interested in protecting the ecosystem health and sustainability in agricultural settings, the migration of pathogens or biocolloids through porous media is of high awareness. Most of the published works are dedicated to predicting how far these pathogens travel through natural soils or other porous media (Bitton and Harvey 1992; Harvey 1997; Taylor et al. 2004; Sen 2011; Bradford and Harvey 2017). Mathematical modelling is possible since their transport behavior can be linked to key microbe physicochemical properties. If the main purpose is to make a prediction and facilitate the understanding on pathogens transport, then it must understand the classical colloid filtration theory. In classical theory of colloid transport in porous media is common to use the advection–dispersion partial differential Eq. 1 for the continuum scale approach.

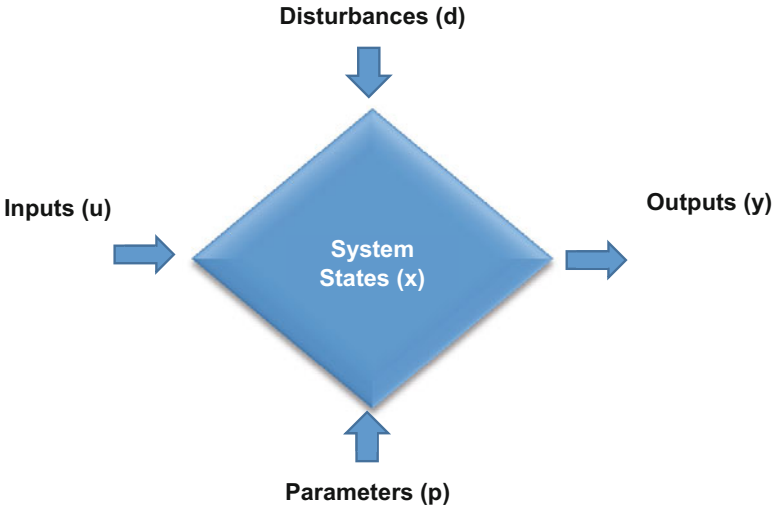

Fig. 7 General system for modeling approach (adapted from Gani et al. 2012)

$$\frac{\partial C}{\partial t}\varepsilon = -\nabla \cdot (vC) + \nabla \cdot (D_d \nabla C)e - \rho_b \frac{\partial S}{\partial t} \tag{1}$$

where the left hand is the rate of increase of the colloids concentration per unit volume, the right hand are the net rate of addition by advection, dispersion and the mass of colloids retained, respectively, per unit volume and t is the time, ε is the medium's porosity, v is the pore velocity vector, D_d is the dispersion tensor, ρ is the bulk density of the medium, and S is the mass of colloids. The colloids transport is given by the driving forces such a colloid concentration, velocity, pressure and other thermodynamic quantities (Stewart et al. 2014).

The dominant mass transport process is the advection and many of the dissolved contaminants move in the groundwater by this mechanism (Domenico and Schwartz 1998). For a simple system when only advection acts the dynamic concentration profiles can be calculated using the pore velocity or seepage velocity.

For the second term on Eq. 1, there are two processes that lead to dispersion and those are molecular diffusion and mechanical dispersion. The first one is the movement of molecules from gradients of high concentration to ones of lower concentration and it is given by the Fick's first law of diffusion (Eq. 2). For the particular case of these changes in concentration with time and the spatial concentration gradients given by the called Fick's second law of diffusion (Bird et al. 2014) or sometimes simply the diffusion equation (Eq. 3).

$$J = -D_d \nabla C \tag{2}$$

$$\frac{\partial C}{\partial t} = D_d \nabla C \tag{3}$$

It has been observed the molecular diffusion is important at very low flow velocities and negligible for modeling contaminant transport. Pollutants are carried with the flow by advection and they do not all move at the groundwater average pore velocity. This is the mechanical dispersion represents the spreading of contaminant substances due to spatial variation of groundwater flow velocity. In this case, the heterogeneity and the tortuosity of the porous medium are very important for the distribution of groundwater velocities within pores. Yao et al. (1971) established the standard conceptual model for filtration process where the colloids are transported into the porous media surfaces, which is comprised of pore spaces and collectors called void and fluid phases, respectively. This collector's surface is on the order of a few nanometers where the colloids are transported via advection, dispersion and diffusion. Colloids arrive to this zone either when any kind of forces: gravity or Brownian or inertial forces or convective from streamline toward the collector (Goltz and Huang 2017; Rajagopalan and Tien 1976). It is important to highlighting that the Brownian motion modifies the individual pathways and affect how far it may be transport through porous system. The interactions between groundwater and collector's surface are complex, however the trajectories of the colloids in a flowing fluid can be calculated using a combination of deterministic external and hydrodynamic forces and stochastic forces given by Brownian forces (Coffey and Kalmykov 2004; Nelson and Ginn 2011). Moreover, there are some unique and important characteristics of colloids which influence transport behavior, for instance, the diffusivity is inversely proportional to fluid viscosity because the magnitude of Brownian motion increases with decreasing particle size. Another one, when the specific surface area is high the colloids are highly reactive because the particle size decreases and the total area available increasing its reactivity and capacity for surface absorption (Klaine et al. 2008).

The deep bed filtration is a process where some colloids are hold on by the collector and other one continues travelling and it is described as a first-order kinetic process with the retention parameter from Eq. 1 which one are rewritten as Eq. 4.

$$\rho_b \frac{\partial S}{\partial t} = k_{ret} C_e \tag{4}$$

In this case, k_{ret} is a kinetic retention parameter and it depends on (1) the colloid–colloid forces of interaction, (2) the properties of the porous medium, (3) the flow regime, and (4) the chemical conditions. Many researchers have been interested in developing mechanistic approaches to calculate k_{ret} without relying on empirically determined parameters (Yao et al. 1971; Rajagopalan and Tien 1976; Paraskeva et al. 1991; Tufenkji and Elimelech 2004; Long and Hilpert 2009; Ma and Johnson 2010; Nelson and Ginn 2011; Messina et al. 2015; Kamai et al. 2015). From the point of view of filtration theory, there are two main approach formulations. First, a mechanistic model proposes to solve a force-torque balance to estimate the trajectory

of a colloid near a collector and in second place, a correlation equation which combines physicochemical properties and dimensionless quantities that arise in connection with experimental data and these summarize the behavior of the mechanistic model.

For the idealized case when a sphere geometry is considered as a sand grain in a granular medium packed bed, k_{ret} is calculated using Eq. 5 (Tufenkji and Elimelech 2004). In this equation, η_0 is derived from colloid filtration theory and denotes the fraction of colloids approaching a collector that are attached.

$$k_{ret} = \frac{3(1-f)\eta_0}{2d_c} \tag{5}$$

As it has been mentioned above the accuracy of prediction of k_{ret} depends of the colloid–collector surface forces, if these are attractive then we have favorable attachment conditions, however there are scenarios where these forces are repulsive and attachment conditions are unfavorable. Unfortunately, in environmental systems predominate the unfavorable attachment conditions and geologic media and natural colloids carry negative electrostatic charges (Molnar et al. 2019) which yield repulsive forces of interaction. Elimelech and Omelia (1990) have proposed to develop and additional parameter, α, called attachment efficiency, which expresses the fraction of colloids that contact the collector and are retained. This attachment efficiency (α) is included in the Eq. 5 as follows (Eq. 6):

$$\eta = \eta_0 \cdot \alpha \tag{6}$$

Where η is the fraction of colloids attached near surface of the collector. Currently, α remains a fitting parameter because there are not successful methods to predict a priori the impact factors. Some of these efforts to predict colloid and microbe attachment efficiency equations are given by Eqs. 7–11. All of these correlations are written on physical and chemical mechanism into the dimensionless numbers as follows: $N_{PE} = d_c\, U/D$, $N_{LO} = H/9\pi\mu {a_p}^2 U$, $N_R = d_p/d_c$, $N_G = 2\, {a_p}^2\, g\, (\rho_p - \rho_f)/9\mu U$, where N_{PE}, N_{LO}, N_R, and N_G are the Péclet, London-van der Waals, interception, and the gravitation dimensionless numbers, respectively. These are defined with the physical properties given by U as the velocity, D is the diffusion coefficient, H is the Hamaker constant, μ is the fluid viscosity, a_p and d_p (L) are radius and diameter of particle, respectively, g is gravitational force, and ρ_p and ρ_f are the particle and fluid density, respectively.

$$\eta \approx \gamma^2 \left(P_1 A_s^{1/3} N_{PE}^{P_2} + P_3 A_s N_{LO}^{P_4} N_R^{P_5} + P_6 A_s N_G^{P_7} N_R^{P_8} \right) \tag{7}$$

This Eq. 7 (Rajagopalan and Tien 1976) is for favorable conditions this is when $\alpha = 1$, and diffusion, interception and sedimentation mechanisms are considered for the first, second and third terms, respectively. We must highlight that the principle of superposition applied to add the diffusion with interception and sedimentation

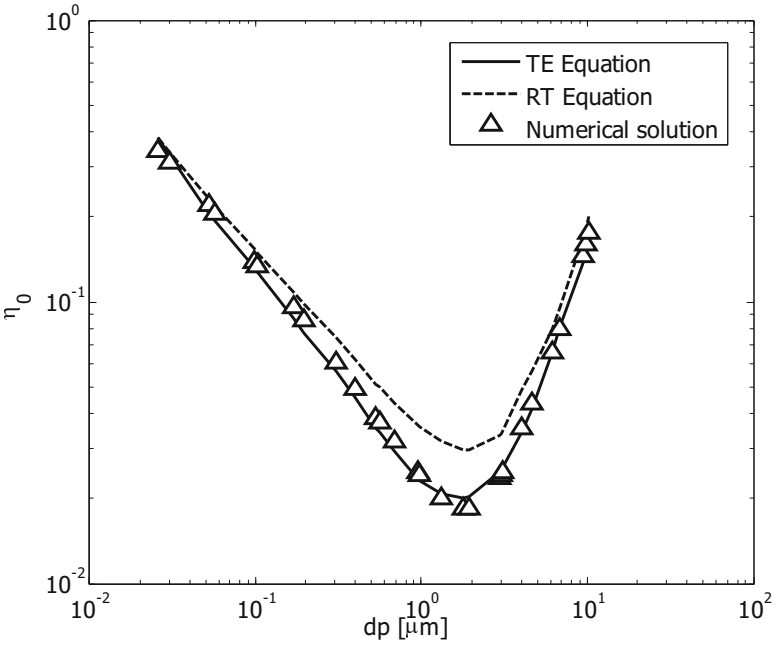

Fig. 8 Comparison of collection efficiency when pathogen diameter changes (Tufenkji and Elimelech 2004). *This figure/table is reprinted with permission from source American Chemical Society (ACS)*

correlation. To improve this equation Tufenkji and Elimelech (2004) solved by numerical methods all of forces for getting their single-collector contact efficiency equation (Eq. 8). In this case, they combined the van der Waals forces and thermal energy into the van der Waals number. Then, they transform the filtration Péclet number to a particle Péclet number.

$$\eta \approx 2.4A_s^{1/3}N_R^{-0.081}N_{PE}^{-0.715}N_{vdW}^{0.052} + 0.55A_sN_A^{0.125}N_R^{1.675} + 0.22A_sN_G^{1.11}N_R^{-0.24}N_{vdW}^{0.053} \quad (8)$$

In Fig. 8, the collection efficiencies of the Rajagopalan-Tien and Tufenkji-Elimelech models and numerical solution of advection-dispersion equation are shown. As it can be seen, these collection efficiencies vary and depend on model assumptions and the processes that are accounted for each model as they have been described at this moment. Models' comparison against numerical solution shows that these prediction capabilities seem modest for this theoretical simple case.

Long and Hilpert (2009) proposed to simulate the diffusion in a 3-D model for adding their result on Tufenkji and Elimelech's model (Eq. 9).

$$\eta \approx (15.56 \pm 0.21)\frac{(1-\varepsilon)^3}{\varepsilon^2} \times \left(N_R^{0.19\pm0.03} N_{PE}^{-0.65\pm0.023} + 0.55 A_s N_A^{0.125} N_R^{1.675} + 0.22 A_s N_G^{-0.24} N_{vdW}^{0.053}\right) \quad (9)$$

In order to consider the collision frequency for hydrodynamic retardation and variable diffusion toward surfaces, Ma and Johnson (2010) used the hemisphere model to get Eq. 10.

$$\eta \approx \gamma^2 \left(2.3 A_s^{1/3} N_R^{-0.080} N_{PE}^{-0.65} N_A^{0.052} + 0.55 A_s N_A^{0.15} N_R^{1.8} + 0.2 N_G^{1.1} N_R^{-0.10} N_A^{0.053} N_{PE}^{0.053}\right) \quad (10)$$

Then Nelson and Ginn (2011) included the small particles and low velocity flow for high gravity and low Péclet numbers expressed for Eq. 11.

$$\eta \approx \gamma^2 \left(2.4 A_s^{1/3} \left(\frac{N_{PE}}{N_{PE}+16}\right)^{0.75} N_{PE}^{-0.68} N_{LO}^{-0.015} N_{Gi}^{0.8} + A_s N_{LO}^{1/8} N_R^{15/8} + 0.7\left(\frac{N_{Gi}}{N_{Gi}+0.9}\right) N_G N_R^{-0.05}\right) \quad (11)$$

Finally, Kamai et al. (2015) reported a new correlation equation for favorable conditions for colloid filtration. They significantly improved the match between theory and experimental data (Eq. 12):

$$\eta \approx \gamma^2 \left(1.354 A_s^{1/3} N_{PE}^{-2/3} + 9.487 \times 10^{-2} A_s N_{LO}^{0.1196} N_R^{1.383} + 1.211 \times 10^{-3} A_s N_G^{1.045} N_R^{-0.5573}\right) \quad (12)$$

In Sect. 3, we have mention that soil consists of three dispersed phases: solid, liquid and gas. Now we are going to describe another important mechanism, sorption. The sorption processes consider (1) adsorption, where the microbial or pathogen is attached to the solid surface as a result of electrostatic attraction; (2) chemisorption, where these species are assured to the surface because a chemical reaction; (3) absorption, the compound break up inside the solid. The common way to model sorption is by assuming that this process is faster than other fate and transport processes. Then it can be modeled as an equilibrium process and simpler model is a linear relationship between concentrations in the dissolved and solid phases. Nonlinear models are also used to describe this mechanism.

3.2 Techniques to Solve Deterministic Models

A mathematical model is a representation of some aspects of a non-mathematical system, and it plays an important role to increase the transport and fate of pathogens in environmental settings (e.g. retention, release, aggregation, survival, etc). These models can be used for several purposes such as simulation, control, steady-state and dynamic process optimization, etc. To improve the numerical robustness of the model, and to reduce potential difficulties related to the numerical integration and optimization, the model must be properly scaled. As this mathematical model may be of different types and form then different techniques are needed to solve them. Some of this kind of model may be expressed as: (1) algebraic systems of equations for steady-state models; (2) ordinary differential algebraic systems of equations for dynamic models and (3) partial differential algebraic systems of equations for distributed models when the time and the distance or surface or volume is considered. All modeling case study can be cast into a systems structure as they are shown in Fig. 9. Here the system includes the principal entities within the boundary and the primary mechanisms that operating in it. States are variables (x), which indicate the condition of the system at a point in time and space. They represent the amount of microorganism concentrations in the system. Inputs variables (u) are associated with properties of the system that can be chosen to affect the behavior of the system and are usually known, for instance, the water flow or any initial concentration. Disturbances (d) reflect the effects on the system that are normally uncontrolled, for example, the temperature ambient conditions. Parameters (p) are associated with geometric, physical or chemical properties, for example, the viscosity. Finally, outputs (y) reflect internal properties of the system and are often linked to the states of the system, for example, the collector efficiencies.

Continuum mathematical models to assess the mobility of biocolloids average the subsurface water flow that occur at the pore-scale over a control volume to obtain effective model parameters. This approach numerically solves the flow and convective diffusive transport equations subject to the appropriate boundary conditions. Eq. 1 is nonlinear because of the nonlinear character of hydraulic function given by the velocity. Therefore, it can be solved by numerical methods only, for instance, the method of finite elements, finite differences or finite volumes. Some of these partial differential and algebraic systems may be readily solved by standard solvers such as those in Matlab™ (Shampine 2005). There are some tools designed to guide and help the model developer user to perform the modeling tasks in a systematic and efficient manner. In this respect, the user thinks about the modeling problem definition and the model data requirements are assigned by the model developer, while model solution, model verification, and model validation are assigned to be performed by the computer. Some of these tools are: (1) HYDRUS™, a computer program widely used to simulate water and solute transport in granular porous media (Šimůnek 2021); (2) SWAP (Soil, Water, Atmosphere and Plant), this simulates transport of water, solutes and heat in unsaturated/saturated soils (Kroes et al. 2017); (3) The MACRO model is one-dimensional, dual-permeability model for water flow

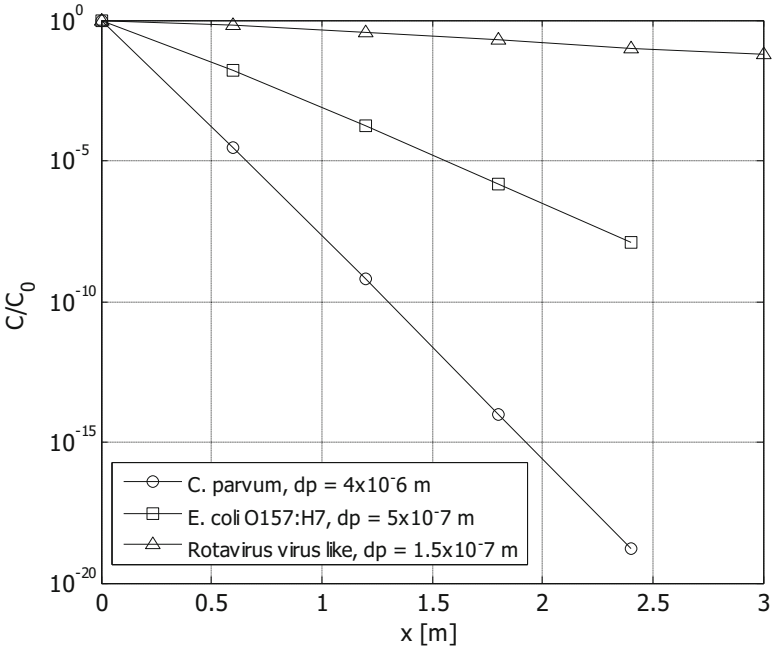

Fig. 9 Dimensionless pathogen concentrations along the porous media for three organisms: *Cryptosporidium Parvum, E. coli O157:H7* and *Rotavirus* virus like

and reactive or non-reactive solute transport in vertical soil environment. The model is used to estimate the effects of macropore flow and contaminant transport in structured soils. Its main application is in risk assessment for groundwater and surface waters (Jarvis and Larsbo 2012); (4) COMSOL Multiphysics™, this software has the governing equations for flow and transport, and these are solved using the finite-element method. This software has a module for model transport phenomena in porous media, which includes multiphase transport, fracture flow, etc. For improving the accurate models, it considers nonisothermal flows in porous media, effective properties for multicomponent systems, porelasticity, and transport of moisture and chemical species. Table 6 shows some studies about migration of pathogens from surface land to groundwater.

As mentioned in Sect. 2.1, pathogen transport studies have been developed as indicators of fecal contamination and bacterial impairment of watersheds. To improve the existing models to predict their transport and assessment of pathogen water contamination, these models must include thermodynamic properties, mechanisms and processes between pathogen and porous media. Marin et al. (2019) have modeled a simple case for vertical flow in a homogeneous unsaturated zone where it is assumed to be uniform at a given depth, around 3 m, and takes place in a continuum system when *Cryptosporidium parvum, E. coli O157:H7* and *Rotavirus* are transported. In Fig. 9, Marin shows the simulation results for dimensionless pathogen concentration along the porous media. The Eq. 1 was solved numerically

Table 6 Deterministic approaches used to predict biocolloid transport

Model equations	Pathogens transported	Methods for solving the model	References
Ordinary differential equations	Non-specific	Analytic solution (Laplace transform)	Goltz and Huang (2017)
	Non-specific	Numerical methods (finite differences in MATLAB™)	Goltz and Huang (2017)
	Bacteria	Numerical method (fourth-order Runge-Kutta)	Nelson and Ginn (2011)
Partial differential equations	*Cryptosporidiumparvum oocyst*	Analytic solution	Bradford and Harvey (2017)
	Non-specific	Analytic solution (Laplace transform)	Goltz and Huang (2017)
	Non-specific	Numerical methods (finite differences in 1,2-D MATLAB™)	Goltz and Huang (2017)
	Rotavirus	Method of characteristics	Bhattarai et al. (2017)
	Several viruses	Finite element method embedded in HYDRUS™	Torkzaban et al. (2019)
	Escherichia coli	COMSOL MULTIPHYSICS™	Engström and Liu (2015)

and the initial and boundary conditions considered the initial pathogen content in the surface soil. As it can be seen, in this figure the size of pathogens plays an important role in their migration, in this case, *Rotavirus* dimensionless concentration decreases slower than *Cryptosporidium parvum* because the first one size is one thousand smaller the second one. This behavior suggests that could lead to estimate of the health risks associated with groundwater contamination close to sources of manure and fecal material.

3.3 Deviations from Colloidal Filtration Theory (CFT)

The characterization of physicochemical interactions is the key in determining the behavior of biocolloids in aqueous suspensions. The well-known DLVO theory expresses the total interaction between two surfaces as the sum of the van der Waals, electrostatic interactions and other forces such as born repulsion and acid base interactions in the extended form of DLVO (XDLVO) approach (Elimelech et al. 1995). The magnitudes of different interaction energies depend on particle size and thus, in the overall interaction energy, size effects have consequences with regards to the stability of particle dispersion and particle mobility.

However, DLVO theory describing particle behavior in aqueous media relies on the assumption that the fluid is a uniform, structureless medium that is well described in terms of bulk properties, such as density, viscosity and dielectric constant. As

illustrated for particles with dimensions similar to ions and complex composition of any real system it might not be possible to simply calculate and sum all of the interactions that occur at the molecular scale. Experimental techniques will need to be used to determine if the stability of different biocolloids and surfaces behave according to the DLVO model (Bradford et al. 2013; Tufenkji 2007).

4 Recommended Measures to Prevent Biological Contamination in Groundwater Aquifers

The main objective should be the prevention of pathogen spreading and several simple practices might reduce the risk of soil and groundwater pollution. Some of those will be briefly exposed below:

- Manure solids are known to contain pathogens that can be spread in hydrologically sensitive areas, however composting temperatures might eliminate most microorganisms. It has been proved that mixing the entire waste mass uniformly and keeping the temperature above 55 °C for at least 3 days is an effective practice to accomplish the goal (U.S. Environmental Protection Agency 1996).
- Vegetative treatment areas are designed land spaces (e.g., perennial grass) that might help to control the transport of microorganisms. Scale studies with rainfall events simulated and natural rain have reported the utility of this approach to create a feasible barrier for groundwater contamination reducing the concentration of coliforms and protozoa in wastewater coming from agricultural settings (Koelsch et al. 2006).
- In a similar fashion, constructed wetlands are cost-effective engineered systems that use natural functions vegetation, soil, and organisms to treat water and wastewater. Although this technology has not been designed specifically for pathogen removal it acts a biofilter eliminating pathogenic bacteria by physicochemical filtration, ultraviolet radiation, natural die-off, or predation by zooplankton in a more controlled as compared to vegetative treatment areas (Stefanakis et al. 2014).
- Engineered systems, such as Riverbank Filtration could also be used to minimize pathogen movement. Riverbank filtration involves river water filtered through riverbanks before passing to the groundwater table. The water quality in the quality is improved by a series of physical filtration and chemical and biological reactions to diminish the pathogen load in surface water and groundwater. When compared with water treatment plants, the efficacy of this technique has proven to be successful to remove oocysts and cysts of *Cryptosporidium sp.* and *Escherichia coli*. (Hu et al. 2016).

5 Conclusions

This chapter summarizes the most important aspects to prevent groundwater contaminated by waterborne pathogens. However, despite decades of different mathematical approaches to model the pathogen migration through the soil matrix, substantial future research opportunities exist. From the colloidal chemistry point of view, there are some of them with self-propelled and they have interesting transport properties. This behavior can modify fluid rheological properties and reflect non-Newtonian responses. Simulation-model development has evolved quickly over the past 25 years and the improved strategies enable the processing of great amounts of data in a short-time. There are many factors that influence the transport properties for pathogens and biocolloids in heterogenous system such as soil. These simulators may be giving us the opportunity to exchange of knowledge and experience among the different disciplines involved in soil physics, hydrology, chemistry, biology and we may cooperate and contribute in more complex problem on pathogens transport from porous media to groundwater.

Microbial pathogens from manure and municipal wastewater are the most common sources of waterborne pathogens in agricultural settings. Thus, different alternatives should be used to decrease the number of viable pathogens that could contaminate the groundwater aquifer. A better understanding of the fundamental principal to prevent pathogen mobility in surface and subsurface water is required to prevent the polluting of our groundwater reserves.

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Current Insights into Phylloplane Fungal Species Diversity in the Western Ghats and Its Perspective

Sabeena Aliyarukunju, Biju Haridas, and Shiburaj Sugathan

Abstract The tropical forests on earth play a vital role in ensuring highest biological diversity and the Western Ghats represents as one of the great diversity centers of the world. One important part of microbial diversity is phylloplane fungi, which includes rusts, smuts, powdery mildews, black mildews, tar spots, etc. Phylloplane fungi (leaf infecting micro fungi) are ectophytic obligate biotrophs infecting a wide range of flowering plants and produce black colonies on the leaf surface. They are distributed in the tropical and subtropical regions of the world. Black mildews shows more abundance than other groups of fungi. These black colony-forming organisms belong to different taxonomic groups, *viz. Meliolales*, *Asterinales,* Schiffnerulales, Hyphomycetous fungi, etc. This chapter deals with the diversity, distribution and host range of phylloplane fungi with respect to black mildews in the Western Ghats.

Keywords Phylloplane fungi · Black mildews · Obligate parasites · Host specificity · Western Ghats

1 Introduction

The fungal kingdom girdles remarkable biological diversity, with members covering a wide array of lifestyles, forms, habitats, and sizes. Fungi are sister to animals and include thousands of ancestries, from the yeasts to mushroom-forming macro-fungi, bread molds to phytopathogenic rusts and smuts, and more or less noticeable critters with exciting morphologies. The fungi world provides an exciting and almost endless source of biological diversity, which is a rich source for exploitation

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(Manoharachary et al. 2005). One-third of the global fungal diversity is existing in India. Out of 2.2 to 3.8 million fungi, only 8% are characterized until now (Hawksworth and Lucking 2017). Fungi have imparative ecological roles, most notably in disintegration processes, but are likewise involved in critical interactions with plants and animals making symbiotic or parasitic associations (Alexopoulos et al. 1996).

Furthermore, fungal assemblages are in very species-rich groups, making the full documentation of fungal diversity in targeted sites a chiefly challenging task. The crucial roles fungi play in the conservation and functioning of ecosystems; such documentation is often united with functional perspectives to recognize fungal ecology. Advances in molecular techniques have made a base for the increase in fungal diversity studies. The fast progress of next-generation sequencing and proteomic technologies promises further progress towards a more thorough understanding of fungal diversity and metabolic patterns.

Currently our information on fungal diversity is limited and complicates a valuation of fungal species' conservation grade and has delayed the development of conservation tools and efforts. Moreover, the lack of expediting and suitable methods to document fungal demographics has made it extremely challenging to fit fungi into the efforts to currently established IUCN conservation categories. Though, recent strenuous efforts have brought fungi to conservation debates, such as the newly created Society for the Conservation of Fungi. By tradition, fungi were classified based on morphological, chemical, and anatomical characters, mainly related with spore-bearing structures (McLaughlin et al. 2009). However, molecular approaches shown repeated trait evolution and, therefore, the dominance of artificial groupings in these traditional classifications.

Fungi have their distinctive cell biology and life cycle and also play critical roles in broader eco-systems. They might show endophytic, parasitic, saprotrophic, and symbiotic associations with plants (Zeilinger et al. 2015; Jayawardena et al. 2018). Fungi are the leading phytopathogens, resulting in a major economic loss by decreasing the quantity and quality of crops (Almeida et al. 2019; Chethana et al. 2019). Many phytopathogenic fungi and interrelated organisms have been studied over the decades to understand its systematic position, developmental biology, pathogenesis, interactions with host plants, and control of their infections (Hyde et al. 2014; Jayawardena et al. 2019a, 2019b). However, due to taxonomic conflicts, correct identification of these pathogens has not been an easy task. Most of the biotrophic pathogens cannot be cultured from their host plants as they develop within the living plant cells. Many plant pathogenic fungi fail to produce sexual morphs in laboratory conditions resulting in a meagre understanding of phytopathogenic fungi (Hyde et al. 2018).

Host plant species, age, micro- and macro-habitats, changes to environmental system, and phyllotaxy are influencing the variety and distribution of phylloplane microbial groups (Kinkel 1997; Talley et al. 2002; Behrendt et al. 2004). Plant genera growing in close vicinity have their characteristic mycota (Kinkel 1997), which is habituated by the nature of the plant exudates, microclimate, and other

members of the mycota (Goodman et al. 1986; Lucas and Knights 1987; Osono and Mori 2004).

The plant-associated fungi colonize either above or below ground level. Corticolous fungi, phylloplane fungi, and Wood rot fungi are above ground communities. Below ground communities include rhizosphere, mycorrhizae, and soil fungi. Soil harbors the fungi belonging to all the major taxonomic groups, outnumbering the other microbes and their biomass (Anderson and Domsch 1978; Kjoller and Struwe 1982).

Fungi exploit right diverse substrates based on their nutritional strategy. Saprobes flourish in soil, water, and decaying animal and plant tissues while the parasitic fungi and mutualistic symbionts nourish on living organisms. Plant associated microorganisms colonize plants from the phyllosphere to the rhizosphere. The phyllosphere includes the leaf surface and its inside (Carroll et al. 1977). The epiphytic microorganisms are found on the exteriors, while endophytes colonize the host organ's interior tissues.

2 Rhizosphere-Endophytes-Phylloplane

The rhizosphere, the micro-habitat immediately surrounding the root/rootlet, is a dynamic zone for microbial activities. Roots of living plants support growth and create a unique habitat for microbes. The influence of roots on soil microorganisms starts instantly after seed germination; it rises as the plant grows and extents the maximum when the plant reaches its vegetative growth (Katznelson et al. 1962). Due to this, the microbial community also will be varied at every stage of plant growth. The roots release a considerable amount of carbohydrates in the form of exudation, and other root debris and dead cells are added to it during root growth. This microhabitat supports a particular population of microflora and microfauna in the rhizosphere resulting in a unique microbial equilibrium. The rhizosphere's symbiotic fungi are ecologically and agriculturally crucial for providing organically bound nitrogen, phosphorus, and other elements. The nutrients released by the plant roots vary at different stages of growth. However, the microbes compete for their food and essential elements (Bowen and Rovira 1999).

The term mycorrhiza denotes fungi-root association. About 95% of vascular plants require mycorrhizal association for their survival. AM fungi are microscopic soil fungi, simultaneously inhabit the roots and rhizosphere, and spread out over several centimeters in branched filaments. This filamentous network spread inside and outside the roots permits the plant to access minerals and water essential for its nutrition. In return, the plant provides sugars, amino acids, and vitamins essential for the fungi (Harley and Smith, 1983). Due to this mutual existence, the plant acquires better growth, increased resistance to environmental hassles such as drought, cold, and root pathogens (Elsen et al. 2001, Sylvia and Williams, 1992). Arbuscular mycorrhizal fungi are non-specific with the majority of vascular plants (Gianinazzi and Gianinazzi-Pearson, 1986). Their services to plants are enormous. They absorb

and translocate minerals and water to plants; produce enzymes, auxins, vitamins, and cytokinins, increase the rootlets' size and longevity; and protect the root system from pathogens by secreting chemical compounds.

Endophytes are those microorganisms inhabiting inside the plant tissues without causing any obvious or detectable symptoms (Bacon and White 2000). In plants, both epiphytic and endophytic myco-populations growing only fragments of millimeters apart. However, they vary significantly in their structure (Santamaría and Bayman 2005). Endophytic microorganisms are known for a long time; their significance becomes obvious only more recently when it was shown that they play specific roles, such as guarding the host-plants against insects and diseases. Unlike mycorrhizal fungi that colonize plant roots and grow into the rhizosphere, endophytes live fully within plant tissues. They may grow inside roots, stems, and leaves, emerging to sporulate at plant or host-tissue senescence (Stone et al. 2004). It has been found that many endophytic microorganisms are an exceptional source of bioactive metabolites.

Endophytes comprise a diverse group of microorganisms that differ in symbiotic and ecological functions. Endophytic fungi can profoundly impact plants' survival and suitability in all terrestrial ecosystems and, therefore, likely play a major role in plant biogeography, evolution, and community structure. As more studies are to be performed to assess host range, colonization and transmission patterns, and symbiotic or ecological function of different endophytes, our perception on endophytes will change. Endophytic fungi seem to be metabolically more advanced than soil fungi concerning bioactive compounds (Schulz et al. 2002) and produce distinctive bioactive metabolites (Mitchell et al. 2008).

Fungal endophytes are a polyphyletic group of highly varied, primarily ascomycetous fungi. They occur inside asymptomatic tissues of vascular and nonvascular plants, distributed from the arctic to the tropics, and from agricultural fields to tropical forests. Their cryptic lifestyle, ubiquity, and abundance within individual plants, together with emerging evidence of their often-overlooked ecological importance, have inspired growing enthusiasm concerning these little known fungi over the past four decades.

Those fungi occur as endophytes in a wide range of plant hosts and diverse geographic locations and mostly dominate the endophyte assemblage of the plant tissue such as leaf so that the overall endophyte diversity in the ecosystem is depressed (Pandey et al. 2003; Murali et al. 2006; Sakalidis et al. 2011; Suryanarayanan et al. 2011; GovindaRajulu et al. 2013; Sudhakara Reddy et al. 2016). Suryanarayanan (2011) opines that because of the cosmopolitan existence and wide geographic distribution of some endophytes, the ratio of generalists and specialists (host-specific) endophytes have to be considered while using endophytes as an indicator group for global fungal diversity.

3 Significant Groups of Phylloplane Fungi

Pioneering taxonomical works of Carl Linnaeus, Christian Hendrik Persoon, and Elias Magnus Fries in the 18th and 19th centuries, fungi have been classified based on their morphology (e.g., characteristics such as spore color or microscopic features) or physiological characteristics. With the help of molecular phylogenetic analyses, a comprehensive phylogenetic classification of the kingdom Fungi by Hibbett et al. (2007). The classification includes one subkingdom (Dikarya: include the Ascomycota and the Basidiomycota), seven phyla, namely Microsporidia, Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, and Basidiomycota. Among these, Ascomycota and Basidiomycota, most species-rich and familiar groups are coming under subkingdom Dikarya. The major phyla or otherwise called fungi divisions, have been classified mainly based on characteristics of their sexual reproductive structures.

3.1 Ascomycetes

Mycelium septate and the fruiting body are asci with ascospores. Ascus (plural: asci) is a sac-like structure comprising a definite number of ascospores (Gr. Askos = goat skin, sac + spore = seed, spore), whih are formed by karyogamy and meiosis. The ascomycetes fungi have two distinct reproductive phases; 1) ascus or sexual phase, and conidial or asexual stage. The ascus phase commonly known as ascigerous or perfect state or teleomorph. The asexual phase is mitotic state and is known as an imperfect state. The classification of ascomycetes is grounded on its perfect state. It is interesting to notice that almost all fungi belonging to Deuteromycetes (fungi imperfecti) are yet to discover those sexual stages or would have either lost their ascus stages due to their evolutionary development. The asci may be unitunicate or bitunicate (Tunica refers to layers). Based on the type of tunia they are grouped into Pyrenomycetes (unitunicate) and Loculoascomycetes (bitunicate). The asci may be naked (asci born but are not enclosed in a fruiting body) or inside different types of fruiting bodies (ascocarps or ascomata) such as apothecium (bowl-shaped), perithecium (a spherical or flask-like form with a pore opening), and cleistothecium (no opening).

3.2 Basidiomycetes

The majority of the fungi belonging to this group are mushrooms, toadstools, rusts, and smuts. Basidiomycota is the second-largest fungal group, representing more than 30,000 described species (Carris et al. 2012). These fungi differ from other groups in the formation of the basidiospores on sterigmata produced from the

basidium. Since the Basidiospores are uninucleate and haploid, a definite number of spores are produced on each basidium. They are produced as a result of plasmogamy, karyogamy, and meiosis. Basidiomycota is characterized by the production of basidiospores on a club-shaped structure called a basidium. Dolipore septum and the clamp connection in the mycelium is also unique in this group. Ustilaginomycotina, Pucciniomycotina, and Agaricomycotina are three sub-phyla in this division (Blackwell et al. 2006, Carris et al. 2012). Pucciniomycotina and Ustilaginomycotina are composed mostly of plant-parasitic species, known as rust and smut fungi, respectively, characterized by thick-walled teliospores. The rust fungi Pucciniomycotina produces as many as five distinct types of spores such as spermatia, aeciospores, urediniospores, teliospores, and basidiospores. Agaricomycotina includes fungi producing various types of fruiting bodies such as mushrooms, shelf fungi, stink-horns, jelly fungi, and bird's nest fungi.

3.3 Fungi Imperfecti(Deuteromycetes)

Fungi imperfecti are the conidial stages of Ascomycetes or Basidiomycetes, whose sexual or perfect stages have not been discovered or no longer exist. These are the fungi that have an incomplete life cycle. The perfect or sexual phase is either not discovered or is lacking.

3.4 Basic Structures

Except for one-celled species, most fungi are composed of thread-like tubular filaments called hyphae. A reasonably rigid wall surrounds each hypha, usually made of chitin, which also forms insects' exoskeletons. Hyphae that are partitioned by dividing cross walls are called septate hyphae, and hyphae without cross walls are called nonseptate hyphae. Fungal cells contain cytoplasm, which is a mixture of internal fluids and nutrients. Cytoplasm flows freely within the hyphae, providing nutrients wherever they are required.

Most fungi grow as hyphae: cylindrical, thread-like structures, 2–10 μm in diameter, and several centimeters in length. Hyphae can be either septate or coenocytic: septate hyphae are divided into compartments separated by cross walls with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized. Septa have pores that allow cytoplasm, organelles, and sometimes nuclei to pass through. Coenocytic hyphae are essentially multinucleate supercells. Many species have developed specialized hyphal structures for nutrient uptake from living hosts; examples include haustoria in plant-parasitic species of most fungal phyla and arbuscules of several mycorrhizal fungi, which penetrate the host cells to consume nutrients. A fungus as single cells in aquatic environments is adapted for the efficient extraction of nutrients. Hyphae are specifically adapted for growth on

concrete surfaces and to invade substrates and tissues. They can exert large penetrative mechanical forces; for example, the plant pathogen forms an appressorium structure, which evolved to puncture plant tissues. Hyphae grow by elongation at the tips and branching to form an interwoven mat known as the mycelium. As the mycelium develops, it may produce large fruiting bodies or other structures that contain reproductive spores. Fruiting bodies are often the most visible structure of a fungus, usually growing above the soil or other surfaces so that air currents or other mechanisms can disperse the spores. In contrast, the mycelium is usually hidden beneath the plant's surface, animal, or other material it is decomposing. For example, a mushroom mycelium is typically buried beneath the soil surface, while its fruiting body, the familiar umbrella-shaped structure, sprouts from the ground (Hosagoudar 2010).

4 How They Reproduce and Multiply

Fungal reproduction is complex, reflecting the differences in lifestyles and genetic makeup within this kingdom of organisms. For example, the reproduction may occur in two well-differentiated stages within a species' life cycle, the teleomorph, and the anamorph. Environmental conditions trigger genetically determined developmental states that lead to the creation of specialized structures for sexual or asexual reproduction. These structures aid reproduction by efficiently dispersing spores or spore-containing propagules.

Fungi reproduce both sexually and asexually by the formation of spores. Many fungi produce more than one type of spores as part of their lifecycle. The asexual spores result from mitosis (mitospores), and the sexual spores involving meiosis (meiospores). Zygospores, Ascospores, and Basidiospores are Meiospores. Ascospores are formed inside a sac-like structure called an ascus, typically eight per ascus that is the unique character of Ascomycetes. Asci are produced in a wide range of fruiting bodies called ascomata. Apothecium, cliestothecium, perithecium, and pseudothecium are the different types of ascomata in ascomycetes. Basidiospores are formed on a basidium, which is a specialized club-shaped cell and is characteristic of basidiomycetes. There are large conspicuous fruiting bodies for the production of spores by various fungi. Mushrooms, puffball, and shelf fungi are the best examples of large fruiting bodies of basidiomycetes. In zygomycetes fungi, two sexually compatible strains must conjugate (mate) in order for sexual reproduction to occur. The terms 'anamorph' and 'teleomorph' convey the asexual and sexual reproductive stages, respectively, of a particular fungus. How meiospores are formed reflects the evolutionary history and classification of fungi.

The parasitic fungi are commonly named as Rusts, Smuts, Powdery mildews, Downy mildews, Black mildews, Sooty molds, etc. based on the mode of their infection, nature, and symptoms produced **Parasites acquire nutrients partly or entirely**. Phylloplane fungi are those associated with the aerial part of plants, especially leaves. Phylloplane fungi (leaf infecting microfungi) are ectophytic

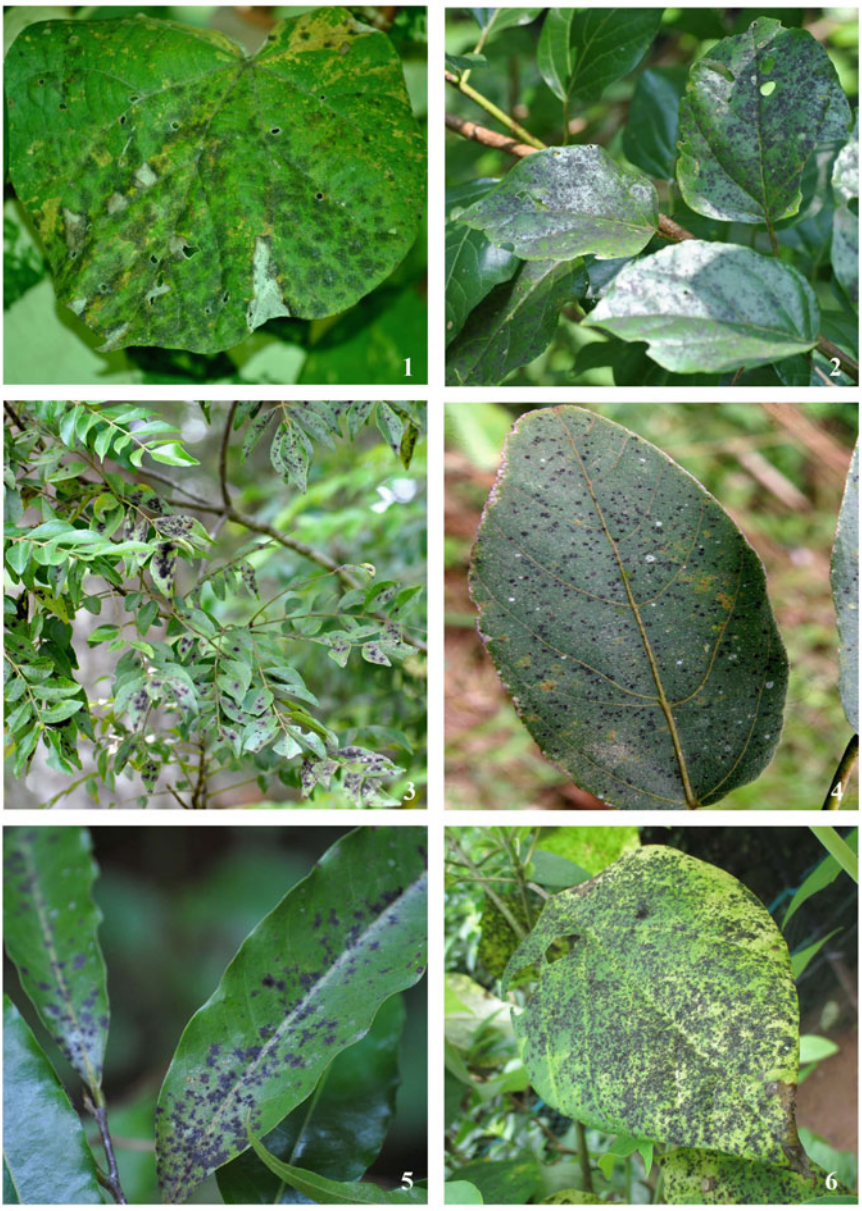
obligate biotrophs infecting a wide range of flowering plants and produce black colonies on the leaf surface (Fig. 1). They are distributed in the tropical and subtropical regions of the world. Black mildews are leaf dwelling fungi and obligate biotrophs on mostly leaves and other parts of the plant like stem, petiole, and in some cases, on fruits. These fungi occur a month or two after the monsoon. However, they occur scantily during summer; they are abundant during the winter season. The Microbiology research team lead by Dr. Hosagouder VB made efforts to study the phylloplane fungi in the Western Ghats of peninsular India. It has taken momentum in the taxonomic account and thorough knowledge and systematic study (http://www.drvbhosagoudar-bioresearch.org/index.php/publications).

5 Obligate and Facultative

Traditionally, the fungi are considered heterotrophs, organisms that rely solely on other organisms' carbon for metabolism. Based on their nutrition, fungi are grouped into three, namely, parasites, saprophytes, and symbionts. Parasites obtain their required nutrients partly or entirely from the living tissues of host organism. The parasites which are exlusively dependent on living organisms are called obligate parasites. The parasites which feed both on living and nonliving organisms are called facultative or opportunistic parasites or saprophytes. Hence, the obligate parasites have to adjust and modify themselves with their partners for their existence. Certain obligate parasites producing toxins or enzymes, that destroy the host tissues and help them to absorbe nutrients from the hosts. These are called necrotrophs (necros-death, trophy-feeding). While certain parasites absorb nutrients from the living tissues, without harming them, by the specialized organs like appressoria, haustoria, or nutritive hyphae. These are called biotrophs (bio-life, trophy feeding). Black mildew fungi are strictly obligate biotrophs and must interact with living plant cells for growth and reproduction.

6 Host Specificity

Host specificity is the relationship in which a fungus is restricted to a single host or a group of related species but does not occur in other unrelated plants in the same habitat (Holliday 1998). According to May (1988, 1991), host specificity among plant-associated organisms (insects, fungi) would be less in tropical forests owing to the high diversity of the plants (hosts). The high diversity decreases their density (contiguity). It reduces the possibility of the plant-associated species to find a specific host - a situation that might lead to the evolution of organisms with the ability to infect a wide range of plants (Basset et al. 1996; Novotny et al. 2006). In accordance with this is the fact that many plant-associated fungal guilds such as



1.***Irenopsis molleriana*** (Wint.) Stev. on *Hibiscus teliaceus*; 2. ***Asterina pusilla*** Sydow & Sydow on *Premna corymbosa*; 3. ***Irenopsis murrayae*** Hosag. & Rajkumar on *Murraya koenigii;* 4. ***Irenopsis benguetensis*** Stev. & Rold on *Ficus exasperata*; 5. ***Prillieuxina polyalthiae*** Hosag. & Abraham on *Polyalthia longifolia*; 6. ***Meliola clerodendricola*** Henn. on *Gmelina arborea*.

Fig. 1 Plant Parts infected with Phylloplane fungi

rotting wood fungi (Parfitt et al. 2010), ectomycorrhizal fungi (Tedersoo et al. 2010), and arbuscular mycorrhizal fungi (Zhao et al. 2003) do not exhibit host specificity.

Black mildew fungi infect numerous plant species and are widely distributed in the tropics and subtropics. As these fungi are strictly obligate biotrophs and must interact with living plant cells for growth and reproduction, they usually host-specific or have a very narrow host range (Hansford 1961; Hosagoudar 1996). These fungi are host specific because they must evade and overcome the host's specific resistance factors. The resistance factors may be a physical barrier, noxious chemicals produced by the host either before or after the response to the infection, and environmental fators. The general answer for constraining the speciation to the host family level was that the pathogens were adapted to a particular species, genus, or family. The plants within that taxon have similar types of defense systems and the fungi has evolved mehanisms to overcome it. Thus, attempts to grow species of Meliolales in pure culture have not been successful, making their DNA extraction challenging (Hansford 1961, Hosagoudar 1996, Vitoria et al. 2010). The assumption of host-specificity means this group highly diverse, and it is imperative to identify the host before attempting to identify a fungal collection (Zeng et al. 2017).

Black mildew fungi are believed to be host-specific, and numerous new species were introduced based on host association (Hansford 1961, Rodríguez Justavino and Piepenbring 2007). They parasitize only indigenous plants of any particular area and are limited to a narrow range of host plants that rarely extends to more than one family of phanerogamic hosts (Ciferri 1954, Hansford 1961). It is also authoritative to identify the host, at least family level, before attempting to identify a new collection (Hansford 1961, Hirata 1972, Saenz & Taylor 1999, Hongsanan et al. 2015). These fungi' species concept is based on the individual host plants and the fungal morphological characters.

- Identification & Confirmation
- Identification of host plants by referring to literature, herbaria, and consulting plant taxonomists.
- Identification of Genera of Black Mildews, by preparing permanent slides and observing micro-morphological characters.
- The digital formula is applied as per Beeli formula in the case of Meliolales.
- Confirmation is done by referring to monographs and consulting subject experts.

7 Digital Formula (Applicable to Both Armatellaceae and Meliolaceae)

Beeli (1920) introduced an eight-digit formula for summarizing key diagnostic characters in a numerical code that has proved an appropriate and valuable tool in expediting the identification of fungi belonging to the pyrenomycete family Meliolaceae. Account of the individual collection identified up to generic level, and it is being converted to a digital formula for classifying fungi up to species level.

Beeli formula consists of eight digits. The the first four digits, before the stop (left side to the stop), represent the morphological characters like ascospore septation, presence or absence, and the nature of the perithecial setae or appendages, presence or absence and the nature of the mycelial setae and the arrangement of appressoria, respectively. The second four digits, after the stop, represent the measurements such as length and breadth of ascospores, the diameter of perithecia, and the length of mycelial setae, respectively. The species having both simple and dentate setae is denoted by ⅓ and the species having straight and uncinate setae are designated as ½ . The Beeli formula is modified here to accommodate the genus *Armatella*, which is having 1–2 septate ascospores. An example of digital formula (Beeli formula) is given below.

1. Morphology (first four digits from left)
   (a) Normal septation of ascospores
      - 1-septate
      - 3-septate
      - 4-septate

   (b) Perithecia
      - Without setae or appendages
      - With larviform, horizontally striated appendages
      - With uncinate or coiled setae
      - With straight setae

   (c) Mycelial setae (often on perithecia and from subiculam)
      - Absent
      - Simple
      - Simple, entire, uncinate or coiled
      - Dentate or shortly furcate (up to 30 μm)
      - Branched (branches more than 30 μm)

   (d) Appressoria
      - Alternate or unilateral (less than 1% opposite)
      - Regularly opposite
      - Both opposite and alternate

2. Measurements (second four digits from the full stop)
   (e) Maximum ascospore length
      - Below 20 μm long
      - Between 21–30 μm long
      - Between 31–40 μm long.
      - Between 41–50 μm long.

- Between 51–60 μm long
- More than 60 μm long

(f) Maximum ascospore width

- Up to 10 μm broad
- Between 11–20 μm broad
- Between 21–30 μm broad
- More than 30 μm broad

(g) Maximum diameter of perithecia

- Up to 100 μm
- Between 101–200 μm
- Between 201–300 μm
- More than 301 μm

(h) Maximum length of mycelial setae

- Up to 300 μm long
- Between 301–500 μm long
- Between 501–1000 μm long
- More than 1000 μm long
- Absent.

8 Diversity in the Western Ghats

The Western Ghats represents rich flora with enormous species diversity and endemism and is therefore accepted as one among the world's hot spots (Mittermeier et al. 2000). Owing to its diverse climatic and altitudinal conditions, India is rich in the Phanerogamic flora, which is the chief host for the parasitic fungi. In general, the fungi that attack the cultivated plants have got much significance because of their direct effect on humankind. Several other fungi groups whose occurrences are mostly restricted to wild plants are less destructive hence received less attention (Hosagoudar 1996). India holds a distinct identity, not only because of its geography, history, and culture but also because of the great diversity of its natural ecosystems. The panorama of Indian forests ranges from evergreen tropical rain forests in the Andaman and Nicobar Islands, the Western Ghats, and the North-Eastern states, to dry alpine scrub to high Himalaya to the north. The Western Ghats are the hill ranges run along the west coast of Peninsular India, stretch to 1600 km from the Tapti river in Gujarat to Kanyakumari in Tamil Nadu through the states of Maharashtra, Goa, Karnataka, Kerala, and Tamil Nadu and merge in Kanyakumari.

The southern Western Ghats are, in particular, rich in Phanerogams and are the centers of Mega biodiversity, represent 4000 flowering plants. The Western Ghats comprise the mountain range that runs the ecosystem of the Western Ghats. There is a great variety of vegetation all along the Ghats: scrub jungles, grasslands along the

lower altitudes, dry and moist deciduous forests, and semi-evergreen and evergreen forests. There are two main centers of diversity, the Agasthyamala Biosphere Reserve and the Silent Valley. The complex topography and the heavy rainfall have made certain areas inaccessible and have helped the region retain its diversity. There are currently seven national parks in the Western Ghats, with a total area of 2073 sq. Km (equivalent to 1.3% of the region) and 39 wildlife sanctuaries cover about 13,862 sq. Km (8.1%). Of about 1.7 million species globally described and recorded in scientific literature, India has about 1,26,200 species. It ranks tenth in the world regarding the richness of flowering plants (17,000 species) and mammals (372 species). Of India's 49,219 plant species, 1600 endemics (40% of the total number of endemics) are found in a 17,000 km2 strip of forest along the Western Ghats' seaward side Maharashtra, Karnataka, Tamil Nadu, and Kerala (Sabeena et al. 2020).

Biodiversity always refers to the genetic or taxonomic variability within a specific area or region. Earth's biodiversity is rapidly decreasing in response to pollution and habitat destruction as forests are logged, prairies are converted to subdivisions, and wetlands are drained for development. With each species' loss, we lose potential sources of new medicines, chemicals, and food. We also lose links in food webs and critical ecosystem processes essential for clean air, clean water, and healthy ecosystems. To conserve this, we need to know more about the various kinds of organisms that exist and co-exist with our forests. The tropical forests are considered to be the house of the most incredible biodiversity on earth. These forests occupy a great variety of edaphically and climatically heterogeneous sites. The destruction and degradation of tropical forests have resulted in the extinction of these forests' microbial diversity.

9 Major Genera and Distribution

Fungi are an essential component of biodiversity in tropical forests. As a significant contributor to the maintenance of the earth's ecosystem, biosphere, and biochemical cycles, fungi perform unique and indispensable activities on which humans depend. Fungi inhabiting the leaves are known as phylloplane fungi. Leaves are the most exposed parts subjected to an interaction with the environment and also with the microbes. Phylloplane fungi are the leaf dweller or leaf infecting microfungi. Here the meaning of phylloplane is mainly restricted to leaf infecting, parasitic microfungi. They produce different symptoms like pustules, black colonies, tar spots, etc. A critical part of microbial diversity is phylloplane fungi, including rusts, smuts, powdery mildews, Black mildews, tar spots, etc. Most of these fungi are obligate or facultative parasites. On the other hand, several fungi groups that do not cause any severe symptomatic appearance on the hosts received less attention. These are certain fungi which maintain a parasitic symbiosis with their host plants.

An extensive collection of microfungi infecting plants have been discovered from the Western Ghats region. The minute nature of microfungi makes their direct

observation in the field is difficult. The success of this approach varies according to the collector's experience. However, even an expert who may achieve targeted collection for some groups often has little idea of what has been collected until the materials being examined microscopically.

Black colony-forming parasitic fungi are known as "Black or dark mildews." These are obligate biotrophic parasites found superficially on the aerial portions of vascular plants commonly known as black or dark mildews. The black colony-forming parasitic fungi belong to several taxonomic groups, viz. Meliolales, Schiffnerula, and its anamorphic forms, Asterinales, Meliolinaceae, Hyphomycetes, etc. The Indian climate, in general, is suitable for the growth of these fungi. Of these, the fungi belonging to Meliolales can be distinguished by their two-celled appressorial mycelium, setae, presence of globose perithecia with setae, appendages, etc. These are the unique group of fungi which are distinguished very easily. The order Meliolales comprises 2403 species (plus 106 uncertain species) distributed in two families and nine genera. The family Meliolaceae comprises eight genera *Amazonia* (64 species), *Appendiculella* (75 species), *Asteridiella* (367 species), *Cryptomeliola* (3 species), *Endomeliola* (1 species), *Irenopsis* (152 species), *Meliola* (1703 species) (Fig. 2), and *Setameliola* (20 species), with 2385 species (plus 106 uncertain species) (Zeng et al. 2017).

This work includes the Phylloplane Fungi from the Western Ghats. It comprises an account of 932 Phylloplane fungal taxa belonging to 35 genera. A list of fungi, Fungal genera, Fungus-Family-Genera-Host Plant-Locality is included below the table (Table 1).

The genus *Meliolina* was proposed by Sydow and Sydow (1914) to accommodate the fungi similar to Meliola but have distantly septate hyphae, lack characteristic appressoria, and having dichotomously branched phialophores. These fungi can be easily identified in the field by their velvet carbonaceous woolly colonies, mostly on the leaves' lower surface. Hughes (1993) revised the genus and gave an account of thirty-eight species, and Hosagoudar (2002) provided the key to species of this genus.

Asterinaceous fungi have mycelium with or without appressoria; ascomata flattened orbicular known as thyriothecia dehisce stellately at the center. The asci are bitunicate, octosporous, and ascospores uni to many septate (Hansford 1961; Hosagoudar 2012, 2013). The fungal taxa are distributed among 20 genera in two families, namely, Asterinaceae: *Asterina, Asterolibertia, Bheemamyces, Gangamyces, Ishwaramyces, Meliolaster, Prillieuxina, Symphaster, Trichasterina, Vishnumyces; Lembosiaceae: Cirsosia, Echidnodella, Echidnoides, Eupelte, Lembosia, Maheshwaramyces. The anamorphs are Asterostomella, Asterostomula, Bramhamyces, and Mahanteshamyces. Asterina* is the type genus of the family Asterinaceae, with more than 600 species worldwide (Fig. 3). These fungi have been extensively studied in the tropics (Theissen 1913, Hansford 1946, Muller & Arx 1962; Hosagoudar & Abraham 2000; Hofmann & Piepenbring 2008). A study made on the Kerala part of Western Ghats revealed 243 *Asterina* species (Sabeena et al. 2020). Although *Prillieuxina* is an important genus that lacks appressoria, it is still included in the family Asterinaceae based on characters such as colonies spread

1. Infected leaves of *Vallaris solanacea*; 2. Colony with perithecium; 3. Appressoriate mycelium with ascospore; 4. Germinating ascospore; 5. Developing perithecium

Fig. 2 *Meliola vallaridis* Hosag. et al. on *Vallaris solanacea*

Table 1 Phylloplane Fungi of Western Ghats

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
1	Hosagoudar (2013a)	***Amazonia abaremae*** Hosag. & Antony	Meliolaceae	Amazonia	*Abarema bigemina* (L.) Kosterm. (Mimosaceae)	Kerala
2	Hosagoudar (2013a)	***Amazonia abutili*** Hosag.	Meliolaceae	Amazonia	*Abutilon ramosum* (Cav.) Guill. & Perr.(Malvaceae)	Kerala
3	Hosagoudar (2013a)	***Amazonia acronychiae*** Hosag.	Meliolaceae	Amazonia	*Acronychia pedunculata* (L.) Miq. .(Rutaceae)	Kerala
4	Hosagoudar (2013a)	***Amazonia actinodaphnes*** Hosag.	Meliolaceae	Amazonia	*Actionodaphne malabarica* Balakr. (Lauraceae)	Kerala
5	Hosagoudar (2013a)	***Amazonia antistrophecola*** Hosag. & Abraham	Meliolaceae	Amazonia	*Antistrophe serratifolia* (Bedd.) Hook.f. (Myrsinaceae)	Kerala
6	Lini et al. (2017)	***Amazonia atlantiicola*** Lini K. Mathew & Neeta N. Nair	Meliolaceae	Amazonia	*Atlantia* sp.(Rutaceae)	Kerala
7	Hosagoudar (2013a)	***Amazonia cinnamomi*** Hosag.	Meliolaceae	Amazonia	*Cinnamomum riparium* Gamble (Lauraceae)	Kerala
8	Hosagoudar (2013a)	***Amazonia dikesinghii*** Hosag. et al.	Meliolaceae	Amazonia	*Pogostemon travancoricus* Bedd. (Lamiaceae),	Kerala
9	Hosagoudar (2013a)	***Amazonia flacourtiae*** Hosag. et al.	Meliolaceae	Amazonia	*Flacourtia* sp. (Flacourtiaceae)	Tamil Nadu
10	Hosagoudar (2013a)	***Amazonia gomphandrae*** Hosag.	Meliolaceae	Amazonia	*Gomphandra coriacea* Wight (Icacinaceae)	Kerala
11	Hosagoudar (2013a)	***Amazonia goosii*** Hosag. & Abraham	Meliolaceae	Amazonia	*Canthium* sp. (Rubiaceae), *Psychotria flavida* Talbot	Kerala
12	Hosagoudar (2013a)	***Amazonia goosii*** Hosag. & Abraham var. ***microspora*** Hosag. et al.	Meliolaceae	Amazonia	*Psychotria globicephala* Gamble (Rubiaceae)	Kerala
13	Hosagoudar (2013a)	***Amazonia gordoniicola*** Hosag.	Meliolaceae	Amazonia	*Gordonia* sp. (Theaceae)	Kerala
14	Hosagoudar (2013a)	***Amazonia gouaniae*** Hosag. & Braun	Meliolaceae	Amazonia	*Gouania microcarpa* DC.	Kerala

15	Hosagoudar (2013a)	***Amazonia kakachiana*** Hosag.	Meliolaceae	Amazonia	*Vaccinium leschenaultii* Wight var. *zeylanica* Clarke	Kerala
16	Hosagoudar (2013a)	***Amazonia karnatakensis* Hosag.** & Manian	Meliolaceae	Amazonia	*Symplocos* sp. (Symplocaceae)	Kerala
17	Hosagoudar (2013a)	***Amazonia leeae*** Hansf. & Thirum	Meliolaceae	Amazonia	*Leea macrophylla* Roxb.(leeaceae), *Leea indica* (Burm.f.) Merr	Kerala
18	Hosagoudar (2013a)	***Amazonia mayteni***Hosag. et al.	Meliolaceae	Amazonia	*Maytenus rothiana* (Walp.) Ramam.	Karnataka
19	Hosagoudar (2013a)	***Amazonia melicopecola*** Hosag. & Abraham	Meliolaceae	Amazonia	*Melicope lunuankenda* (Gaertn.) T.G. Hartley(Rutaceae)	Kerala
20	Hosagoudar (2013a)	***Amozonia paloquii*** Y. B. Hosagoudar et P. J. Robin	Meliolaceae	Amazonia	*Palaquium* sp. (Sapotaceae),	Kerala
21	Hosagoudar (2013a)	***Amazonia patilii* Hosag.**	Meliolaceae	Amazonia	*Maytenus emerginata* (Willd.) Ding Hu (*Gymnosporia montana* (Roth) Benth.) Celastraceae	Maharashtra
22	Hosagoudar (2013a)	***Amazonia peregrina*** Syd. & Syd	Meliolaceae	Amazonia	*Embelia viridiflora* Bl. (Myrsinaceae), *Embelia basal* (Rem. & Schultes) A. DC.	Maharashtra
23	Hosagoudar (2013a)	***Amazonia psychotriae*** (Henn.) Theiss	Meliolaceae	Amazonia	*Psychotria* sp., (Rubiaceae) *Saprosma corymbosum* (Bedd.) Bedd	Kerala
24	Hosagoudar (2013a)	***Amazonia psychotriae*** (Henn.) Theiss. var. ***macrospora*** Hosag. et al.	Meliolaceae	Amazonia	Rubiaceae member	Kerala
25	Hosagoudar (2013b)	***Amazonia symploci*** Hosag.	Meliolaceae	Amazonia	*Symplocos* sp. (Symplocaceae)	Kerala
26	Hosagoudar (2013a)	***Amazonia syzygii*** Hosag	Meliolaceae	Amazonia	*Syzygium* sp., *Syzygium cumini* Skeels(Myrtaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
27	Hosagoudar (2013a)	***Amazonia vaccinii*** Hosag. et al.	Meliolaceae	Amazonia	*Vaccinium* sp. (Vacciniacea)	Kerala
28	Hosagoudar (2013a)	***Appendiculella calophylli*** (Stev.) Toro var. ***apetali*** Hosag. et al	Meliolaceae	*Appendiculella*	*Calophyllum apetalum* Willd	Karnataka
29	Hosagoudar (2013a)	***Appendiculella calostroma*** (Desm.) Höhn	Meliolaceae	*Appendiculella*	*Rubus ellipticus* Sem.(Rosaceae), *Rubus vulgaris* Meikle, *Crataegus crenulata* Roxb, *Rubus calycinus* Don.	Karnataka Kerala
30	Hosagoudar (2013a)	***Appendiculella elaeocarpicola*** Hosag. & Robin	Meliolaceae	*Appendiculella*	*Elaeocarpus tuberculatus* Roxb (Elaeocarpaceae)	Kerala
31	Hosagoudar (2013a)	***Appendiculella shettyi*** Hosag. et al.	Meliolaceae	*Appendiculella*	*Gordonia* sp. (Theaceae), *Gordonia obtusa* Wall. ex. Wight & Arn.	Kerala
32	Hosagoudar (2013a)	***Appendiculella vacciniorum*** Hosag. et al.	Meliolaceae	*Appendiculella*	*Vaccinium leschenaultii* Wight. (Vacciniaceae)	Kerala
33	Hosagoudar (2013a)	***Appendiculella vivekananthanii*** Hosag. et al.	Meliolaceae	*Appendiculella*	*Gordonia* sp. (Theaceae), *Gordonia obtusa* Wall. ex. Wight & Arn.	Kerala
34	Hosagoudar (2013a)	***Armatella actinodaphnes*** Hosag. et al.	Meliolaceae	*Armatella*	*Actinodaphne* sp. (Lauraceae)	Kerala
35	Hosagoudar (2013a)	***Armatella apolloniadis*** Hosag. et al.	Meliolaceae	*Armatella*	*Apollonias arnottii* Nees (Lauraceae)	Kerala
36	Hosagoudar and Divya (2013)	***Armatella apollonigena*** Hosag. & A. Sabeena	Meliolaceae	*Armatella*	*Apollonias* sp. (Lauraceae)	Kerala
37	Hosagoudar (2013a)	***Armatella balakrishnanii*** Hosag.	Meliolaceae	*Armatella*	*Cinnamomum malabatrum* (Burm. f.) Blume (Lauraceae)	Kerala

38	Hosagoudar (2013a)	***Armatella caulicola*** Hosag. et al.	Meliolaceae	*Armatella*	*Litsea* sp. (Lauraceae)	Kerala
39	Hosagoudar (2013a)	***Armatella cinnamomi*** Hansf. & Thirum.	Meliolaceae	*Armatella*	*Cinnamomum zeylanicum* L. (Lauraceae) *C. macrocarpum* Hook.f. *Cinnamomum* sp	Kerala
40	Hosagoudar (2013a)	***Armatella cinnamomicola*** Hansf.	Meliolaceae	*Armatella*	*Cinnamomum macrocarpum* Hook. f. (Lauraceae), *C. malabatrum* (Burm.f.) Blume *Cinnamomum* sp.	Kerala
41	Hosagoudar (2013a)	***Armatella cryptocaryae*** Hosag.	Meliolaceae	*Armatella*	*Cryptocarya bourdillonii* Gamble (Lauraceae), *Cryptocarya* sp. *Litsea coriacea* (Heyne ex Meissner) Hook.f. *L. stocksii* Hook.f. *L. wightii ana sensu* Hook.f. *L. glabra* (Wallich ex Nees) Hook.f. *L. glabrata* (Wallich ex Nees) Hook. f. *L. deccanensis* Gamble *Litsea* sp.	Kerala
42	Hosagoudar (2013a)	***Armatella indica*** Hosag.	Meliolaceae	*Armatella*	*Cinnamomum malabatrum* (Burm. f.) Blume (Lauraceae), *C. sulphuratum* *Cinnamomum* sp	Kerala
43	Hosagoudar (2013a)	***Armatella kakachiana*** Hosag. & Goos	Meliolaceae	*Armatella*	*Apollonias arnottii* Nees (Lauraceae)	Kerala
44	Hosagoudar (2013a)	***Armatella katumotoi*** Hosag.	Meliolaceae	*Armatella*	*Litsea stocksii* Hook.f (Lauraceae) *Persea macrantha* (Nees) Kosteerm. (*Machilus macrantha* Nees), *Litsea* sp., *Persea* sp.	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
45	Hosagoudar (2013a)	***Armatella litseae*** (Henn.) Theiss. & Syd.	Meliolaceae	*Armatella*	Daphnedium pulcherrima (Lauraceae) *Neolitsea fischeri* Gamble, *N. scrobiculata* (Meisner) Gamble, *N. zeylanica* Merr.	
46	Hosagoudar (2013a)	***Armatella litseae*** (Henn.) Theiss. & Syd. var. ***boninensis*** Katumoto & Harada	Meliolaceae	*Armatella*	*Neolitsea* sp. (Lauraceae), *Neolitsea scrobiculata* (Meisner) Gamble	Kerala
47	Hosagoudar (2013a)	***Armatella phoebecola*** Hosag.	Meliolaceae	*Armatella*	*Phoebe lanceolata* Nees. (Lauraceae), *Actinodaphne* sp., *Litsea* sp.	Kerala
48	Hosagoudar (2013a)	***Asteridiella acronychiae-pedunculatae*** Hosag.	Meliolaceae	*Asteridiella*	*Acronychia pedunculata* (L.) Miq. (Rutaceae)	Kerala
49	Hosagoudar (2013a)	***Asteridiella americana*** Hansf.	Meliolaceae	*Asteridiella*	*Chionanthus mala-elengi* (Dennst.) Green (*Linociera malabarica* Wall. ex G. Don)(Oleaceae)	Kerala
50	Hosagoudar (2013a)	***Asteridiella amomi*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Amomum subulatum* Roxb (Zingiberaceae)	Kerala
51	Hosagoudar (2013a)	***Asteridiella anamalaiana*** Hosag.	Meliolaceae	*Asteridiella*	*Sterculia urens* Roxb.(Sterculiaceae)	Tamil Nadu,
52	Hosagoudar (2013a)	***Asteridiella antidesmaticola*** Hosag. & Abraham	Meliolaceae	*Asteridiella*	*Antidesma alexiteria* L. (Stilaginaceae)	Kerala
53	Hosagoudar (2013a)	***Asteridlella anacolosae*** Hosag. & Sabeena	Meliolaceae	*Asteridiella*	*Anacolosa* sp. (Olacaceae)	Kerala
54	Hosagoudar (2013a)	***Asteridiella anastomosans*** (Wint.) Hansf.	Meliolaceae	*Asteridiella*	*Leucas* sp. (Lamiaceae)	Kerala

55	Hosagoudar (2013a)	***Asteridiella atricha*** (Speg.) Hansf.	Meliolaceae	*Asteridiella*	*Eugenia* sp.(Myrtaceae)	Maharashtra
56	Hosagoudar (2013a)	***Asteridiella callista*** (Rehm) Hansf.	Meliolaceae	*Asteridiella*	*Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae)	Kerala
57	Hosagoudar (2013a)	***Asteridiella caseariicola*** Hosag.	Meliolaceae	*Asteridiella*	*Casearia esculenta* Roxb. (Flacourtiaceae)	Tamil Nadu
58	Hosagoudar (2013a)	***Asteridiella chowrirae*** Hosagoudar, Thimmaiah & Jayashankara,	Meliolaceae	*Asteridiella*	*Euphorbia pulcherrima* Willd. ex Klotz. (*Poinsettia pulcherima* Graham) (Euphorbiaceae),	Karnataka
59	Hosagoudar (2013a)	***Asteridiella colebrookiae*** Jana et al.	Meliolaceae	*Asteridiella*	*Colebrookia oppositifolia* Sm. (Lamiaceae)	Kerala
60	Hosagoudar (2013a)	***Asteridiella crotonicola*** Hosag. & Abraham	Meliolaceae	*Asteridiella*	*Croton zeylanicus* Muell.-Arg. Euphorbiaceae)	Kerala
61	Hosagoudar (2013a)	***Asteridiella crotonis*** Hosag.	Meliolaceae	*Asteridiella*	*Croton zeylanicus* Muell.-Arg. Euphorbiaceae)	Kerala
62	Hosagoudar (2013a)	***Asteridiella crotonis-caudati*** Hosag. & Riju	Meliolaceae	*Asteridiella*	*Croton caudatus* Geisel. (Euphorbiaceae)	Kerala
63	Hosagoudar (2013a)	***Asteridiella cyrtandrae*** (Stev.) Hansf. var. ***didymocarpi*** Hosag.	Meliolaceae	*Asteridiella*	*Didymocarpus humboldtianus* Gard. (Gesneriaceae)	Tamil Nadu,
64	Hosagoudar (2013a)	***Asteridiella depokensis*** Hansf	Meliolaceae	*Asteridiella*	*Vitex negundo* L. (Verbenaceae)	Karnataka
65	Hosagoudar (2013a)	***Asteridiella clerodendricola*** Hosag.	Meliolaceae	*Asteridiella*	*Clerodendrum viscosum* Vent. (Verbenaceae)	Kerala
66	Hosagoudar (2013a)	***Asteridiella dilleniae*** Hosag. & Kamar.	Meliolaceae	*Asteridiella*	*Dillenia pentagyna* Roxb. Dilleniaceae	Kerala
67	Hosagoudar (2013a)	***Asteridiella elaeocarpi-tuberculati*** Hosag.	Meliolaceae	*Asteridiella*	*Elaeocarpus tuberculatus* Roxb (Elaeocarpaceae)	Kerala
68	Hosagoudar (2013a)	***Asteridiella emciciana*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Scutia myrtina* (Burm. f.) Kurz (Rhamnaceae)	Tamil Nadu,

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
69	Hosagoudar (2013a)	***Asteridiella entebbeensis*** (Hansf. & Stev.) Hansf. var. ***glochidii*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Glochidion* sp. (Euphorbiaceae)	Kerala
70	Hosagoudar (2013b)	***Asteridiella fagraeae*** Hosag. & Sabeena	Meliolaceae	*Asteridiella*	*Fagraea ceilanica* Thunb. (Loganicaceae)	Kerala
71	Hosagoudar (2013a)	***Asteridiella ficicola*** Hosag.	Meliolaceae	*Asteridiella*	*Ficus microcarpa* L.(Moraceae)	Kerala
72	Hosagoudar (2013a)	***Asteridiella formosensis*** (Yamam.) Hansf.	Meliolaceae	*Asteridiella*	*Callicarpa tomentosa* L. (Verbenaceae)	Kerala
73	Hosagoudar (2013a)	***Asteridiella glycosmidis*** Hosag., C.K. Biju & Abraham	Meliolaceae	*Asteridiella*	*Glycosmis pentaphylla* (Retz.) DC (Rutaceae)	Kerala
74	Hosagoudar (2013a)	***Asteridiella grewiae*** C.R.Patil ex Hosag.	Meliolaceae	*Asteridiella*	*Grewia subinaequalis* DC. (*Grewia asiatica sensu* Mast.)	Maharashtra
75	Hosagoudar (2013a)	***Asteridiella gaultheriae*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Gaultheria indica* Wall. (Ericaceae)	Kerala
76	Hosagoudar (2013a)	***Asteridiella heritieriicola*** (Thite & Patil) Hosag.	Meliolaceae	*Asteridiella*	*Herietaria littoralis* Drynand. (Strculiaceae)	Maharashtra
77	Hosagoudar (2013a)	***Asteridiella homaligena*** Hosagoudar, Thimmaiah & Jayashankara,	Meliolaceae	*Asteridiella*	*Homalium zeylanicum* Benth. (Flacourtiaceae)	Karnataka
78	Hosagoudar (2013b)	***Asterdiella hydnocarpigena*** Hosag. & Jagath Timmaih	Meliolaceae	*Asteridiella*	*Hydnocarpus pentendra* (Ham.) Oken (Flacourtiaceae),	Karnataka
79	Hosagoudar (2013a)	***Asteridiella ixorae*** Hosag. & Archana	Meliolaceae	*Asteridiella*	*Ixora* sp. (Rubiaceae),	Kerala
80	Hosagoudar (2013a)	***Asteridiella julostylidis*** Hosag. & Abraham	Meliolaceae	*Asteridiella*	*Julostylis polyandra* Ravi & Anil (Malvaceae)	Kerala
81	Hosagoudar (2013a)	***Asteridiella justiciae*** Hosag. & Rajkumar	Meliolaceae	*Asteridiella*	*Justicia* sp. Acanthaceae	Kerala

82	Hosagoudar (2013a)	***Asteridiella kapoorii*** Hosag. & Raghu	Meliolaceae	*Asteridiella*	*Diospyros* sp. (Ebenaceae)	Karnataka
83	Hosagoudar (2013a)	***Asteridiella knemae*** Hansf. var. ***macrospora*** Hosag. & Abraham	Meliolaceae	*Asteridiella*	*Knema attenuata* (Wall. ex Hook.f.) Thoms. (Myristicaceae)	Kerala
84	Hosagoudar (2013a)	***Asteridiella kodaikanalensis*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Symblocos anamallayana* Bedd. (Symplocaceae)	Tamil Nadu
85	Hosagoudar (2013a)	***Asteridiella kombeensis*** Hosag.	Meliolaceae	*Asteridiella*	*Mallotus philippensis* (Lam.) Muell.-Arg. (Euphorbiaceae)	Kerala
86	Hosagoudar (2013a)	***Asteridiella macarangicola*** Hosag.	Meliolaceae	*Asteridiella*	*Macaranga peltata* Muell.-Arg. (Euphorbiaceae)	Kerala
87	Hosagoudar (2013a)	***Asteridiella madikeriensis*** Hosagoudar, Thimmaiah & Jayashankara	Meliolaceae	*Asteridiella*	*Premna* sp. (Verbenaceae),	Karnataka
88	Hosagoudar (2013a)	***Asteridiella malloti*** (Hansf. & Thirum.) Hansf.	Meliolaceae	*Asteridiella*	*Mallotus* sp. (Euphorbiaceae)	Karnataka Kerala
89	Hosagoudar (2013a)	***Asteridiella meliosmae*** Kar & Maity	Meliolaceae	*Asteridiella*	*Meliosma simplicifolia* (Roxb.) Walp. (Sabiaceae)	Kerala
90	Hina Mohamed and JacobThomas (2020)	***Asteridiella micheliifolia*** Hosag., Archana. & Agarwal var. ***macrospora*** JacobThomas and HinaMohamed	Meliolaceae	*Asteridiella*	*Michelia champaka* L. (Magnoliaceae)	Kerala
91	Hosagoudar (2013a)	***Asteridiella millettiae*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Millettia rubiginosa* Wight & Arn. (Fabaceae)	Kerala
92	Hosagoudar (2013a)	***Asteridiella myristicacearum*** Hosag.	Meliolaceae	*Asteridiella*	Myristicaceae	Kerala
93	Hosagoudar (2013a)	***Asteridiella oreocnidecola*** Hosag.	Meliolaceae	*Asteridiella*	*Oreocnide integrifolia* (Gaud. ex Wedd.) Miq.	Kerala
94	Hosagoudar and Sabeena (2012)	***Asterediella pittosporacearum*** Hosagoudar & Sabeena,	Meliolaceae	*Asteridiella*	*Pittospo-rum neelgherrense* Wight & Arn. (Pittosporaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
95	Hosagoudar (2013a)	***Asteridiella pitya*** (Sacc.) Hansf.	Meliolaceae	*Asteridiella*	*Taxus* sp. (Pinacea)	Kerala
96	Hosagoudar (2013a)	***Asteridiella pothodis*** (Hansf. & Thirum.) Hansf.	Meliolaceae	*Asteridiella*	*Pothos scandense* L.	Karnataka
97	Hosagoudar (2013b)	***Asteridiella premnigena*** Hosag.	Meliolaceae	*Asteridiella*	*Premna* sp. (Verbenaceae)	Kerala
98	Hosagoudar (2013a)	***Asteridiella pygei*** Hansf. var. ***microspora*** Hosag**.**	Meliolaceae	*Asteridiella*	*Rubus* sp. (Rosaceae)	Kerala
99	Hosagoudar (2013a)	***Asteridiella resinosi*** Hosag.	Meliolaceae	*Asteridiella*	*Madhuca longifolia* (L.) Macbr. var. *latifolia* (Roxb) A. Chev. (Euphorbiaceae)	Kerala
100	Hosagoudar (2013a)	***Asteridiella sapotacearum*** Hansf.	Meliolaceae	*Asteridiella*	*Madhuca longifolia* (L.) Macbr. var. *latifolia* (Roxb.) A. Chev (Sapotaceae)	Kerala
101	Hosagoudar (2013a)	***Asteridiella schumannianthi*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Schumannianthus* sp. (Marantaceae), *Schumannianthus virgatus* (Roxb.) Rolfe	Kerala
102	Hosagoudar (2013a)	***Asteridiella scolopiae*** Hosag.	Meliolaceae	*Asteridiella*	*Scolopia crenata* (Wight & Arn.) Clos. (Flacourtiaceae)	Tamil Nadu
103	Hosagoudar (2013a)	***Asteridiella scolopiae*** var**.** ***indica*** Hosag**.** et Riju	Meliolaceae	*Asteridiella*	*Scolopia* sp. (Flacourtiaceae),	Kerala
104	Hosagoudar (2013a)	***Asteridiella sebastianiae*** Hosagoudar, Sabeena *et* Jacob-Thomas	Meliolaceae	*Asteridiella*	*Sebastiania chamaelea* (L.) Mull. (Euphorbiaceae)	Kerala
105	Hosagoudar (2013a)	***Asteridiella shenbganurensis*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Symblocos anamallayana* Bedd. (Symplocaceae)	Tamil Nadu

106	Hosagoudar (2013a)	***Asteridiella strebli*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Streblus asper* Lour. (Moraceae)	Kerala
107	Hosagoudar (2013a)	***Asteridiello symploct-microphyllae*** Hosag. & Sabeena	Meliolaceae	*Asteridiella*	*Symplocos macrophylla* Wallich. ex DC.(Symplocaceae)	Kerala
108	Hosagoudar (2013a)	***Asteridella solani*** McAlpine var. ***kodaikanlensis*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Solanum viburnum* (Solanaceae)	Tamil Nadu,
109	Hosagoudar (2013a)	***Asteridiella tarlacence*** Petrak	Meliolaceae	*Asteridiella*	*Wendlandia notianiana* Wall. (Rubiaceae)	Maharashtra
110	Hosagoudar (2013a)	***Asteridiella theae*** Patil & Maham.	Meliolaceae	*Asteridiella*	*Thea sinensis* L.	Maharashtra
111	Hosagoudar (2013a)	***Asteridiella toddaliae*** Hosag. & Riju	Meliolaceae	*Asteridiella*	*Toddalia asiatica* (L.) Lam. (Rutaceae)	Kerala
112	Hosagoudar (2013b)	***Asteridiella tragiae*** Hosag. & Jagath Timmaih	Meliolaceae	*Asteridiella*	*Tragia* sp. (Euphorbiaceae)	Karnataka
113	Hosagoudar (2013a)	***Asteridiella vacciniicola*** Hansf.	Meliolaceae	*Asteridiella*	*Vaccinum neilgherrense* Wight Vacciniaceae	Kerala
114	Hosagoudar (2013a)	***Asteridiella viticis-negundoi*** Hosagoudar, Thimmaiah & Jayashankara	Meliolaceae	*Asteridiella*	*Vitex negundo* L. (Verbenaceae)	Karnataka
115	Hosagoudar (2013a)	***Asteridiella vivekananthanii*** Hosag.	Meliolaceae	*Asteridiella*	*Clerodendrum viscosum* Vent (Verbenaceae)	Kerala
116	Hosagoudar (2013b)	***Asteridiella xyliae*** Hosag.	Meliolaceae	*Asteridiella*	*Xylia xylocarpa* Roxb. (Mimosaceae),	Kerala
117	Hosagoudar (2013a)	***Asteridiella wyanadensis*** Hosag. et al.	Meliolaceae	*Asteridiella*	*Mallotus* sp. (Euphorbiaceae)	Kerala
118	Hosagoudar (2013a)	***Asteridiella websteri*** Hosag.	Meliolaceae	*Asteridiella*	*Olea dioica* Roxb. (Oleaceae)	Maharashtra
119	Bhise et al. (2014)	***Asteridiella websteri*** Hosag. var. *oleae* Bhise and Patil	Meliolaceae	*Asteridiella*	*Olea dioica* Roxb. (Oleaceae)	Maharashtra

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
120	Hosagoudar (2013a)	***Asterina acronychiae*** Hosag. & Goos	Asterinaceae	*Asterina*	*Acronychia pedunculata* (L.) Miq. (Rutaceae)	Kerala, Tamil Nadu, Karnataka
121	Hosagoudar and Fathima (2013)	***Asterina acronychigena*** Hosag. & Fathima	Asterinaceae	*Asterina*	*Acronychia* sp. (Rutaceae)	Kerala
122	Hosagoudar (2013a)	***Asterina acrotremae*** Hosag. & Chandr. ex Hosag.	Asterinaceae	*Asterina*	*Acrotrema arnottianum* Wight	Kerala
123	Hosagoudar (2013a)	***Asterina adeniicola*** Hosag. & Kamar.	Asterinaceae	*Asterina*	*Adenia hondala* (Gaertn.) Wilde (Passifloraceae),	Kerala
124	Hosagoudar (2013a)	***Asterina aganosmae*** Syd	Asterinaceae	*Asterina*	*Aganosma cymosum* (Roxb.) G. Don. Apocynaceae),	Tamil Nadu
125	Hosagoudar (2013a)	***Asterina aglaiae*** Hosag.	Asterinaceae	*Asterina*	*Aglaia* sp. (Meliaceae),	Karnataka Kerala
126	Hosagoudar (2013a)	***Asterina anamirtae*** Hosag.	Asterinaceae	*Asterina*	*Anamirta cocculus* (L.) Wight & Arn. (Menispermaceae),	Tamil Nadu
127	Hosagoudar et al. (2013a)	***Asterina antidesmatis*** Petrak	Asterinaceae	*Asterina*	*Antidesma* sp. (Stylaginaceae	Karnataka
128	Hosagoudar (2013a)	***Asterina aporusae*** Hansf.	Asterinaceae	*Asterina*	*Aporusa lindleyana* (Wight) Baill. (Euphorbiaceae),	Karnataka Kerala
129	Hosagoudar (2013a)	***Asterina arecacearum*** Hosag. et al.	Asterinaceae	*Asterina*	*Calamus* sp. (Arecaceae),	Kerala
130	Hosagoudar (2013a)	***Asterina araliae*** Patil & Pawar	Asterinaceae	*Asterina*	*Brassaiopsis actinophila F.* Muell (Araliaceae)	Maharashtra
131	Hosagoudar (2013a)	***Asterina ardisiae*** Hansf.	Asterinaceae	*Asterina*	*Ardisia solanacea* Roxb. (Myrsinaceae)	Kerala

132	Hosagoudar (2013a)	***Asterina ardisiicola*** Hosag. & Chandr. ex Hosag.	Asterinaceae	*Asterina*	Ardisia sonchifolia *Mez.* (Myrsinaceae),	Kerala
133	Hosagoudar (2013a)	***Asterina aristolochiae*** Hosag. & Jacob-Thomas	Asterinaceae	*Asterina*	*Aristolochia tagala* Cham. (Aristolochiaceae	Kerala
134	Hosagoudar et al. (2013b)	***Asterina arkemibeyi*** Hosagoudar, Sabeena & Sam P. Mathew	Asterinaceae	*Asterina*	*Flacourtia ontana* Graham (Flacourtiaceae	Kerala
135	Hosagoudar (2013a)	***Asterina argyreiae*** Hansf.	Asterinaceae	*Asterina*	*Argyreia* sp. (*Merremia* sp.) (Convolvulaceae),	Karnataka
136	Hosagoudar (2013a)	***Asterina asclepiadis*** Hosag. & Goos	Asterinaceae	*Asterina*	*Asclepias curassavica* L. (Asclepiadaceae),	Tamil Nadu
137	Hosagoudar and Sabeena (2013)	***Asterina artocarpi*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Artocarpus hirsutus* Lam. (Moraceae)	Kerala
138	Hosagoudar (2013a)	***Asterina atalantiae*** Hosag. & Agarwal	Asterinaceae	*Asterina*	*Atalantia rotundifolia* (Thw.) Tanaka (Rutaceae),	Kerala
139	Hosagoudar (2013a)	***Asterina averrhoae*** Hosag. et al.	Asterinaceae	*Asterina*	*Averrhoa carambola* L. (Averrhoaceae),	Kerala
140	Hosagoudar (2013a)	***Asterina balakrishnanii*** Hosag.	Asterinaceae	*Asterina*	*Solanum torvum* Sw. (Solanaceae),	Kerala
141	Hosagoudar (2013a)	***Asterina banguiensis*** Yates	Asterinaceae	*Asterina*	*Glycosmis pentaphylla (*Retz.) DC. (Rutaceae)	Tamil Nadu
142	Hosagoudar (2013a)	***Asterina betonicae*** Hosag. & Goos	Asterinaceae	*Asterina*	*Justicia betonica* L. (Acanthaceae)	Kerala Tamil Nadu
143	Hosagoudar (2013a)	***Asterina bottomleyae*** Doidge	Asterinaceae	*Asterina*	*Ilex walkeri* Wight & Gard. ex Thw. (Aquifoliaceae), *Ilex wightiana* Wallich ex Wight,	Tamil Nadu
144	Hosagoudar (2013a)	***Asterina cannonii*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Eurya japonica* Thunb. (Theaceae),	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
145	Hosagoudar (2013a)	***Asterina caseariae-esculentae*** (Hosag. & Hosagoudar, V.B. 2013Goos) Hosag. & Abraham	Asterinaceae	*Asterina*	*Cipadessa baccifera* (Roth.) Miq. (Meliaceae),	Karnataka Kerala Tamil Nadu
146	Hosagoudar (2013a)	***Asterina cassiicola*** Hosag. & Archana	Asterinaceae	*Asterina*	Cassia fistula *L.* (Caessalpiniaceae)	Kerala
147	Hosagoudar (2013a)	***Asterina cansjerae*** Ryan	Asterinaceae	*Asterina*	*Cansjera rheedi* Gmel. (Opiliaceae)	Karnataka
148	Hosagoudar (2013a)	***Asterina cansjericola*** Hansf. & Thirum.	Asterinaceae	*Asterina*	*Cansjera rheedi* Gmel. (Opiliaceae)	Karnataka
149	Hosagoudar (2013a)	***Asterina cansjericola*** Hansf. & Thirum. var. ***indica*** Hosag. et al.	Asterinaceae	*Asterina*	*Cansjera rheedi* Gmel. (Opiliaceae)	Tamil Nadu
150	Hosagoudar (2013a)	***Asterina canthii-dicocci*** Hosag.	Asterinaceae	*Asterina*	*Canthium dicoccum* (Gaertn.) Teijsm. & Binn. (Rubiaceae),	Karnataka, Kerala
151	Hosagoudar (2013a)	***Asterina canthiigena*** Hosag. et al.	Asterinaceae	*Asterina*	*Canthium* sp. (Rubiaceae),	Karnataka
152	Hosagoudar (2013a)	***Asterina capparidis*** Syd. & Butler	Asterinaceae	*Asterina*	*Capparis zeylanica* L. (Capparaceae), *Capparis horrida* L. f.	Tamil Nadu, Karnataka
153	Hosagoudar and Fathima (2013)	***Asterina cassiifolia*** Hosag. & Fathima	Asterinaceae	*Asterina*	*Cassiaoccidentalis* L. (Caesalpiniaceae)	Kerala
154	Hosagoudar et al. (2013a)	***Asterina cassiigena*** Hosag., Jagath Thimmaiah & Sabeena	Asterinaceae	*Asterina*	*Cassia glauca* Lam. (Caesalpiniaceae),	Karnataka
155	Hosagoudar et al. (2013a)	***Asterina chrysophylligena*** Hosag., Jagath Thimmaiah & Sabeena	Asterinaceae	*Asterina*	*Chrysophyllum roxburghii* G. Don (Sapotaceae)	Karnataka

156	Hosagoudar (2013a)	***Asterina chukrasiae*** Hosag.	Asterinaceae	*Asterina*	*Chukrasia tabularis A.* Juss. (Meliaceae),	Kerala
157	Hosagoudar (2013a)	***Asterina cinnamomi*** Syd	Asterinaceae	*Asterina*	*Cinnamomum* sp. (Lauraceae)	Kerala
158	Hosagoudar (2013a)	***Asterina cinnamomicola*** Hansf.	Asterinaceae	*Asterina*	*Cinnamomum* sp. (Lauraceae)	Kerala
159	Hosagoudar (2013a)	***Asterina cipadessae*** Yates	Asterinaceae	*Asterina*	*Cipadessa baccifera* (Roth.) Miq. (Meliaceae)	Karnataka, Kerala Tamil Nadu
160	Hosagoudar (2013a)	***Asterina cissi*** Hughes	Asterinaceae	*Asterina*	*Cissus* sp. (*Vitis* sp.) (Vitaceae)	Kerala
161	Hosagoudar (2013a)	***Asterina claviflori*** Kar & Maity	Asterinaceae	*Asterina*	*Syzygium mundagam* (Bourd.) Chitra (Myrtaceae), *S. jambolana* (Lam.) DC., *Syzygium cumini (*L.) Skeels *Syzygium zeylanicum* (L.) DC.	Karnataka, Kerala Tamil Nadu
162	Hosagoudar (2013a)	***Asterina clematidis*** Hansf.	Asterinaceae	*Asterina*	*Clematis* sp. (Ranunculaceae),	Tamil Nadu
163	Hosagoudar (2013a)	***Asterina clusiacearum*** Hosag. & Jagath Thimmaiah	Asterinaceae	*Asterina*	*Garcinia gummigutta* (L.) Robs. (Clusiaceae)	Karnataka,
164	Hosagoudar (2013a)	***Asterina clausenicola*** Doidge	Asterinaceae	*Asterina*	*Melicope lunu-ankenda* (Gaertn.) T. Hartley (*Euodia lunu-ankenda* (Gaertn.) Merr. (Rutaceae),	Kerala Tamil Nadu
165	Hosagoudar (2013a)	***Asterina combreti*** Syd.	Asterinaceae	*Asterina*	*Calycopteris floribunda* (Roxb.) Poiret (Combretaceae), *Calycopteris* sp. *Terminalia arjuna* (Roxb. ex DC.), *Terminalia cuneata* Roth.	Kerala
166	Hosagoudar (2013a)	***Asterina congesta*** Cooke	Asterinaceae	*Asterina*	*Santalum* sp. (Santalaceae), *Santalum album* L.	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
167	Hosagoudar (2013a)	***Asterina crebra*** Syd.	Asterinaceae	*Asterina*	*Opilia amentacea* Roxb. (Opiliaceae),	Karnataka
168	Hosagoudar (2013a)	***Asterina cryptocariicola*** Hosag. et al.	Asterinaceae	*Asterina*	*Cryptocarya wightiana* Thwaites (*C. bourdillonii* Gamble) (Lauraceae),	Kerala Tamil Nadu
169	Hosagoudar (2013a)	***Asterina cynanchi*** Hosag. & Shiburaj	Asterinaceae	*Asterina*	*Cyananchum callialatum* Buch. Ham. ex Wight & Arn. (Asclepiadaceae)	Kerala
170	Hosagoudar (2013a)	***Asterina cynanchicola*** Hosag. & Archana	Asterinaceae	*Asterina*	*Cynanchum alatum* Wight & Arn. (Ascleipidaceae),	Kerala
171	Hosagoudar (2013a)	***Asterina dallasica*** Petrak	Asterinaceae	*Asterina*	*Trema orientalis* (L.) Blume (Ulmaceae)	Kerala
172	Hosagoudar et al. (2013b)	***Asterina derridicola*** Hosagoudar, Sabeena & Sam P. Mathew	Asterinaceae	*Asterina*	*Derris* sp. (Fabaceae),	Kerala
173	Hosagoudar et al. (2013b)	***Asterina desmosicola*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Desmos lawii* (Hook. f. & Thoms.) Saff. {(*Unona lawii* Hook. f. & Thoms.} (Annonaceae	Kerala
174	Hosagoudar (2013a)	***Asterina deightonii*** Syd.	Asterinaceae	*Asterina*	*Dendrophthoe* sp. (*Loranthus* sp.) (Loranthaceae),	Kerala
175	Hosagoudar (2013a)	***Asterina dhivaharanii*** Hosag. & Nithyatharani	Asterinaceae	*Asterina*	*Impatiens viscida* Wight (Balsaminaceae),	Tamil Nadu
176	Hosagoudar (2013a)	***Asterina dichapetali*** Hansf. & Thirum	Asterinaceae	*Asterina*	*Dichapetalum gelaniodes* (Roxb.) Engl. (Dichapetalaceae),	Karnataka
177	Hosagoudar (2013a)	***Asterina diospyri*** Hosag. & C.K. Pradeep	Asterinaceae	*Asterina*	*Diospyros* sp. (Ebenaceae),	Kerala

178	Hosagoudar (2013a)	***Asterina diplocarpa*** Cooke	Asterinaceae	*Asterina*	*Sida cordata* (Burm. f.) Borssum (Malvaceae) *Sida glutinosa auct non.* Cav., *Abutilon* sp. (Malvaceae), *Solanum ferox* L. (Solanaceae)	Kerala
179	Hosagoudar (2013a)	***Asterina disciferae*** Hosag.	Asterinaceae	*Asterina*	*Eugenia discifera* Gamble (Myrtaceae)	Tamil Nadu
180	Hosagoudar (2013a)	***Asterina dissiliens*** (Syd.) Doidge	Asterinaceae	*Asterina*	*Pleurostylia opposita* (Wall.) Alston (Celastraceae), *Maytenus ovata* (Wallich ex Wight & Arn.) Loes. (Celastraceae), *Gymnosporia rothiana* (Walp) Lawson (Celastraceae)	Tamil Nadu Karnataka Maharashtra
181	Hosagoudar and Thomas (2013)	***Asterina drypetigena*** **Hosagou. & Sony Thomas**	Asterinaceae	*Asterina*	*Drypetes roxburghii* (Wallich) Hurusawa (Euphorbiaceae),	Karnataka
182	Hosagoudar (2013a)	***Asterina elaegni*** (Syd.) Syd. & Petrak	Asterinaceae	*Asterina*	*Elaegnus kologa* Schlecht. (Elaegnaceae),	Karnataka Tamil Nadu
183	Hosagoudar (2013a)	***Asterina elaeocarpicola*** Hansf.	Asterinaceae	*Asterina*	*Elaeocarpus munronii* (Wight) Masters (Elaeocarpaceae),	Kerala, Karnataka Tamil Nadu
184	Hosagoudar (2013a)	***Asterina elaeocarpi*** Syd. var. ***ovalis*** Kar & Maity	Asterinaceae	*Asterina*	*Elaeocarpus tuberculatus* Roxb (Elaeocarpaceae)	Kerala, Karnataka Tamil Nadu
185	Hosagoudar (2013a)	***Asterina elatostematis*** Hosag. & Goos	Asterinaceae	*Asterina*	*Elatostema lineolatum* Wight (Urticaceae),	Kerala,
186	Hosagoudar (2013a)	***Asterina emciciana*** Hosag. et al.	Asterinaceae	*Asterina*	*Maerua apetala* (Spreng.) M. Jacobs (Capparaceae),	Tamil Nadu

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
187	"	***Asterina enicostematis*** Hosag. & Chandr.	Asterinaceae	*Asterina*	*Enicostema axillare* (Lam.) A. Raynal. (Gentianaceae)	Kerala,
188	Hosagoudar (2013a)	***Asterina erysiphoides*** Kalch. & Cooke	Asterinaceae	*Asterina*	*Jasminum retchiei* Clarke (Oleaceae), *Jasminum rigidum* Zenker, *J. angustifolium* (L.) Willd. Don, *Jasminum cordifolium Wallich* ex G.	Kerala, Karnataka Tamil Nadu
189	Hosagoudar (2013a)	***Asterina erythropalicola*** Hosag. & Goos	Asterinaceae	*Asterina*	*Erythropalum populifolium* (Arn.) Masters (Erythropalaceae),	Kerala, Tamil Nadu
190	Hosagoudar (2013a)	***Asterina escharoides*** Syd.	Asterinaceae	*Asterina*	*Quisqualis indica* L. (Combretaceae)	
191	"	***Asterina euonymi*** Hosag. & Goos	Asterinaceae	*Asterina*	*Euonymous crenulatus* Wall. ex Wight & Arn. (Celastraceae)	Tamil Nadu
192	Hosagoudar (2013a)	***Asterina flacourtiacearum*** Hosag. & Ravikumar	Asterinaceae	*Asterina*	*Scolopia crenata* (Wgiht & Arn.) D. Clox. (Flacourtiaceae)	Karnataka Tamil Nadu
193	"	***Asterina gamsii*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Elaeocarpus variabilis* Zmarzty (*Elaeocarpus tectorius* (Lour.) Poir.) (Elaeocarpaceae)	Kerala, Tamil Nadu
194	Hosagoudar (2013a)	***Asterina garciniae*** Hansf.	Asterinaceae	*Asterina*	*Garcinia sp.* (Clusiaceae), *Garcinia travancorica* Bedd	Kerala
195	Hosagoudar (2013a)	***Asterina garciniicola*** Ouyang & Song	Asterinaceae	*Asterina*	*Garcinia* sp. (Clusiaceae),	Kerala
196	Hosagoudar (2013a)	***Asterina girardiniae*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Girardinia diversifolia* (Link) Fries (Urticaceae),	Kerala Tamil Nadu
197	"	***Asterina glycosmidigena*** Hosag. & Jacob- Thomas	Asterinaceae	*Asterina*	*Glycosmis pentaphylla* (Retz.) DC. (Rutaceae),	Karnataka Kerala

198	Hosagoudar (2013a)	***Asterina glycosmidis*** Hosag. & Rajkumar	Asterinaceae	*Asterina*	*Glycosmis* sp. (Rutaceae	Kerala
199	"	***Asterina glyptopetali*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Glyptopetalum zeylanicum* Thw. (Celastraceae),	Kerala
200	Aliyarukunju et al. (2020)	***Asterina gordoniae*** Sabeena, H. Biju, Dhanusha & Shiburaj	Asterinaceae	*Asterina*	*Gordonia obtusa* Wallich ex Wight & Arn.(Theaceae	Kerala
201	Hosagoudar (2013a)	***Asterina goosii*** Hosag. & Balakr.	Asterinaceae	*Asterina*	*Mahonia leschenaultii* (Wallich ex Wight & Arn.) Takeda ex Gamble (Berberidaceae),	Kerala Tamil Nadu
202	Hosagoudar (2013a)	***Asterina girardiniae*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Girardinia diversifolia* (Link) Fries (Urticaceae),	Kerala Tamil Nadu
203	"	***Asterina gomphandrae*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Gomphandra* sp. (Icacinaceae)	Kerala
204	Hosagoudar (2013a)	***Asterina gopalakrishnanii*** Nair & Kaul	Asterinaceae	*Asterina*	*Syzygium cumini* (L.) Skeels (Myrtaceae)	Maharashtra
205	"	***Asterina granulosa*** (Hansf.) Hosag. et al.	Asterinaceae	*Asterina*	*Scolopia crenata* (Wight & Arn.) Clos. (Flacourtiaceae),	Kerala Tamil Nadu
206	Hosagoudar (2013a)	***Asterina gymnemae*** Hosag. & Jacob-Thomas	Asterinaceae	*Asterina*	*Gymnema sylvestre* R.Br. (Asclepiadaceae),	Kerala Tamil Nadu
207	"	***Asterina gymnosporiae*** Castellani	Asterinaceae	*Asterina*	*Gymnosporia puberula* Laws. (Celastraceae),	Kerala
208	Hosagoudar (2013a)	***Asterina hakgalensis*** Hansf.	Asterinaceae	*Asterina*	*Rhododendron arboreum* J. E. Smith ssp. *nilagiricum* (Zenk.) Tagg. (Ericaceae)	Tamil Nadu
209	"	***Asterina helicteris*** Ouyang & Hu	Asterinaceae	*Asterina*	*Helicteres isora* L. (Sterculiaceae)	Kerala Tamil Nadu
210	Hosagoudar et al. (2013a)	***Asterina hemidesmi*** Hosag., Jagath Thimmaiah & Sabeena	Asterinaceae	*Asterina*	*Hemidesmus* sp. (Periplocaceae	Karnataka

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
211	Hosagoudar (2013a)	***Asterina hibisci*** (Doidge) Hosag.	Asterinaceae	*Asterina*	*Hibiscus sp.* (Malvaceae)	Kerala
212	Hosagoudar (2013a)	***Asterina homaligena*** Hosag. & Jagath Thimmaiah	Asterinaceae	*Asterina*	*Homalium zeylanica* (Gardner) Benth. (Flacourtiaceae)	Karnataka
213	Hosagoudar (2013a)	***Asterina homonoiae*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Homonoia riparia* Lour. (Euphorbiaceae),	Kerala
214	"	***Asterina hopeae*** Hosag. & Kamar.	Asterinaceae	*Asterina*	*Hopea ponga* (Dennst.) Moberly (Dipterocarpaceae),	Kerala
215	"	***Asterina hopiicola*** Hosag. & Abraham	Asterinaceae	*Asterina*	*Hopea parviflora* Bedd. (Dipterocarpaceae)	Kerala
216	Hosagoudar (2013a)	***Asterina hugoniae*** Hosag. et al.	Asterinaceae	*Asterina*	*Hugonia mystax* L. (Linaceae)	Kerala
217	"	***Asterina hydnocarpi*** Hosag. & Abraham	Asterinaceae	*Asterina*	*Hydnocarpus macrocarpa* (Bedd.) Warb. (Flacourtiaceae),	Kerala
218	"	***Asterina hydrocotyles*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Hydrocotyle* sp. (Apiaceae), *Hydrocotyle javanica* Thumb.,	Kerala
219	Hosagoudar (2013a)	***Asterina hyptidicola*** Hosag.	Asterinaceae	*Asterina*	*Hyptis sauveolense* (L.) Poit. (Lamiaceae),	Karnataka
220	"	***Asterina indica*** Syd.	Asterinaceae	*Asterina*	*Symplocos sp.* (Symplocaceae), *Symplocos cochinchinensis* ssp. *laurina (*Retz.) Nooteb. (Symplocaceae)	Kerala Tamil Nadu
221	Hosagoudar (2013a)	***Asterina ixorae*** Ryan	Asterinaceae	*Asterina*	*Ixora lanceolariae* Colebr. (Rubiaceae),	Maharashtra

222	Hosagoudar (2013a)	***Asterina jambolana*** Kar & Maity	Asterinaceae	*Asterina*	*Syzygium cumini* (L.) Skeels (Myrtaceae), *Syzygium mundagam* (Bourd.) Chithra, *Syzygium densiflorum* Wallich ex Wight & Arn., *S. jambolanum* (Lam.) DC., *Syzygium heyneanum* (Duthie) Wall. Ex Gamble	Kerala Karnataka Tamil Nadu
223	Hosagoudar (2013a)	***Asterina jasmini*** Hansf. var. ***indica*** Hosag.	Asterinaceae	*Asterina*	*Jasminum bignoniacearum* Wall. ex G. Don (Oleaceae)	Kerala Karnataka
224	"	***Asterina jasminicola*** Yates	Asterinaceae	*Asterina*	*Jasminum pubescens* Willd. (Oleaceae)	Kerala
225	Hosagoudar et al. (2012)	***Asterina kannurensis*** Hosag., Archana, Khaleel & Ramya	Asterinaceae	*Asterina*	*Clrodendrum Viscosum* Vent. (Verbinaceae)	Kerala
226	Hosagoudar (2013a)	***Asterina knemae-attenuatae*** Hosag. et al.	Asterinaceae	*Asterina*	*Knema attenuata* (Wallich ex Hook. f. & Thomson) Warb. (Myristicaceae),	Kerala
227	Hosagoudar (2013a)	***Asterina kodajadriensis*** Hosag. et al.	Asterinaceae	*Asterina*	*Capparis clegghornii* Dunn ex Gamble (Capparaceae)	Karnataka
228	"	***Asterina kukkalensis*** Hosag. et al.	Asterinaceae	*Asterina*	*Premna sp.* (Verbenaceae),	Tamil Nadu
229	Hosagoudar (2013a)	***Asterina lanneae*** Hosag. & Manoj.	Asterinaceae	*Asterina*	*Lannea coromandelica* (Houtt.) Merr. (Anacardiaceae),	Kerala
230	"	***Asterina laxiuscula*** Syd	Asterinaceae	*Asterina*	*Xanotis tomentosum* (Roxb.) Rafin. (Sapotaceae),	Maharashtra
231	Hosagoudar (2013a)	***Asterina lobeliacearum*** Hosag. et al.	Asterinaceae	*Asterina*	*Lobelia nicotinifolia* Roth ex Schultes (Lobeliaceae)	Kerala
232	"	***Asterina lycianthedis*** Hosag. & Abraham	Asterinaceae	*Asterina*	*Lycianthes laevis* (Dunnal) Bitter (Solanaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
233	Hosagoudar (2013a)	***Asterina lawsoniae*** Henn. & Nyn.	Asterinaceae	*Asterina*	*Lawsonia inermis* L. (Lythraceae)	Karnataka Kerala Tamil Nadu Maharashtra
234	"	***Asterina lepianthis*** (Hosag., Balakr. & Goos) Hosag.	Asterinaceae	*Asterina*	*Lepianthes umbellata*, (L.) Rafin. [Hackeria subpeltata (Willd.) Kunth] (Piperaceae	Karnataka Kerala Tamil Nadu
235	Hosagoudar (2013a)	***Asterina leucadis*** Hosag. & Robin	Asterinaceae	*Asterina*	*Leucas* sp. (Lamiaceae)	Karnataka
236	"	***Asterina ligustricola*** Hosag. & Kamar.	Asterinaceae	*Asterina*	*Ligustrum travencoricum* Gamble (Oleaceae),	Kerala
237	Hosagoudar (2013a)	***Asterina litseae*** Yates	Asterinaceae	*Asterina*	*Litsea deccanensis* Gamble (Lauraceae),	Tamil Nadu
238	"	***Asterina litseae-ligustrinae*** Hosag. et al.	Asterinaceae	*Asterina*	*Litsea ligustrina* (Nees) Hook. f. (Lauraceae),	Tamil Nadu
239	Hosagoudar (2013a)	***Asterina lobulifera*** Syd.	Asterinaceae	*Asterina*	*Glochidion sp.* (Euphorbiaceae),	Kerala
240	"	***Asterina lobulifera*** Syd. var. ***indica*** Hosag. & Chandr.	Asterinaceae	*Asterina*	*Glochidion* sp. (Euphorbiaceae),	Kerala
241	Hosagoudar (2013a)	***Asterina loranthigena*** Hosag. et al.	Asterinaceae	*Asterina*	*Dendrophthoe* sp. (*Loranthus* sp.) (Loranthaceae),	Karnataka Kerala Tamil Nadu
242	"	***Asterina loeseneriellae*** Hosag. & Goos	Asterinaceae	*Asterina*	*Loeseneriella obtusifolia* (Roxb.) A. C. Smith (Celastraceae),	Tamil Nadu
243	Hosagoudar (2013a)	***Asterina mabae*** M. S. Patil & Pawar	Asterinaceae	*Asterina*	*Maba nigrescens* Dalz. (Ebenaceae),	Maharashtra

244	“	***Asterina madikeriensis*** Hosag.	Asterinaceae	*Asterina*	*Memecylon sp.* (Melastomataceae),	Karnataka
245	Hosagoudar (2013a)	***Asterina mallotigena*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Malotus sp.* (Euphorbiaceae)	Kerala
246	Hosagoudar and Sabeena (2013)	***Asterina mananthavadiensis*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Argyreia* sp. (Convolvulaceae)	Kerala
247	Hosagoudar and Thomas (2013)	***Asterina melastomatigena*** Hosag. & Sony Thomas	Asterinaceae	*Asterina*	Melastomataceae member(*Osbeckia* / *Melastoma*)	Kerala
248	Hosagoudar (2013a)	***Asterina melicopecola*** Hosag. & Abraham	Asterinaceae	*Asterina*	*Melicope lunuankanda* (Gaertn.) T. G. Hartley (Rutaceae), *Euodia luna-ankenda* (Gaertner) Merr.	Kerala
249	“	***Asterina meliosmae-simplicifoliae*** Hosag. et al.	Asterinaceae	*Asterina*	*Meliosma simplicifolia* (Roxb.) Walp. (Sabiaceae),	Karnataka Kerala
250	Hosagoudar (2013a)	***Asterina memecylonis*** Ryan	Asterinaceae	*Asterina*	*Memecylon edule* Roxb. (Melastomataceae), *Memecylon sylvaticum* *Memecylon* sp.,	Karnataka Kerala Maharashtra
251	Hosagoudar (2013a)	***Asterina micheliifolia*** Hosag. & Riju	Asterinaceae	*Asterina*	*Michelia chempaka* L. (Magnoliaceae)	Kerala
252	Hosagoudar (2013a)	***Asterina micheliigena*** Hosag. & Riju	Asterinaceae	*Asterina*	*Michelia chempaka* L. (Magnoliaceae),	Kerala
253	Hosagoudar (2013a)	***Asterina morellae*** Hosag. et al.	Asterinaceae	*Asterina*	*Garcinia morella* (Gaertn.) Descr. (Clusiaceae)	Kerala
254	“	***Asterina mezonevronis*** Hosag. & Jagath Thimmaiah	Asterinaceae	*Asterina*	*Mezonevron cucullatum* (Roxb.) Wight & Arn. (Caesalpiniaceae),	Karnataka
255	Hosagoudar (2013a)	***Asterina microtropidicola*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Microtropis latifolia* Wight & Lawson (Celastraceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
256	"	***Asterina microtropidis*** Hosag. et al.	Asterinaceae	*Asterina*	*Microtropis latifolia* Wight & Lawson (Celastraceae	Kerala
257	Hosagoudar (2013a)	***Asterina microtropidis*** Hosag. et al.	Asterinaceae	*Asterina*	*Microtropis latifolia* Wight & Lawson (Celastraceae),	Kerala
258	"	***Asterina miliusae*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Miliusa* sp. (Annonaceae)	Kerala
259	Hosagoudar (2013a)	***Asterina millettiae*** Hosag. et al	Asterinaceae	*Asterina*	*Millettia rubiginosa Wight* & Arn. (Fabaceae),	Kerala
260	"	***Asterina mimusopsidicola*** Hosag. et al.	Asterinaceae	*Asterina*	*Mimusops elengi* L. (Sapotaceae),	Kerala
261	Hosagoudar (2013a)	***Asterina munnarensis*** Hosag.	Asterinaceae	*Asterina*	*Cinnamomum sp.* (Lauraceae	Kerala
262	"	***Asterina murrayae*** Hansf.	Asterinaceae	*Asterina*	of *Murraya koenigii* (Rutaceae),	Kerala
263	Hosagoudar (2013a)	***Asterina murrayicola*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Murraya exotica* (Rutaceae)	Kerala
264	Hosagoudar (2013a)	***Asterina myristicacearum*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Myristica malabarica* Lam. (Myristicaceae),	Kerala
265	"	***Asterina myristicae*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Myristica sp.* (Myristicaceae)	Kerala
266	Bhise et al. (2014)	***Asterina myrtacearum*** Bhise & Patil	Asterinaceae	*Asterina*	*Syzygium caryophyllatum*(Myrtaceae).	Maharashtra
267	Hosagoudar (2013a)	***Asterina mysorensis*** Hansf.	Asterinaceae	*Asterina*	*Ficus* sp. (Moraceae),	Karnataka
268	"	***Asterina naraveliae*** Hosag. et al.	Asterinaceae	*Asterina*	*Naravelia zeylanica* (L.) DC. (Ranunculaceae),	Karnataka Kerala
269	Hosagoudar (2013a)	***Asterina neolitsiicola*** Hosag. et al.	Asterinaceae	*Asterina*	*Neolitsea* sp. (Lauraceae), *Neolitsea scrobiculata* (Meissner) Gamble	Kerala Tamil Nadu

270	Hosagoudar (2013a)	***Asterina nothopegiae*** Ryan	Asterinaceae	*Asterina*	*N. travancorica* Bedd. ex Hook. f., *Nothopegia colebrookiana* (Wight) Blume (Anacardiaceae), *Nothopegia racemosa* (Dalz.) Ramam *N. aureo-fulva* Bedd. ex Hook. f.,	Karnataka Kerala Tamil Nadu
271	"	***Asterina olacicola*** Hansf.	Asterinaceae	*Asterina*	*Olax scandens* Roxb. (Olacaceae)	Karnataka Kerala Maharashtra
272	Hosagoudar (2013a)	***Asterina oreocnidecola*** Hosag. et al.	Asterinaceae	*Asterina*	*Oreocnide integrefolia* (Gaud. ex Wedd.) Miq. (Urticaceae), (*Villebrunea integrifolia Gaudich.*) (Urticaceae), *Villebrunea* sp.	Kerala Tamil Nadu
273	Hosagoudar (2013a)	***Asterina palaquii*** Hosag. & Goos	Asterinaceae	*Asterina*	*Palaquium ellipticum* (Dalz.) Baillon (Sapotaceae),	Tamil Nadu
274	"	***Asterina pavoniae*** Werd.	Asterinaceae	*Asterina*	*Sida rhombifolia* L. (Malvaceae)	Tamil Nadu
275	Hosagoudar (2013a)	***Asterina parsonsiae*** Hosag.	Asterinaceae	*Asterina*	*Parsonsia alboflavescens* (Dennst.) Mabberley (Apocynaceae),	Karnataka
276	Hosagoudar (2013a)	***Asterina perpusilla*** Syd.	Asterinaceae	*Asterina*	*Alangium salvifolium* (L.F) Wans) Alangiaceae	Karnataka Kerala
277	Hosagoudar and Divya (2013)	***Asterina persigena*** Hosag. & B. Divya	Asterinaceae	*Asterina*	*Persea* sp. (Lauraceae	Kerala
278	Hosagoudar (2013a)	***Asterina phyllanthi-beddomei*** Hosag. & Jacob-Thomas	Asterinaceae	*Asterina*	*Phyllanthus beddomi* (Gamble) M. Mohanan (Euporbiaceae)	Kerala
279	"	***Asterina phyllanthigena*** Hosag.	Asterinaceae	*Asterina*	*Phyllanthus* sp. (Euphorbiaceae),)	Kerala
280	Hosagoudar (2013a)	***Asterina physalidis*** Hosag. & Archana	Asterinaceae	*Asterina*	Physalis sp. (Solanaceae),	Kerala
281	"	***Asterina piperina*** Syd.	Asterinaceae	*Asterina*	*Piper* sp. (Piperaceae),	Kerala Karnataka

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
282	Hosagoudar (2013a)	***Asterina pittospori*** Hansf.	Asterinaceae	*Asterina*	*Pittosporum dasycaulon* Miq. (Pittosporaceae),	Kerala
283	"	***Asterina plectranthi*** Hosag. et al.	Asterinaceae	*Asterina*	*Plectranthus* sp. (Lamiaceae)	Kerala Tamil Nadu
284	Hosagoudar (2013a)	***Asterina plurisporus*** Ryan	Asterinaceae	*Asterina*	*Shorea talura* Roxb. (Dipterocarpaceae),	Karnataka
285	"	***Asterina pogostemonis*** Petrak	Asterinaceae	*Asterina*	*Sentellaria violacea* Heyne ex Benth. (Lamiaceae), *Pogostemon bengalensis* (Burm.f.) Kuntze (Lamiaceae)	Kerala Tamil Nadu
286	Hosagoudar (2013a)	***Asterina polygalae*** Hosag. et al.	Asterinaceae	*Asterina*	*Polygala arillata* Buch.-Ham. ex D. Don (Polygalaceae),	Tamil Nadu
287	Hosagoudar (2013a)	***Asterina pongalaparensis*** Hosag. et al	Asterinaceae	*Asterina*	*Jasminum* sp. (Oleaceae), *J. sambac* (L.) Aiton	Kerala
288	"	***Asterina prataparajii*** Hosag. & Jacob-Thomas	Asterinaceae	*Asterina*	*Tylophora dalzelli* (Burm.f.) Merr. (Asclepiadaceae),	Maharashtra
289	Hosagoudar (2013a)	***Asterina psychotriicola*** Hosag. & Archana	Asterinaceae	*Asterina*	*Psychotria* sp. (Rubiaceae),	Karnataka
290	"	***Asterina pusilla*** Syd. & Syd.	Asterinaceae	*Asterina*	*Premna corymbosa* Rottl. & Willd. (Verbenaceae), *P. serratifolia* L, *P. latifolia* Roxb.	Kerala
291	Hosagoudar (2013a)	***Asterina rhodomyrti*** Hosag. et al.	Asterinaceae	*Asterina*	*Rhodomyrtus tomentosa* (Ait.) Hassk. (Myrtaceae),	Kerala
291	Hosagoudar (2013a)	***Asterina sabiacearum*** Hosag. & Goos	Asterinaceae	*Asterina*	*Meliosma simplicifolia* (Roxb.) Walp. ssp. *pungens* (Wall ex Wight & Arn.) Beus (Sabiaceae),	Kerala Tamil Nadu
293	Hosagoudar (2013a)	***Asterina saccopetali*** Thite & Kulkarni	Asterinaceae	*Asterina*	*Saccopetalum tomentosum H.f.* & T. (Annonaceae),	Maharashtra

294	"	***Asterina samaderae*** Hosag. & Manoj.	Asterinaceae	*Asterina*	*Samadera indica Gaertn.* (Simaroubaceae),	Kerala
295	Hosagoudar (2013a)	***Asterina saracae*** Hosag. et al.	Asterinaceae	*Asterina*	*Saraca asoca* (Roxb.) de Willd. (Caesalpiniaceae),	Kerala
296	"	***Asterina sarcandrae*** Hosag. & Kamar	Asterinaceae	*Asterina*	*Sarcandra chloranthoides* Gard. (Chloranthaceae),	Kerala
297	Hosagoudar (2013a)	***Asterina scleropyri*** Hosag. & Chandr	Asterinaceae	*Asterina*	*Scleropyrum pentandrum* (Dennst.) Mabb. (Santalaceae),	Kerala
298	Hosagoudar and Sabeena (2013)	***Asterina shastavunadaensis*** Hosag. & Sabeena	Asterinaceae	*Asterina*	*Myristica* sp. (Myristicaceae)	Kerala
299	Hosagoudar (2013a)	***Asterina songii*** Hosag.	Asterinaceae	*Asterina*	*Eurya nitida* Korth. (Theaceae)	Kerala Tamil Nadu
300	"	***Asterina suttonii*** Hosag. et al.	Asterinaceae	*Asterina*	*Symplocos* sp. (Symplocaceae),	Kerala Tamil Nadu
301	Hosagoudar (2013a)	***Asterina talacauveriana*** Hosag.	Asterinaceae	*Asterina*	*Scolopia sp.* (Flacourtiaceae),	Tamil Nadu
302	Hosagoudar (2013a)	***Asterina tertia*** Racib.	Asterinaceae	*Asterina*	*Adhatoda zeylanica* Medikus (Acanthaceae),	Kerala Karnataka Tamil Nadu
303	Hosagoudar (2013a)	***Asterina tertia*** Racib. var**.** ***africana*** Doidge	Asterinaceae	*Asterina*	*Adhatoda beddomei* C. B. Clarke (Acanthaceae),	Kerala Tamil Nadu
304	Hosagoudar and Thomas (2013)	***Asterina terminaliae-paniculatae*** Hosag. & Sony Thomas	Asterinaceae	*Asterina*	*Terminalia paniculata* Roth. (Combretaceae),	Kerala
305	Hosagoudar (2013a)	***Asterina theacearum*** Hosag. et al.	Asterinaceae	*Asterina*	*Eurya* sp. (Theaceae), *Eurya japonica* Thunb.,	Kerala
306	Hosagoudar et al. (2013a)	***Asterina thevalakkaraensis*** Hosag. & Bindu	Asterinaceae	*Asterina*	*Hydnocarpus* sp. (Flacourtiaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
307	Hosagoudar (2013a)	***Asterina thotteae*** Hosag. & Hanlin	Asterinaceae	*Asterina*	*Thottea siliquosa* (Lam.) Ding Hou. (Aristolochiaceae),	Kerala Karnataka Tamil Nadu
308	"	***Asterina thunbergiicola*** Hansf. var. *indica* Hosag. & Jacob-Thomas	Asterinaceae	*Asterina*	*Thunbergia* sp. (Thunbergiaceae),	Kerala
309	Hosagoudar (2013a)	***Asterina tinosporae*** Hansf.	Asterinaceae	*Asterina*	*Tinospora cordifolia* (Willd.) Miers (Menispermaceae),	Kerala
310	"	***Asterina toddaliae*** Kar & Ghosh	Asterinaceae	*Asterina*	*Toddalia asiatica (*L.) Lam. (Rutaceae),	Kerala Tamil Nadu
311	Hosagoudar (2013a)	***Asterina toddaliicola*** Hosag. et al.	Asterinaceae	*Asterina*	*Toddalia sp.* (Rutaceae)	Karnataka
312	"	***Asterina toxocarpi*** Hosag. & C.K. Biju	Asterinaceae	*Asterina*	*Toxocarpus sp.* (Asclepiadaceae)	Kerala
313	Hosagoudar (2013b)	***Asterina tragiae*** Hosag. & Jagath Timmaih	Asterinaceae	*Asterina*	*Tragia* sp. (Euphorbiaceae)	Karnataka
314	Hosagoudar (2013a)	***Asterina travancorensis*** Syd. & Syd	Asterinaceae	*Asterina*	*Wattakaka volubilis* (L. f.) Stapf. (*Marsdenia volubilis* (L. f.) Cooke) (Asclepiacaceae),	Tamil Nadu Kerala
315	"	***Asterina trichiliae*** Doidge	Asterinaceae	*Asterina*	*Trichilia connaroides* (Wight & Arn.) Bentv. (Meliaceae)	Tamil Nadu
316	Hosagoudar (2013a)	***Asterina triumfetticola*** Yamam.	Asterinaceae	*Asterina*	*Triumfetta* sp. (Tiliaceae),	Kerala
317	"	***Asterina tylophorae-indicae*** Hosag. et al.	Asterinaceae	*Asterina*	*Tylophora indica* (Burm. f.) Merr. (Asclepiadaceae),	Kerala
318	Hosagoudar and Fathima (2013)	***Asterina urticacearum*** Hosag. & Fathima	Asterinaceae	*Asterina*	Urticaceae member,	Kerala

319	Hosagoudar et al. (2013a)	***Asterina ushae*** Hosag.,Jagath Thimmaiah & Archana Hosag. & Fathima	Asterinaceae	*Asterina*	*Glochidion bourdillonii* Gamble (Euphorbiaceae)	Karnataka
320	Hosagoudar (2013a)	***Asterina viburnicola*** Hosag	Asterinaceae	*Asterina*	*Viburnum cylindricum* Buch.-Ham. ex D. Don (Caprifoliaceae),	Tamil Nadu
321	Hosagoudar et al. (2013a)	***Asterina vitacearum*** Hosag., Jagath Thimmaiah & Sabeena	Asterinaceae	*Asterina*	*Cissus repens* Lam. (Vitaceae)	Karnataka
322	Hosagoudar (2013a)	***Asterina visci*** Hosag.	Asterinaceae	*Asterina*	*Viscum* sp. (Loranthaceae),	Kerala
323	Hosagoudar (2013a)	***Asterina wingfieldii*** Hosag. et al.	Asterinaceae	*Asterina*	*Grewia* sp. (Tiliaceae),	Kerala
324	"	***Asterina woodfordiae*** Sahni	Asterinaceae	*Asterina*	Woodfordia fruticosa (L.) Kurz (Lythraceae),	Maharashtra
325	Hosagoudar (2013a)	***Asterina wrightiae*** Syd.	Asterinaceae	*Asterina*	*Wrightia tinctoria* (Roxb.) R. Br. (Apocynaceae),	Kerala
326	Hosagoudar (2013a)	***Asterina xanthophylli*** Hosag. et al.	Asterinaceae	*Asterina*	*Xanthophyllum arnottianum* Wight (*X. flavescens auct. Non* Roxb.) (Xanthophyllaceae),	Kerala
327	Hosagoudar (2013b)	***Asterina xyliae*** Hosag.	Asterinaceae	*Asterina*	*Xylia xylocarpa* Roxb. (Mimosaceae),	Kerala
328	Hosagoudar (2013a)	***Asterolibertia hydnocarpi*** Hosag. & Abraham	Asterinaceae	*Asterolibertia*	*Hydnocarpus macrocarpus* (Bedd.) Warb. (Flacourtiaceae),	Kerala
329	Hosagoudar (2013a)	***Asterolibertia mangiferae*** Hansf. & Thirum.	Asterinaceae	*Asterolibertia*	*Mangifera indica* L. (Anacardiaceae),	Kerala
330	"	***Asterolibertia nothopegiae*** Hosag. & Abraham	Asterinaceae	*Asterolibertia*	*Nothopegia colebrookiana* (Wight) Blume (Anacardiaceae), *N. travancorica* Bedd. ex Hook. f.,	Kerala
331	Hosagoudar (2013a)	***Asterolibertia vateriae*** Hosag.	Asterinaceae	*Asterolibertia*	*Vateria indica* L. (Dipterocarpaceae)	Karnataka Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
332	Hosagoudar (2013a)	***Asterostomella* anogeissi** Hosag. & Archana	Asterinaceae	*Asterostomella*	*Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Guill. & Perr. (Combretaceae)	Maharashtra
333	Hosagoudar (2013a)	***Asterostomella baliospermi*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Baliospermum montanum* (Willd.) Muell. - Arg. (Euphorbiaceae),	Kerala
334	Hosagoudar (2013a)	***Asterostomella boehmeriae*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Boehmeria glomerulifera* Miq. (Urticaceae),	Kerala, Tamil Nadu
335	"	***Asterostomella ceropegiae*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Ceropegia* sp. (Asclepiadaceae),	Kerala
336	Hosagoudar (2013a)	***Asterostomella daphniphylli*** Hosag. & Ravikumar	Asterinaceae	*Asterostomella*	*Daphniphyllum neilgherrense* (Wight) K. Rosenthal (Daphniphyllaceae	Kerala, Tamil Nadu
337	Hosagoudar et al. (2013a)	***Asterostomella derridicola*** Hosag., Jagath Thimmaiah & Sabeena	Asterinaceae	*Asterostomella*	*Derris canarensis* (Dalz.) Baker (Fabaceae	Karnataka
338	Hosagoudar (2013a)	***Asterostomella dilleniacearum*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Dillenia pentagyna* Roxb. (Dilleniaceae)	Kerala
339	"	***Asterostomella elaeocarpi-serrati*** Hosag.	Asterinaceae	*Asterostomella*	*Elaeocarpus serratus* L. (Elaeocarpaceae),	Kerala Karnataka
340	Hosagoudar (2013a)	***Asterostomella excoecariicola*** Hosag. & Goos	Asterinaceae	*Asterostomella*	*Excoecaria crenulata* Wight (Euphorbaceae),	Tamil Nadu
341	Hosagoudar (2013a)	***Asterostomella flacourtiae-montanae*** Hosag. & Sabeena	Asterinaceae	*Asterostomella*	*Flacourtia montana* Graham (Flacourtiaceae)	Kerala
342	"	***Asterostomella isonadrae*** Hosag. & Goos	Asterinaceae	*Asterostomella*	*Isonandra lanceolata* Wight forma *anfractuosa* (Clarke) Jewken (Sapotaceae	Tamil Nadu
343	Hosagoudar (2013a)	***Asterostomella ligustri*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Ligustrum bamlei* Ramam. (Oleaceae),	Tamil Nadu

344	“	***Asterostomella meliosmigena*** Hosag.	Asterinaceae	*Asterostomella*	*Meliosma simplicifolia* (Roxb.) Walp. (Sabiaceae),	Kerala Tamil Nadu
345	Hosagoudar (2013a)	***Asterostomella micheliae*** Hosag. & Goos	Asterinaceae	*Asterostomella*	*Michelia nilagirica* Zenker (Magnoliaceae)	Tamil Nadu
346	“	***Asterostomella otonephelii*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Otonephelium stipulaceum* (Bedd.) Radlk. (Sapindaceae),	Kerala
347	Hosagoudar (2013a)	***Asterostomella radermacherae*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Radermachera xylocarpa* (Roxb.) K. Schum. (Bignonaceae),	Kerala
348	Sabeena and Hosagoudar (2018a, b)	***Asterostomella salacigena*** Sabeena & Hosag.	Asterinaceae	*Asterostomella*	*Salacia* sp. (Celastraceae),	Kerala
349	Hosagoudar (2013a)	***Asterostomella scolopiae-crenatae*** Hosag. & Abraham	Asterinaceae	*Asterostomella*	*Scolopia crenata* (Wight & Arn.) D. Clos (Flacourtiaceae),	Kerala
350	Hosagoudar (2013a)	***Asterostomella strombosiae***Hosag. etal	Asterinaceae	*Asterostomella*	*Strombosia ceylanica* Garden. (Olaceae),	Karnataka
351	Hosagoudar (2013a)	***Asterostomella terminaliae*** Hosag. et al.	Asterinaceae	*Asterostomella*	*Terminalia paniculata* Roth. (Combertaceae), *T. arjuna* (Roxb. ex DC.) Wight & Arn	Tamil Nadu
352	Hosagoudar (2013a)	***Asterostomella ziziphina*** Hosag. & Archana	Asterinaceae	*Asterostomella*	*Zizyphus rugosa* Lam. (Rhamnaceae)	Kerala
353	Hosagoudar et al. (2013a)	***Asterostomella vernoniae*** Hosag., Jagath Thimmaiah & Archana	Asterinaceae	*Asterostomella*	*Vernonia monosis* Benth. ex C.B. Clarke (Asteraceae)	Karnataka
354	Hosagoudar (2013a)	***Asterostomula loranthi*** Theiss.	Asterinaceae	*Asterostomulla*	*Loranthus* sp. (Loranthaceae),	Kerala
355	“	***Asterostomula syzygii*** Hosag. et al.	Asterinaceae	*Asterostomula*	*Syzygium* sp. (Myrtaceae),	Kerala
356	Hosagoudar (2013a)	***Balladyna salaciae*** Hosag. et al.	Parodiellinaceae	*Balladyna*	*Salacia oblonga* Wallich ex Wight & Arn. (Hippochrataceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
357		***Basavamyces litseae*** Hosag. et al.	Meliolaceae	*Basavamyces*	*Litsea* sp. (Lauraceae)	Kerala
358	Hosagoudar (2013a)	***Basavamyces patilii*** Hosag.	Meliolaceae	*Basavamyces*	*Litsea* sp. (Lauraceae)	Kerala
359	Hosagoudar (2013a)	***Bheemamyces argyreicola*** Hosag. & Sabeena & Riju	Asterinaceae	***Bheemamyces***	*Argyreia nervosa* (Burm.f.) Bojer. (Convolvulaceae),	Kerala
360	Hosagoudar (2013a)	***Bheemamyces argyreiae*** (Hansf.) Hosag.	Asterinaceae	***Bheemamyces***	*Argyreia nervosa* (Burm.f.) Bojer. (Convolvulaceae)	Kerala
361	Hosagoudar (2013a)	***Bheemamyces capparidis*** Hosag. & Sabeena	Asterinaceae	*Bheemamyces*	*Capparis* sp. (Capparaceae)	Kerala
362	Hosagoudar and Thomas (2013)	***Bheemamyces jasmini*** V.B. Hosagoudar & Sony Thomas	Asterinaceae	*Bheemamyces*	*Jasminum* sp. (Oleaceae)	Kerala
363	Hosagoudar and Fathima (2013)	***Bheemamyces oleae*** Hosag. & Fathima	Asterinaceae	*Bheemamyces*	*Olea polygama* Wight (Oleaceae),	Kerala
364	Hosagoudar (2013a)	***Bramhamyces ilecis*** Hosag. & Chandr.	Asterinaceae	*Bramhamyces*	*Ilex wightiana* Wall. (Aquifoliaceae),	Kerala
365	Hosagoudar (2013a)	***Cirsosia arecacearum*** Hosag. & Pillai	Lembosiaceae	*Cirsosia*	*Calamus thwaitesii* Beccary ex Hook f. (Arecaceae)	Karnataka
366	Hosagoudar (2013a)	***Cirsosia globuliferae*** (Pat.) Arn.	Lembosiaceae	*Cirsosia*	*Calamus pseudotenuis* (Arecaceae)	Kerala Tamil Nadu
367	"	***Cirsosia hopeae*** Hosag. & Jacob-Thomas	Lembosiaceae	*Cirsosia*	*Hopea ponga* (Dennst.) Mabb. (Dipterocarpaceae),	Kerala
368	Mathew et al. (2017)	***Cirsosia humboldtigena*** Lini, Neeta and Swapna	Lembosiaceae	*Cirsosia*	*Humboldtiavahliana*Wight (Caesalpiniaceae)	Kerala
369	Hosagoudar (2013a)	***Cirsosia litseae*** Hosag. & Archana	Lembosiaceae	*Cirsosia*	*Litsea travancorica* (Lauraceae),	Kerala

370	“	***Cirsosia vateriae*** Hosag.	Lembosiaceae	*Cirsosia*	*Vateria indica* L. (Dipterocarpaceae),	Karnataka Kerala
371	Hosagoudar (2013a)	***Dysrhynchis palmicola*** (Syd.) Arx	Parodiellinaceae	*Dysrhynchis*	*Elaeis guineensis* Jacq.(Arecaceae)	Kerala
372	Hosagoudar (2013a)	***Dysrhynchis uncinata*** (Syd.) Arx	Parodiellinaceae	*Dysrhynchis*	*Ochlandra travancorica* Benth. ex Gamble(Poaceae)	Kerala
373	Hosagoudar (2013a)	***Echidnodella hopeae*** Hosag. et al.	Lembosiaceae	*Echidnodella*	*Hopea ponga* (Dennst.) Mabberley (Dipterocarpaceae),	Kerala
374	“	***Echidnodella manilkarae*** Hosag. & T. Sabu	Lembosiaceae	*Echidnodella*	*Manilkara hexandra* (Roxb.) Dubard (Saportaceae)	Kerala
375	Hosagoudar (2013a)	***Echidnodella memecyli*** Hosag. & Abraham	Lembosiaceae	*Echidnodella*	*Memecylon* sp., Melastomataceae)	Kerala Maharastra
376	Hosagoudar et al. (2013a)	***Echinodella mimusopsidis*** Hosag., Jagath Thimmaiah & Sabeena	Lembosiaceae	*Echidnodella*	*Mimusops elengi* L. (Sapotaceae),	Karnataka
377	Hosagoudar and Divya (2013)	***Echidnodella myristicacearum*** Hosag. & Divya	Lembosiaceae	*Echidnodella*	Myristicaceae member	Kerala
378	Hosagoudar (2013a)	***Echidnodella polyalthiae*** Hosag.	Lembosiaceae	*Echidnodella*	*Polyalthia* sp. (Annonaceae), *P. longifolia* (Sonn.) Thwaites,	Kerala
379	Hosagoudar (2013a)	***Echidnoides pandanicola*** Hosag. & Hanlin	Lembosiaceae	*Echidnodella*	*Pandanus* sp. (Pandanaceae),	Kerala Tamil Nadu
380	“	***Echidnodella vateriae*** Hosag. & Kamar.	Lembosiaceae	*Echidnodella*	*Vateria indica* L. (Diptocarpaceae),	Kerala
381	Hosagoudar (2013a)	***Ectendomeliola walsurae*** Hosag. & Agarwal	Meliolaceae	*Ectendomeliola*	*Walsura trifolia* (A.Juss.) Harms (Meliaceae)	Kerala
382	Hosagoudar (2013a)	***Englerula coscinii*** VB Hosagoudar, A Sabeena & M Kamarudeen	Asterinaceae	*Englerula*	*Coscinium fenestratum* (Gaertn.) Colebr. (Menispermaceae),	Kerala
383	Hosagoudar (2013a)	***Eupelte amicta*** Syd.	Lembosiaceae	*Eupelte*	*Olea dioica* Roxb. (Oleaceae)	Kerala Karnataka

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
384	"	***Irenopsis benguetensis*** Stev. & Rold. ex Hansf.	Meliolaceae	*Irenopsis*	*Ficus tinctoria* Forst *F. exasperata* Vahl *F. gibbosa* Blume *F. gibbosa* (Blume) Corner var. *cuspidifera* (Miq.) Chitra *F. hispida* L.f. *F. nervosa* Heyne ex Roth, *Ficus asperrima* Roxb.(Moraceae)	Kerala
385	"	***Irenopsis boehmeriae*** Gosh et al.	Meliolaceae	*Irenopsis*	*Boehmeria platyphylla* D. Don. (Urticaceae)	Kerala
386	Hosagoudar (2013a)	***Irenopsis chukrasiae*** Hosag.	Meliolaceae	*Irenopsis*	*Chukrasia tabularis* A. Juss. (Meliaceae)	Kerala
387	"	***Irenopsis coimbatorica*** Hosag. et al.	Meliolaceae	*Irenopsis*	*Grewia* sp. (Tiliaceae)	Tamil Nadu
388	Hosagoudar (2013a)	***Irenopsis crotonis*** (Stev. & Tehon) Stev.	Meliolaceae	*Irenopsis*	*Pavetta indica* L.	Maharastra
389	Hosagoudar (2013a)	***Irenopsis eriolaenae*** Hosag.	Meliolaceae	*Irenopsis*	*Eriolaena quinquelocularis* (Wight & Arn.) Wight. (Sterculiaceae)	Kerala
390	Hosagoudar (2013a)	***Irenopsis gordoniae*** Hosag. et al.	Meliolaceae	*Irenopsis*	*Gordonia* sp. (Thaceae)	Kerala
391	Hosagoudar (2013a)	***Irenopsis helicteridis*** Hosag.	Meliolaceae	*Irenopsis*	*Helicteres isora* L.	Kerala
392	Hosagoudar (2013a)	***Irenopsis indica*** (Anahosur) Hosag.	Meliolaceae	*Irenopsis*	*Aphanamixis polystachya* (Wall.) Parker	Karnataka
393	"	***Irenopsis kleinhoviae*** Hosag. & Archana	Meliolaceae	*Irenopsis*	*Kleinhovia hospita* L. (Sterculiaceae)	Kerala
394	Hosagoudar (2013a)	***Irenopsis leeae*** Hansf. var. ***javensis*** Hansf.	Meliolaceae	*Irenopsis*	*Leea indica* (Burm.f.) Merr. (Leeaceae)	Kerala

395	“	***Irenopsis ligustri* Patil & Maham.**	Meliolaceae	*Irenopsis*	*Ligustrum neilgherrense* Wight (Oleaceae)	Kerala
396	Hosagoudar (2013a)	***Irenopsis loranthicola*** Hosag. et Riju	Meliolaceae	*Irenopsis*	*Loranthus* sp. (Loranthaceae)	Kerala
397	Hosagoudar and Thomas (2013)	***Irenopsis macarangigena*** Hosag. & Sony Thomas	Meliolaceae	*Irenopsis*	*Macaranga peltata* (Roxb.) Muel-Arg. (Euphorbiaceae),	Kerala
398	Hosagoudar (2013a)	***Irenopsis molleriana*** (Wint.) Stev.	Meliolaceae	*Irenopsis*	*H. molleriana* *Hibiscus hispidissimus* Griffith, *Hibiscus furcatus* Roxb. (Malvaceae)	Kerala
399	Hosagoudar (2013a)	***Irenopsis mudumalaiensis*** Hosag.	Meliolaceae	*Irenopsis*	*Kydia calycina* Roxb. (Malvaceae)	Kerala
400	Hosagoudar (2013a)	***Irenopsis mysorensis*** Hansf. & Thirum.	Meliolaceae	*Irenopsis*	*Gnidia glauca* (Fresen.) Gilg [*Lasiosiphon Ieriocephalus* (Meisner) Decne] (Thymeleaceae)	Karnataka
401	Hosagoudar (2013a)	***Irenopsis nephelii*** Patil & Maham.	Meliolaceae	*Irenopsis*	*Nephelium longan* Lour. (Sapindaceae)	Kerala
402	Hosagoudar (2013a)	***Irenopsis pavoniae*** Hosag. et al.	Meliolaceae	*Irenopsis*	Pavonia sp. *(Malvaceae)*	Kerala
403	Hosagoudar (2013a)	***Irenopsis pterigotae*** Hosag. et al.	Meliolaceae	*Irenopsis*	*Pterigota alata* (Roxb.) R.Br.	Kerala
404	“	***Irenopsis rubi*** Patil & Maham.	Meliolaceae	*Irenopsis*	*Rubus moluccanussensu* Hook.f. (Rosaceae)	Kerala
405	Hosagoudar (2013a)	***Irenopsis sidae*** (Rehm) Hughes	Meliolaceae	*Irenopsis*	*Sida cordata* (Burm. f.) Borssum (Malvaceae)	Kerala
406	Hosagoudar (2013a)	***Irenopsis sidae*** (Rehm) Hughes var. ***indica*** Hosag. & Manoj.	Meliolaceae	*Irenopsis*	*Sida cordata* (Burm. f.) Borssum (Malvaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
407	Hosagoudar (2013a)	***Irenopsis thespesiae*** Hansf.	Meliolaceae	*Irenopsis*	*Thespesia lampas* (Cav.) Dalz. ex Dalz. & Gibs(malvaceae)	Kerala
408	"	***Irenopsis tjibodensis*** Hansf.	Meliolaceae	*Irenopsis*	*Pterospermum diversifolium* Blume (Sterculiaceae)	Kerala
409	Hosagoudar (2013a)	***Irenopsis trichiliae*** Hosag. & Riju	Meliolaceae	*Irenopsis*	*Trichilia* sp. (Meliaceae)	Kerala
410	Hosagoudar (2013a)	***Irenopsis triumfettae*** (Stev.) Hansf. & Deight.	Meliolaceae	*Irenopsis*	*Triumfetta pilosa* Roth (Tiliaceae), *Triumfetta bartramia* L., *Triumfetta rhomboidea* Jacq.	Kerala
411	Hosagoudar (2013a)	***Irenopsis triumfettae*** Stev. var. ***indica*** Hosag. & Abraham	Meliolaceae	*Irenopsis*	*Triumfetta* sp. (Tiliaceae)	Kerala
412	Hosagoudar (2013a)	***Irenopsis vaccinii*** Hosag. et al.	Meliolaceae	*Irenopsis*	*Vaccinium leschenaultii* Wight	Kerala
413	"	***Irenopsis xanthophylli*** Hosag.	Meliolaceae	*Irenopsis*	*Xanthophyllum flavescens* Roxb. (Xanthophyllaceae)	Kerala
414	Hosagoudar (2013a)	***Irenopsis xeromphidis*** Hosag. & Sabeena	Meliolaceae	*Irenopsis*	*Xeromphis uliginosa* (Thunb.) Keay (Rubiaceae),	Kerala
415	**"**	***Ishwaramyces flacourtiae*** Hosag. et al.	Asterinaceae	*Ishwaramyces*	*Flacourtia montana* Graham (Flacourtiaceae), *F. indica* (Burm. f.) Merr.	Kerala
416	Hosagoudar (2013a)	***Lembosia araliacearum*** Hosag. & Kamar.	Lembosiaceae	Lembosia	Araliaceae member	Kerala
417	"	***Lembosia decalvans*** Pat	Lembosiaceae	Lembosia	*Pachygone ovata* (Poir.) Miers ex Hook. f. & Thoms. (Menispermaceae),	Tamil Nadu
418	Hosagoudar (2013a)	***Lembosia garciniae*** Hosag. & Jagath Thimmaiah	Lembosiaceae	Lembosia	*Garcinia gummigutta* (L.) Robs. (Clusiaceae),	Karnataka

419	Hosagoudar and Divya (2013)	***Lembosia hopiigena*** Hosag. & A. Sabeena	Lembosiaceae	Lembosia	*Hopea* sp. (Dipterocarpaceae)	Kerala
420	Hosagoudar (2013a)	***Lembosia hosagoudarii*** Sivanesan & Shivas	Lembosiaceae	Lembosia	*Syzygium tamilnadensis* Rathakr. & Chitra (Myrtaceae), *S. arnottianum* Walp.,	Karnataka Kerala
421	Hosagoudar (2013a)	***Lembosia humboldtiae*** Hosag. & Abraham	Lembosiaceae	Lembosia	*Humboldtia vahliana* Wight (Caesalpiniaceae),	Kerala
422	Hosagoudar (2013a)	***Lembosia humboldtiicola*** Hosag. et al.	Lembosiaceae	Lembosia	*Humboldtia unijuga* Bedd. (Caesalpiniaceae),	Kerala
423	"	***Lembosia humboldtiigena*** Hosag. et al.	Lembosiaceae	Lembosia	*Humboldtia vahliana* Wight (Caesalpiniaceae),	Kerala
424	Hosagoudar (2013a)	***Lembosia incisa*** (Syd.) Theiss	Lembosiaceae	Lembosia	*Webera corymbosa* Willd. (Rubiaceae),	Karnataka
425	"	***Lembosia lagerstroemiae*** Hosag. & Abraham	Lembosiaceae	Lembosia	*Lagerstroemia microcarpa* Wight (Lytheracae)	Kerala
426	Hosagoudar (2013a)	***Lembosia linocierae*** Hosag.	Lembosiaceae	Lembosia	*Linociera* sp. (Oleaceae),	Kerala
427	Bhise et al. (2014)	***Lembosia mahabaleshwarensis*** Bhise & Patil	Lembosiaceae	Lembosia	*Syzygium rubicundum*(Myrtaceae).	Maharashtra
428	Hosagoudar (2013a)	***Lembosia malabarensis*** (Syd. & Syd.) Hosag. & Goos	Lembosiaceae	Lembosia	*Pothos scandens* L. (Araceae),	Kerala
429	Hosagoudar (2013a)	***Lembosia memecylicola*** Hosag.	Lembosiaceae	Lembosia	*Memecylon* sp. (Melastomataceae),	Karnataka
430	"	***Lembosia ormosiae*** Yamam.	Lembosiaceae	Lembosia	*Ormosia travancorica* Bedd. (Fabaceae),	Kerala
431	Hosagoudar (2013a)	***Lembosia pandanacearum*** Hosag. & Jagath Thimmaiah	Lembosiaceae	Lembosia	*Pandanus sp.* (Pandanaceae),	Karnataka
432	Hosagoudar (2013a)	***Lembosia perseae*** Orejuela	Lembosiaceae	Lembosia	*Persea macrantha* (Nees) Kosterm. (Lauraceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
433	"	***Lembosia salaciae*** Hosag. & Archana	Lembosiaceae	Lembosia	*Salacia* sp. (Hippocrataceae),	Kerala.
434	Hosagoudar (2013a)	***Lembosia shoreae*** (Ryan) Hosag. & B. Song	Lembosiaceae	Lembosia	*Shorea talura* Roxb. (Dipterocarpaceae),	Karnataka
435	"	***Lembosia terminaliae-chebulae*** Hosag., Abraham & Crane	Lembosiaceae	Lembosia	*Terminalia chebula* Retz. (Combretaceae),	Kerala.
436	Hosagoudar (2013a)	***Mahanteshamyces agrostachydis*** Hosag. & C.K. Biju	Asterinaceae	*Mahanteshamyces*	*Agrostistachys indica* Dalz. (Euphorbiaceae),	Kerala.
437	"	***Maheshwaramyces coculi*** Hosag. & Archana	Asterinaceae	*Mahanteshamyces*	*Coculus laurifolius* (Menispermaceae),	Kerala.
438	Hosagoudar et al. (2013a)	***Mahanteshamyces litseae*** Hosag., Jagath Thimmaiah & Sabeena	Asterinaceae	*Mahanteshamyces*	*Litsea* sp. (Lauraceae)	Karnataka
439	Hosagoudar (2013a)	***Maheshwaramyces pachygones*** Hosag. et al.	Asterinaceae	*Mahanteshamyces*	*Pachygone ovata* (Poir.) Miers ex Hook. f. & Thoms. (Menispermaceae),	Kerala
440	Hosagoudar (2013a)	***Meliola abdulkalamii*** Hosag. et Riju	Meliolaceae	*Meliola*	*Aralia* sp. (Araliaceae),	Kerala
441	"	***Meliola abri*** Hosag. et Riju	Meliolaceae	*Meliola*	*Abrus pulchellus* Wallich ex Thwaites (Fabaceae),	Kerala
442	Hosagoudar (2013a)	***Meliola abrupta*** Sydow & Sydow	Meliolaceae	*Meliola*	*Derris* sp. (Fabaceae)	Kerala
443	Hosagoudar (2013a)	***Meliola actinodaphnecola*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Actinodaphne malabarica* Balakr. (Lauraceae)	Kerala
444	Hosagoudar (2013a)	***Meliola actephilae*** Hosag. et al.	Meliolaceae	*Meliola*	*Actephila excelsa* (Dalz.) Muell.-Arg. (Euphorbiaceae)	Kerala
445	Hosagoudar (2013a)	***Meliola adenanthericola*** Hosag. et al.	Meliolaceae	*Meliola*	*Adenanthera pavonia* L. (Mimosaceae)	Kerala

446	Hosagoudar (2013a)	***Meliola aethiops*** Sacc. var. ***longiseta*** Deight.	Meliolaceae	*Meliola*	*Mimosa intsia* L. (Mimosaceae)	Kerala
447	"	***Meliola africana*** Hansf	Meliolaceae	*Meliola*	*Canthium angustifolium* Roxb.	Kerala
448	Hosagoudar (2013a)	***Meliola aganopes*** Hosag. et al.	Meliolaceae	*Meliola*	*Aganope thyrsiflora* (Benth.) Polh (Fabaceae)	Kerala
449	"	***Meliola aglaicola*** Hansf.	Meliolaceae	*Meliola*	*Aglaia minutiflora* Bedd.(Meliaceae)	Kerala
450	Hosagoudar (2013a)	***Meliola agrostistachydis*** Hosag. & Rajkumar	Meliolaceae	*Meliola*	*Agrostistachys borneensis* Becc. (Euphorbiaceae)	Kerala
451	Hosagoudar (2013a)	***Meliola ailanthi*** Sharma et al.	Meliolaceae	*Meliola*	*Ailanthus triphysa* (Dennst.) Alston (Simaroubaceae)	Tamil Nadu Kerala
452	Hosagoudar (2013a)	***Meliola ailanthicola*** Hosag. & Riju	Meliolaceae	*Meliola*	*Ailanthus triphysa malabarica* (Dennst.) Alston (Simaroubaceae)	Kerala
453	Hosagoudar (2013a)	***Meliola altissimae*** Hosag.	Meliolaceae	*Meliola*	*Vitex altissima* L. (Verbenaceae)	Kerala
454	Hosagoudar (2013a)	***Meliola allophyligena*** Hosag.	Meliolaceae	*Meliola*	*Allophylus serrulatus* Radlk.	Kerala
455	"	***Meliola allophyli-concanici*** Hosag.	Meliolaceae	*Meliola*	*Allophyllus concanicus* Radlk. var. *lanceolatus* Gambl (Sapindaceae)	Kerala
456	Hosagoudar (2013a)	***Meliola allophyli-serrulati*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Allophylus serrulatus* Raddlk (Sapindaceae)	Kerala
457	"	***Meliola anceps*** Syd. & Syd.	Meliolaceae	*Meliola*	*Mussaenda belilla* Buch.- Ham. (*M. laxa* Hook.f.) Hutch. ex Gamble (Rubiaceae)	Kerala
458	Hosagoudar (2013a)	***Meliola anisophylleae*** Hansf. & Deight. var. ***caralliae*** Hosag. et al.	Meliolaceae	*Meliola*	*Carallia integrerrhima* DC. (Rhizophoraceae)	Kerala
459	Hosagoudar (2013a)	***Meliola aphanamixidis*** Hosag.	Meliolaceae	*Meliola*	*Aphanamixis polystachya* (Wall.) Parker (Meliaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
460	"	***Meliota aporusoe*** V. B. Hosagoudar et P. J. Robin	Meliolaceae	*Meliola*	*Aporusa* sp. (Euphorbiaceae)	Kerala
461	Hosagoudar (2013a)	***Meliola ardisiicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Ardisia missionis* Wall. ex A. DC. (Myrsinaceae)	Kerala
462	"	***Meliola ardisiigena*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Ardisia* sp.(Myrsinaceae)	Kerala
463	Hosagoudar (2013a)	***Meliola arkevermae*** Hosagoudar & Sabeena	Meliolaceae	*Meliola*	Meliaceae member	Kerala
464	Hosagoudar (2013a)	***Meliola aristolochigena*** Hosag. & Archana	Meliolaceae	*Meliola*	*Aristolochia tagala* Cham. (Aristolochiaceae)	Kerala
465	Hosagoudar (2013a)	***Meliola artocarpi*** Yates	Meliolaceae	*Meliola*	*Artocarpus heterophyllus* Lam. (Moraceae)	Kerala
466	"	***Meliola artocarpi*** Yates var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Artocarpus gomezianus*Wall. ex Tree. subsp. *zeylanicus* Jarret (Moraceae)	Karnataka
467	Hosagoudar (2013a)	***Meliola atalantiae*** Hosag.	Meliolaceae	*Meliola*	*Atalantia wightii* Tanaka (Rutaceae)	Kerala
468	Hosagoudar (2013a)	***Meliola attayarica*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Memecylon* sp. (Melastomataceae)	Kerala
469		***Meliola atylosiae*** Hosag.	Meliolaceae	*Meliola*	*Atylosia lineata* Wight & Arn. (Fabaceae)	Kerala
470	Hosagoudar (2013a)	***Meliola bakeri*** Sydow	Meliolaceae	*Meliola*	*Cayrtia pedata* (Lam.) A. L. Juss ex Gagnepain (Vitaceae);	Kerala
471	"	**Meliola banasuranii** *Hosag.* et al.	Meliolaceae	*Meliola*	*Actinodaphne* sp. (Lauraceae)	Kerala
472	Hosagoudar (2013a)	***Meliola beilschmediicola*** Hosag.	Meliolaceae	*Meliola*	*Beilschmiedia wightii* (Nees) Benth. ex Hook.f. (Lauraceae)	Kerala

473	Hosagoudar (2013a)	***Meliola beilschmiediae*** Yamam. var. ***cinnamomicola*** Hosag.	Meliolaceae	*Meliola*	*Cinnamomum malabatrum* (Burm. f.) Blume (Lauraceae)	Kerala
474	Lonkar et al. (2018)	***Meliola bhimashankarensis*** Lonkar, Patil & Salunkhe	Meliolaceae	*Meliola*	*Dichapetalum gelonioides* (Roxb.) Engl (Dichapetalaceae)	Maharashtra
475	Hosagoudar (2013a)	***Meliola bangalorensis*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Ficus* sp.(Moraceae)	Karnataka
476		***Meliola banosensis*** Syd.	Meliolaceae	*Meliola*	*Puereria tuberosa* DC. (Fabaceae)	Tamil Nadu
477	Hosagoudar (2013a)	***Meliola banosensis*** Syd. var. ***puerariae*** Hosag.	Meliolaceae	*Meliola*	*Puereria* sp. (Fabaceae)	Tamil Nadu
478	Hosagoudar (2013a)	***Meliola banosensis*** Syd. var**.** ***puerariicola*** Hosag.	Meliolaceae	*Meliola*	*Pueraria tuberosa* DC.(Fabaceae)	Tamil Nadu
479	Hosagoudar (2013a)	***Meliola bantanensis*** Hansf. var. ***keralensis*** Hosag.	Meliolaceae	*Meliola*	*Desmodium gyrans* DC (Fabaceae)	Kerala
480	Hosagoudar (2013a)	***Meliola bataanensis*** Syd. & Syd.	Meliolaceae	*Meliola*	*Millettia rubiginosa* Wight & Arn. (Fabaceae)	Kerala
481	Hosagoudar (2013a)	***Meliola bataanensis*** Syd. var. ***indica*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Millettia rubiginosa* Wight & Arn. (Fabaceae)	Kerala
482	Hosagoudar (2013a)	***Meliola bicornis*** Wint.	Meliolaceae	*Meliola*	*Desmodium telifolia, D. triquetrum* (L.) DC *D. velutinum* (Willd.) DC. *Dolichos trilobus* L. (*D. fulcatus* Kein ex Willd.) (Fabaceae*)*	
483	Hosagoudar (2013a)	***Meliola buteae*** Hafiz et al.	Meliolaceae	*Meliola*	*Butea monosperma* (Lam.) Taub. (*B. frondosa* Roxb.)(Fabaceae)	Kerala
484	Hosagoudar (2013a)	***Meliola butleri*** Syd.	Meliolaceae	*Meliola*	*Citrus* sp. (Rutaceae), *Citrus aurantifolia*(Christm.) Swingle	Kerala
485	Hosagoudar (2013a)	***Meliola cadambae*** Hosag. & C.K. Biju	Meliolaceae	*Meliola*	*Neolamarkia cadamba* (Roxb.) Bosser (Rubiaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
486	Hosagoudar (2013a)	***Meliola cadigensis*** Yates var. ***glycosmidis*** (Kapoor) Hosag.	Meliolaceae	*Meliola*	*Glycosmis macrocarpa* Wight (Rutaceae), *Glycosmis mauritiana* (Lam.) Tanaka (*G. pentaphylla* Correa) (Rutaceae)	Kerala
487	Hosagoudar (2013a)	***Meliola cadigensis*** Yates var**.** ***toddaliae*** Hosag. et al.	Meliolaceae	*Meliola*	*Toddalia sp.* (Rutaceae)	Kerala
488	Hosagoudar (2013a)	***Meliola caesalpiniae*** Hansf. & Deight. var. ***indica*** Hosag. & H. Biju	Meliolaceae	*Meliola*	*Caesalpinia sappan* L. Caesalpiniaceae	Kerala
489	Hosagoudar et al. (2012)	***Meliola caesalpiniicola*** Deight.	Meliolaceae	*Meliola*	*Caesalpinia banduc* (L.)Roxb. (Caesalpiniaceae)	Kerala
490	Hosagoudar (2013a)	***Meliola calopogonii*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Calopogonium mucunoides* Desv. (Fabaceae)	Kerala
491	Hosagoudar (2013a)	***Meliola canavaliae*** Hosag. et Riju	Meliolaceae	*Meliola*	*Canavalia* sp. (Fabaceae),	Kerala
491	"	***Meliola cannonii*** Hosag.	Meliolaceae	*Meliola*	*Strychnos nux-vomica* L. (Strychnaceae)	Kerala
492	Hosagoudar (2013a)	***Meliola canthii*** Hansf.	Meliolaceae	*Meliola*	*Canthium rheedii* DC. (Rubiaceae) *Canthium parviflorum* Lam. (*Plectronia parviflora* Bedd.)	Kerala
493	Hosagoudar (2013a)	***Meliola canthii-angustifolii*** Hosag.	Meliolaceae	*Meliola*	*Canthium angustifolium* Roxb. (Rubiaceae)	Kerala
494	Hosagoudar (2013a)	***Meliola canthiicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Canthium rheedii* DC. (Rubiaceae)	Kerala
495	Hosagoudar (2013a)	***Meliola cannonicola*** Hosag. & C.K. Biju	Meliolaceae	*Meliola*	*Toddalia asiatica* (L.) Lam. (Rutaceae)	Kerala

496	Hosagoudar (2013a)	***Meliola capensis*** (Kalch. & Cooke) Theiss. var. ***allophylicola*** Hansf. & Deight	Meliolaceae	*Meliola*	*Allophylus cobbe* (L.) Raeusch. (Sapindaceae)	Kerala
497	"	***Meliola capensis*** (Kalch. & Cooke) Theiss. var. ***dimocarpi*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Dimocarpus longan* Lour. (Sapindaceae)	Kerala
498	Hosagoudar (2013a)	***Meliola capensis*** (Kalch. & Cooke) Theiss.var. ***ermerginati*** Hosag. et al.	Meliolaceae	*Meliola*	*Sapindus emerginataus* Vahl (Sapindaceae)	Kerala
499	"	***Meliola capensis*** (Kalch. & Cooke) Theiss. var***. indica*** Hosag. et al.	Meliolaceae	*Meliola*	Sapindaceae member	Kerala
500	Hosagoudar (2013a)	***Meliola capensis*** (Kalch. & Cooke) Theiss. var.***malayensis*** Hansf.	Meliolaceae	*Meliola*	*Nephelium longan* Lour. (Sapindaceae)	Kerala
501	Hosagoudar (2013a)	***Meliola capensis*** (Kalch. & Cooke) Theiss. var. ***schleicherae*** Hosag. & Pillai	Meliolaceae	*Meliola*	*Schleichera oleosa* (Lour.) Oken (Sapindaceae)	Kerala
502	Hosagoudar (2013a)	***Meliola careyae*** (Stev.) Hosag.	Meliolaceae	*Meliola*	*Careya arborea* Roxb. (Lecythidaceae)	Kerala
503	Hosagoudar (2013a)	***Meliola careyae*** (Stev.) Hosag. var. ***indica*** Hosag.	Meliolaceae	*Meliola*	*Careya arborea* Roxb. (Lecythidaceae)	Kerala
504	Hosagoudar (2013a)	***Meliola carissae*** Doidge var. ***spinari*** Hosag.	Meliolaceae	*Meliola*	*Carissa spinarum* L. (Apocynaceae)	Tamil Nadu
505	Hosagoudar (2013a)	***Meliola cayratiae*** Hosag. et al.	Meliolaceae	*Meliola*	*Cayratia pedata* (Lam.) A.L. Juss. ex Gagnepain(Vitaceae)	Kerala
506	Hosagoudar (2013b)	***Meliola celastrigena*** Hosag.	Meliolaceae	*Meliola*	Celastrarceae member	Kerala
507	Hosagoudar (2013a)	***Meliola chandolensis*** C.R. Patil ex Hosag.	Meliolaceae	*Meliola*	*Ixora parviflora* Vahl (Rubiaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
508	Hosagoudar (2013a)	***Meliola chandrasekharanii*** Hosag.	Meliolaceae	*Meliola*	*Nothapodytes nimmoniana* (Graham) Mabb. (Icacinaceae)*Apodytes dimidiata* E. Meyer ex Arn.	Kerala
509	"	***Meliola chassaliicola*** Hosag.	Meliolaceae	*Meliola*	*Chassalia* sp. (Rubiaceae)	Kerala
510	Hosagoudar (2013a)	***Meliola chukrasiae*** Hosag.	Meliolaceae	*Meliola*	*Chukrasia* sp. (Meliaceae)	Kerala
511	Hosagoudar (2013a)	***Meliola cholakadensis*** Hosag. et al.	Meliolaceae	*Meliola*	Lauraceae member	Kerala
513	Hosagoudar (2013a)	***Meliola cinnamomi*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Cinnamomum* sp. (Lauraceae)	Kerala
514	Hosagoudar (2013a)	***Meliola cissampelicola*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Cissampelos convolvulacea* Willd. (Menispermaceae)	Kerala
515	Hosagoudar (2013a)	***Meliola cipadessae*** Hosag. et al.	Meliolaceae	*Meliola*	*Cipadessa bacciferra* (Roth) Miq. (Meliaceae)	Kerala
516	"	***Meliola citricola*** Syd. & Syd.	Meliolaceae	*Meliola*	*Citrus aurantium* L. (Rutaceae)	Kerala
517	Hosagoudar (2013a)	***Meliola clausenae*** Hosag.	Meliolaceae	*Meliola*	*Clausena dentate* (Willd.) M. Roem. (Rutaceae), *Clausena indica* (Dalz.) Oliver	Kerala
518	"	***Meliola clausenigena*** Hosag. & Riju	Meliolaceae	*Meliola*	*Clausena* sp. (Rutaceae)	Kerala
519	"	***Meliola clerodendricola*** Henn.	Meliolaceae	*Meliola*	*Clerodendrum viscosum* Vent.	Kerala
520	Hosagoudar (2013a)	***Meliola clerodendricola*** Henn. var. ***micromera*** (Syd. & Syd.) Hansf.	Meliolaceae	*Meliola*	*Gmelina arborea* Roxb. (Verbenaceae), *Gmelina asiatica* L	Kerala
521	Hosagoudar (2013a)	***Meliola clitoriae*** Hosag.	Meliolaceae	*Meliola*	*Clitoria ternatea* L. (Fabaceae)	Kerala
522	"	***Meliola colubrinicola*** Hosagoudar & Sabeena	Meliolaceae	*Meliola*	*Colubrina travancorica* Bedd. (Rhamnaceae),	Kerala

523	Hosagoudar (2013a)	***Meliola commixta*** Syd.	Meliolaceae	*Meliola*	*Nephelium longan* Lour. (Sapindaceae)	Kerala
524	Hosagoudar (2013a)	***Meliola cookeana*** Speg	Meliolaceae	*Meliola*	*Vitex leucoxylon* L.f. (Verbenaceae),	Kerala
525	Hosagoudar (2013a)	***Meliola cookeana*** Speg. var. ***viticis*** Hansf. (*M. castlerockensis* Srivasulu)	Meliolaceae	*Meliola*	*Clerodendrum serratum* (L.) Moon (Verbenaceae)	Kerala
526	Hosagoudar (2013a)	***Meliola crotonis-malabarici*** Hosag.	Meliolaceae	*Meliola*	*Croton malabaricus* Bedd. (Euphorbiaceae)	Kerala
527	Hosagoudar (2013a)	***Meliola cryptocariicola*** Hosag. & Raghu	Meliolaceae	*Meliola*	*Cryptocarya bourdillonii* Gamble (Lauraceae)	Kerala
528	Hosagoudar (2013a)	***Meliola cycleae*** Hosag.	Meliolaceae	*Meliola*	*Cyclea peltata* Cooke (Menispermaceae)	Kerala
529	"	***Meliola cymbopogonis*** Kapoor	Meliolaceae	*Meliola*	*Cymbopogon flexuous* (Nees ex Steud.) Wats.(Poaceae), *C. nardus* (L.) Rendle	Kerala
530	Sabeena and Hosagoudar (2018b)	***Meliola cymbopogonigena*** Sabeena & Hosag.	Meliolaceae	*Meliola*	*Cymbopogon* sp. (Poaceae),	Kerala
531	Hosagoudar (2013a).	***Meliola cyperacearum*** Hosag. et al.	Meliolaceae	*Meliola*	Cyperus *sp.*(Cyperaceae)	Tamil Nadu
532	"	***Meliola daviesii*** Hansf.	Meliolaceae	*Meliola*	*Jasminum* sp.	Maharashtra
533	Hosagoudar (2013a)	***Meliola daviesii*** Hansf. var. ***longiseta*** Hosag.	Meliolaceae	*Meliola*	*Jasminum rottlerianum* Wall. ex A. DC. *(*Oleaceae*)*	Kerala
534	Hosagoudar (2013a)	***Meliola daviesii*** *Hansf.* var. ***kodaikalensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Jasminum brevilobum(*Oleaceae*)*	Tamil Nadu
535	Hosagoudar (2013a)	***Meliola dendropthoeicola*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Dendropthoe falcata* (L.f.) Etting (Loranthaceae), *Helixanthera* sp. *Loranthus* sp.	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
536	Hosagoudar (2013a)	***Meliola densa*** Cooke	Meliolaceae	*Meliola*	*Syzygium laetum* (Buch.- Ham.) Gandhi, *S. munroni* (Walp.) Chandr. *Eugenia* sp.	Kerala
537	Hosagoudar and Sabeena (2013)	***Meliola desmodii-heterocarpi*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Desmodium heterocarpon* (L.). DC. (Fabaceae),	Kerala
538	Hosagoudar (2013a)	***Meliola desmodii-laxiflori*** Deight. var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Desmodium laxiflorum* DC. (Fabaceae)	Kerala
539	Hosagoudar (2013a)	***Meliola desmodii-pulchelli*** Hosag. et al.	Meliolaceae	*Meliola*	*Desmodium pulchellum* (L.) Benth (Fabaceae)	Kerala
540	Hosagoudar (2013a)	***Meliola desmodii-triangularis*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Desmodium triangulare* (Retz.) Merr. (Fabaceae)	Kerala
541	Hosagoudar (2013a)	***Meliola desmodii-triquetri*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Desmodium triquetrum* (L.) DC. (Fabaceae)	Kerala
542	Hosagoudar (2013a)	***Meliola desmodii-velutini*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Desmodium velutinum* (Willd.) DC. (Fabaceae)	Kerala
543	Hosagoudar (2013a)	***Meliola devikulamensis*** Hosag., H. Biju & Manoj.	Meliolaceae	*Meliola*	*Toddalia* sp. (Rutaceae)	Kerala
544	Hosagoudar (2013a)	***Meliola dimidiatae*** Hosag.	Meliolaceae	*Meliola*	*Nothapodytes nimmoniana* (Graham) Mabb. *Apodytes dimidiata* E. Meyer ex Arn.	Kerala
545	Bhise et al. (2014)	***Meliola dioicae*** Bhise and Patil	Meliolaceae	*Meliola*	*Olea dioica* Roxb. (Oleaceae)	Maharashtra
546	Hosagoudar (2013a)	***Meliola dioscoreacearum*** Hosag. & Jacob Thomas	Meliolaceae	*Meliola*	*Dioscorea* sp.	Kerala
547	"	***Meliola dioscoregena*** Hosag. & Jacob Thomas	Meliolaceae	*Meliola*	*Dioscorea* sp.	Kerala

548	Hosagoudar (2013a)	***Meliolo diospyri-buxifoliae*** Hosag. et al.	Meliolaceae	*Meliola*	*Diospyros buxifolia* (Blume) Hiern	Kerala
549	Hosagoudar (2013a)	***Meliola dolichi*** Hosag.	Meliolaceae	*Meliola*	*Dolichus trilobus* L. (Fabaceae), *D. trifolius*	Kerala
550	"	***Meliola dorsteniae*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Dorstenia indica* Wight(Moraceae)	Kerala
551	Hosagoudar (2013a)	***Meliola drypeticola*** Hosag.	Meliolaceae	*Meliola*	*Epiprinus mallotiformis* (Muell.-Arg.) Croizat *(*Euphorbiaceae*) Drypetes macrophylla* (Bl.) Pax & Hoffm	Tamil Nadu
552	Hosagoudar (2013a)	***Meliola dysoxyli-malabarici*** Hosag. & Kamar.	Meliolaceae	*Meliola*	*Dysoxylum malabaricum* Bedd. ex Hiern. (Meliaceae)	Kerala
553	"	***Meliola drepanochaeta*** Syd. var. ***insignis*** Hosag.	Meliolaceae	*Meliola*	*Litsea insignis* Gamble(Lauraceae)	Kerala
554	Hosagoudar (2013a).	***Meliola dysoxyligena*** Hosag. et Riju	Meliolaceae	*Meliola*	*Dysoxylum* sp. (Meliaceae),	Kerala
555	Hosagoudar (2013a)	***Meliola ebeni*** Hosag. & Archana	Meliolaceae	*Meliola*	*Diospyros ebenum* Koenig (Ebenaceae)	Kerala
556	"	***Meliola emespatilii*** Hosag. et al.	Meliolaceae	*Meliola*	*Thea sinensis* L. (Theaceae)	Maharashtra
557	Hosagoudar (2013a)	***Meliola entadicola*** Deight.	Meliolaceae	*Meliola*	*Entada rheedii* Sprengel (Mimosaceae)	Maharashtra
558	Hosagoudar (2013a)	***Meliola erycibes-paniculatae*** **Hosag.**	Meliolaceae	*Meliola*	*Erycibe paniculata* Roxb. Convolvulaceae	Kerala
559	"	***Meliola erumeliensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Drypets elata* (bedd) Pax & Hofm. *(Euphorbiaceae)*	Kerala
560	Hosagoudar (2013a)	***Meliola erythrinae*** Syd.	Meliolaceae	*Meliola*	*Erythrina variegata* L. (*E. indica* Lam.) (Fabaceae) *E. stricta* Roxb.	Kerala
561	"	***Meliola erythrinae*** Syd. var**.** ***indica*** Hosag. & Jacob Thomas	Meliolaceae	*Meliola*	*Erythrina variegate* L. (Fabaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
562	Hosagoudar (2013a)	***Meliola erythropali*** Hosag.	Meliolaceae	*Meliola*	*Erythropalum populifolium* (Arn.) Mast. Erythropalaceae	Kerala
563	Hosagoudar (2013a)	***Meliola eugeniae-jamboloidis*** Hansf.	Meliolaceae	*Meliola*	*Eugenia floccosa* Bedd. *Syzygium munroni* (Walp.) Chandr. (Myrtaceae)	Kerala
564	"	***Meliola eugeniae-stocksii*** Hosag.	Meliolaceae	*Meliola*	*Eugenia stocksii* Duthie(Myrtaceae)	Maharashtra
565	Hosagoudar (2013a)	***Meliola eugeniicola*** Stev.	Meliolaceae	*Meliola*	*Eugenia eucalyptoides* F. Muell. (Myrtaceae)	Maharashtra
566		***Meliola exaci*** Hosag.	Meliolaceae	*Meliola*	*Exacum tetragonum* Roxb. (*E. bicolor*) (Gentianaceae)	Maharashtra
567	Hosagoudar (2013a)	***Meliola ficicola*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Ficus* sp. (Moraceae)	Kerala
568	Hosagoudar and Sabeena (2013)	***Meliola ficigena*** Hosag. & A. Sabeena	Meliolaceae	*Meliola*	*Ficus* sp. (Moraceae),	Kerala
569	Hosagoudar (2013a)	***Meliola filicii*** Hosag.	Meliolaceae	*Meliola*	*Filicium decipiens* (Wight & Arn.) Thw.(Sapindaceae)	Kerala
570	Hosagoudar (2013a)	***Meliola filiciicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Filicium decipiens* (Wight & Arn.) Thw.(Sapindaceae)	Kerala
571	"	***Meliola filicii*** *Hosag. var.* ***indica*** Hosag. & Archana	Meliolaceae	*Meliola*	*Ilicium decipiens* (Wight & Arn.) Thw.	Kerala
572	Hosagoudar (2013a)	***Meliola flemingiicola*** Hosag.	Meliolaceae	*Meliola*	Flemingia semialata (Fabaceae)	Kerala
573	Hosagoudar (2013a)	***Meliola floridensis*** Hansf.	Meliolaceae	*Meliola*	*Persea macrantha* (Nees) Kosterm. (Lauraceae)	Tamil Nadu
574	Hosagoudar (2013a)	***Meliola floridensis*** Hansf. var. ***pudukadensis*** Hosag.	Meliolaceae	*Meliola*	*Persea macrantha* (Nees) Kosterm. (Lauraceae)	Tamil Nadu

575	Hosagoudar (2013a)	***Meliola franciscana*** Hansf.	Meliolaceae	*Meliola*	*Ormosia travancorica* Bedd. (Fabaceae)	Kerala
576	"	***Meliola furcata*** Lev.	Meliolaceae	*Meliola*	*Vitis* sp. (Vitaceae)	Kerala
577	Hosagoudar (2013a)	***Meliola gamblei*** Hosag.	Meliolaceae	*Meliola*	*Smilax zeylanica* L.. (Smilacaceae)	Kerala
578	Hosagoudar (2013a)	***Meliola gamsii*** Hosag. & Shiburaj	Meliolaceae	*Meliola*	*Strychnos nux-vomica* L. (Strychnaceae)	Kerala
579	Hosagoudar and Sabeena (2013)	***Meliola garcinigena*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Garcinia morella* (Gaertner) Desr. (Clusiacee)	Kerala
580	Hosagoudar (2013a)	***Meliola gardneriae*** Hansf. & Thirum	Meliolaceae	*Meliola*	*Gardneria* sp.Loganiaceae	Karnataka
581	Hosagoudar (2013a)	***Meliola gardneriae*** Hansf. & Thirum. var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Gardneria ovata* Wall. (Loganiaceae)	Tamil Nadu
582	Hosagoudar (2013a)	***Meliola garugae*** Stev. & Rold.	Meliolaceae	*Meliola*	*Garuga pinnata* Roxb(Burseraceae)	Kerala
583	Hosagoudar (2013a)	***Meliola geissaspidis*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Geissaspis cristata* Wight & Arn (Fabaceae)	Kerala
584	"	***Meliola geniculata*** Syd. & Butler	Meliolaceae	*Meliola*	*Lannea coromandelica* (Houtt.) Merr. (Anacardiaceae)	Kerala
585	Hosagoudar (2013a)	***Meliola gemellipoda*** Doidge	Meliolaceae	*Meliola*	*Jasminum ozoricum* L.. (Oleaceae), *Jasminum malabaricum* Wight, *Jasminum flexile* Vahl	Kerala
586	Hosagoudar (2013a)	***Meliola gersoppaensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Syzygium* sp. (Myrtaceae)	Kerala
587	"	***Meliola glanduliferae*** Hosag. et al.	Meliolaceae	*Meliola*	*Olea glandulifera* Wall. ex. G. Don. (Oleaceae)	Kerala
588	Hosagoudar (2013a)	***Meliola gliricidiicola*** Hosag. & Agarwal	Meliolaceae	*Meliola*	*Gliricidia sepium* (Jacq.) Walp (Fabaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
589	Hosagoudar (2013b)	***Meliola glochidiifolia*** Hosag.	Meliolaceae	*Meliola*	*Glochidion* sp. (Euphorbiaceae),	Kerala
590	Hosagoudar (2013a)	***Meliola gluticola*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Gluta travancorica* L**.** (Anacardiaceae);	Kerala
591	Hosagoudar (2013a)	***Meliola gneti*** Hansf.	Meliolaceae	*Meliola*	*Gnetum ula* Brogn. (Gnetaceae)	Kerala
592	"	***Meliola gooseana*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Actinodaphne* sp. (Lauraceae)	Kerala
593	Hosagoudar (2013a)	***Meliola gordoniae*** Hosag.	Meliolaceae	*Meliola*	*Gordonia obtusa* Wall. ex Wight & Arn.(Theaceae)	Kerala
594	Hosagoudar (2013b)	***Meliola goniothalamigena*** Hosag. & Jagath Timmaih	Meliolaceae	*Meliola*	*Goniothalamus cardiopetalus* (Dalz.) Hook. f. & Thomson (Annonaceae)	Karnataka
595	Hosagoudar (2013a)	***Meliolo gouaniicola*** V. B. Hosagoudar et P. J. Robin	Meliolaceae	*Meliola*	*Gouania microcorpa*DC. (Rhaminaceae)	Karnataka
596	Hosagoudar (2013a)	***Meliola gouaniae*** *Hansf. var.* ***keralica*** Hosag. & Robin	Meliolaceae	*Meliola*	*Gouania* sp.(Rhamnaceae)	Kerala
597	"	***Meliola grewiae*** Hansf. var. ***longispora*** Hosag. & Raju	Meliolaceae	*Meliola*	*Grewia teliaefolia* Vahl (Tiliaceae)	Maharashtra
598	Hosagoudar (2013a)	***Meliola groteana*** Syd. var. ***maesae*** Hosag. et al.	Meliolaceae	*Meliola*	*Maesa indica* (Roxb.) DC. (Myrsinaceae)	Kerala
599	Hosagoudar (2013a)	***Meliola hemidesmi*** Kamal & Gupta	Meliolaceae	*Meliola*	*Hemidesmus indicus* (L.) R. Br. (Periplocaceae)	Kerala
600	"	***Meliola hemidesmicola*** Hosag.	Meliolaceae	*Meliola*	*Hemidesmus indicus* (L.) R. Br. (Periplocaceae)	Kerala
601	Hosagoudar (2013a)	***Meliola hemidesmicola*** Hosag. var. ***indica*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Hemidesmus indicus* (L.) R. Br. (Periplocaceae)	Kerala

602	Hosagoudar (2013a)	***Meliola henryi*** Hosag.	Meliolaceae	*Meliola*	*Canthium rheedii* DC. (Rubiaceae), *Canthium parviflorum* Lam.	Kerala
603	Hosagoudar (2013a)	***Meliola henryi*** Hosag. var. ***oldenlandiae*** Hosag. et al.	Meliolaceae	*Meliola*	*Hedyotis stylosa* R. Br. ex. Wight & Arn., (Rubiaceae), *Canthium rheedii* DC.	Kerala
604	Hosagoudar (2013a)	***Meliola heudelotii*** Gaill.	Meliolaceae	*Meliola*	*Memecylon talbotium* Brandis (Melastomataceae) *Memecylon edule* Roxb.	Kerala
605	Hosagoudar (2013a)	***Meliola heyneae*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Trichilia connaroides* (Wight & Arn.) Bentvelzen (Meliaceae)	Kerala
606	"	***Meliola homonoiae*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Homonoia riparia* Lour. (Euphorbiaceae)	Kerala
607	Hosagoudar (2013a)	***Meliola hoveniae*** Hosag. et al.	Meliolaceae	*Meliola*	*Hovenia acerba Lindle.* (Rhamnaceae)	Tamil Nadu
608	"	***Meliola hugoniae*** Hanford & Deighton	Meliolaceae	*Meliola*	*Hugonia belli* Sedgwick (Linaceae)	Kerala
609	Hosagoudar (2013a)	***Meliola hydei*** Hosag. et al.	Meliolaceae	*Meliola*	*Psychotria* sp. (Rubiaceae),	Kerala
610	Hosagoudar (2013a)	***Meliola hydnocarpi*** Hansf. var. ***indica*** Hosag. & Kamar.	Meliolaceae	*Meliola*	*Hydnocarpus pentandra* (Buch-Ham) Oken (Flacourtiaceae), *Hydnocarpus laurifolius*	Kerala
611	Hosagoudar (2013a)	***Meliola hyptidis*** Syd.	Meliolaceae	*Meliola*	*Hyptiscapitata* Jacq. (Lamiaceae) *Hyptis suaveilense* (L.) Poit.	Kerala
612	Hosagoudar (2013a)	***Meliola indica*** Syd.	Meliolaceae	*Meliola*	*Barringtonia acutangula* Gaertn. (Lecythidaceae)	Kerala
613	Hosagoudar (2013a)	***Meliola indica*** Syd. var. ***careyae*** Stev.	Meliolaceae	*Meliola*	*Careya arborea* Roxb. (Lecythidaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
614	Hosagoudar (2013a)	***Meliola integrifolii*** C.R. Patil ex Hosag.	Meliolaceae	*Meliola*	*Artocarpus heterophyllus* Lam. (*A. integrifoliaauct. non* L.f.) (Moraceae)	Kerala
615	"	***Meliola invisiae*** Hosag. & H. Biju	Meliolaceae	*Meliola*	*Mimosa invisia* Mart. ex Colla var. *inermis* Adelb.(Mimosaceae)	Kerala
616	Hosagoudar (2013a)	***Meliola ixorae*** Yates	Meliolaceae	*Meliola*	*Ixora* sp., (Rubiaceae), *Ixora polyantha* Wight, Canthium wightii,	Kerala
617	"	***Meliola ixorae*** Yates var. ***macrospora*** Hosag.	Meliolaceae	*Meliola*	*Ixora nigricans* R. Br. (Rubiaceae), *Ixora elongata* Heyne, *Ixora coccinea* L.	Kerala
618	Hosagoudar (2013a)	***Meliola ixorae*** Yates var. ***psychotriae*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Psychotria macrocarpa* Hook.f. (Rubiaceae), *Ixora coccinea* L.	Kerala
619	Hosagoudar (2013a)	***Meliola ixorae-coccineae*** Hosag. & Pillai	Meliolaceae	*Meliola*	*Ixora coccinea* L. (Rubiaceae), *Ixora* sp.	Kerala
620	Hosagoudar (2013a)	***Meliola jasmini*** Hansf. & Stev.	Meliolaceae	*Meliola*	*Jasminum sambac* (L.) Ait. (Oleaceae), *Jasminum* sp.	"
621	Hosagoudar (2013a)	***Meliola jasmini*** Hansf. & Stev. var. ***microspora*** Hosag. et al.	Meliolaceae	*Meliola*	*Jasminum* sp. (Oleaceae)	Kerala
622	Hosagoudar (2013a)	***Meliola jasminicola*** Henn. var. ***indica*** Kapoor	Meliolaceae	*Meliola*	*Jasminum rottlerianum* Wall. ex A. DC. (Oleaceae), *Jasminum auriculatum* Vatil	"
623	Hosagoudar (2013b)	***Meliola jasminigena*** Hosag.	Meliolaceae	*Meliola*	*Jasminum bignoniaceum* Wallich ex DC. (Oleaceae)	Kerala
624	Hosagoudar (2013a)	***Meliola jayachandranii*** Hosag.	Meliolaceae	*Meliola*	*Isonandra lanceolata* Wight forma *anfractuosa* (Clarke) Jeuken (Sapotaceae)	Kerala

625	Hosagoudar (2013a)	***Meliola kakachiana*** Hosag.	Meliolaceae	*Meliola*	*Cryptocarya beddomei* Gamble (Lauraceae)	Tamil Nadu
626	Hosagoudar (2013a)	***Meliola kakachiana* Hosag. var. *poochipa-raensis*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Litsea* sp. (Lauraceae)	Kerala
627	Hosagoudar (2013a)	***Meliola kamettiae*** Hosag. & Riju	Meliolaceae	*Meliola*	*Kamettia caryophyllata* Roxb. (Apocynaceae)	Kerala
628	"	***Meliola kannurensis*** V B Hosagoudar, G R Archana, K M Khaleel and M Soumya,	Meliolaceae	*Meliola*	*Ficus gibbosa* Bl. (Moraceae),	Kerala
629	Hosagoudar (2013a)	***Meliola kanniyakumariana*** Hosag.	Meliolaceae	*Meliola*	*Hedyotis albo-nervia* Bedd., (Rubiaceae), *Hedyotis gamblei* Henry & Subram.	Kerala
630	Hosagoudar (2013a)	***Meliola kanniyakumariana*** Hosag. var. ***brahmagiriense*** Hosag. et al.	Meliolaceae	*Meliola*	*Knoxia* sp., (Rubiaceae)	Kerala
631	Hosagoudar (2013a)	***Meliola kapoorii*** Hosag. & P.A. Raghu	Meliolaceae	*Meliola*	*Pandanus* sp. (Pandanaceae)	Kerala
632	Hosagoudar (2013a)	***Meliola karnatakensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Glochidion* sp. (Euphorbiaceae)	Karnataka
633	Hosagoudar (2013a)	***Meliola kaveriappai*** Hosag. et al.	Meliolaceae	*Meliola*	*Cinnamomum* sp. (Lauraceae)	Karnataka
634	"	***Meliolo knemae*** Hosag. & Robin	Meliolaceae	*Meliola*	*Knema attenuata* (Wall.ex Hook.f.) Thoms	Kerala
635	Hosagoudar (2013a)	***Meliola knoxiicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Knoxia* sp., *Knoxia sumatrensis* (Retz.) DC. (Rubiaceae)	Kerala
636	Hosagoudar (2013a)	***Meliola kulathupuzhaensis*** Hosag. & Justin P. Jacob	Meliolaceae	*Meliola*	*Bauhinia* sp. Caesalpiniaceae	Kerala
637	Hosagoudar (2013a)	***Meliola kydiae-calycinae*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Kydia calycina* Roxb. (Malvaceae)	Karnataka

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
638	Hosagoudar (2013a)	***Meliola laxa*** Gaill. var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Syzygium zeylanicum* (L.) DC. (Myrtaceae)	Kerala
639	Hosagoudar (2013a)	***Meliola lepianthedis*** Hosag. & Kamar.	Meliolaceae	*Meliola*	*Lepianthes umbellata* (L.) Rafin (Piperaceae)	Kerala
640	Bhise et al. (2016)	***Meliola litseae*** Syd.	Meliolaceae	*Meliola*	*Litsea josephii* S. M. Almeida (Lauraceae)	Maharashtra
641	Hosagoudar (2013a)	***Meliola ligustri*** Hosag.	Meliolaceae	*Meliola*	*Ligustrum walkeri* Roxb. ssp. *walkeri* (Decne) Green (*Ligustrum walkeri* Decne) (Oleaceae)	
642	Hosagoudar (2013a)	***Meliola ligustricola*** Hosag. et al.	Meliolaceae	*Meliola*	*Ligustrum perrottettii* DC. (Oleaceae)	Kerala
643	"	***Meliola linderae*** Yamam.	Meliolaceae	*Meliola*	*Actinodaphne hookeri* Meissn. (Lauraceae)	Kerala
644	Hosagoudar (2013a)	***Meliola linderae*** Yamam. var. ***microspora*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Actinodaphne malabarica* Balakr (Lauraceae)	Kerala
645	Hosagoudar (2013a)	***Meliola linocierae-malabaricae*** Hosag.	Meliolaceae	*Meliola*	*Chionanthus mala-elengi* (Dennst.) Green (*Linociera malabarica* Wall. ex Don)(Oleaceae)	Kerala
646	Hosagoudar (2013a)	***Meliola litseae*** Syd. & Syd. var. ***floribundae*** Hosag.	Meliolaceae	*Meliola*	*Litsea floribunda* (Bl.) Gamble (Lauraceae)	Kerala
647	Hosagoudar (2013a)	***Meliola litseae*** Syd. & Syd. var. ***insignis*** Hosag.	Meliolaceae	*Meliola*	*Litsea insignis* Gamble (Lauraceae)	Kerala
648	Hosagoudar (2013a)	***Meliola litsea*** Syd. var. ***keralensis*** Hosag.	Meliolaceae	*Meliola*	*Litsea stocksii* (Meisner) Hook.f. (Lauraceae)	Kerala
649	Hosagoudar (2013a)	***Meliola litseae*** Syd. & Syd. var. ***microspora*** Hosag	Meliolaceae	*Meliola*	*Litsea floribunda* (Bl.) Gamble (Lauraceae)	Kerala

650	Hosagoudar (2013a)	***Meliola litseae*** Syd. & Syd. var. ***rotundipoda*** Hansf	Meliolaceae	*Meliola*	*Litsea coriacea* (Heyne ex Meisner) Hook.f. (Lauraceae)	Kerala
651	"	***Meliola lobeliae*** Stev.	Meliolaceae	*Meliola*	*Lobelia nicotianifolia* Roth ex Schultes.(Lobeliaceae)	Maharashtra
652	Hosagoudar (2013a)	***Meliola longiseta*** Hoeh. *var.* ***umbellata*** (Wight) Sant. & Merch.	Meliolaceae	*Meliola*	*Canthium dicoccum* (Gaertn.) Teijsm. & Binn. (Rubiaceae)	Kerala
653	Hosagoudar (2013a)	***Meliola lophopetaligena*** Hosag. & Robin	Meliolaceae	*Meliola*	*Lophopetalum wightiana* Arn. (Celastraceae)	Kerala
654	Hosagoudar (2013a)	***Meliola loranthacearum*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Dendropthoe falcata* (L.f.) Etting (Loranthaceae)	Kerala
655	Hosagoudar (2013a)	***Meliola luculiae*** Hosag. et al.	Meliolaceae	*Meliola*	*Lucculia grandifolia* Ghose (Rubiaceae)	Tamil Nadu
656	"	***Meliola luvungae*** Hosag.	Meliolaceae	*Meliola*	*Evodia luna-ankenda* (Gaertn.) Merr. (Rutaceae), *Luvunga sermentosa* (Blume) Kurz *(L. elutherandra* Dalz.)	Kerala
657	Hosagoudar (2013a)	***Meliola machili*** Yamam.	Meliolaceae	*Meliola*	*Persea macrantha* (Nees) Kosteerm. (Lauraceae)	Kerala
658	Hosagoudar (2013a)	***Meliola maduraiensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Syzygium lanceolatum* (Lam.) Wight & Arn. (Myrtaceae)	Tamil Nadu
659	Hosagoudar (2013a)	***Meliola macropoda*** Syd.	Meliolaceae	*Meliola*	*Vepris bilocularis* (Wight & Arn.) Engl. (Rutaceae)	Kerala
660	Hosagoudar (2013a)	***Meliola mahabaleshwarensis*** Srinivasulu	Meliolaceae	*Meliola*	*Solanum giganteum* Jacq	Maharashtra
661	"	***Meliola mahamulkarii*** Hosag. et al.	Meliolaceae	*Meliola*	*Osyris arborea* wall. Asclepiadaceae)	Maharashtra
662	Hosagoudar (2013a)	***Meliola malabarensis*** Hansf.	Meliolaceae	*Meliola*	*Olea dioica* Roxb. *Olea* sp. (Oleaceae),	Kerala
663	Hosagoudar (2013a)	***Meliola malloticola*** Hosag.	Meliolaceae	*Meliola*	*Mallotus philippensis* (Lam.) Muell.-Arg. (Euphorbiaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
664	Hosagoudar (2013a)	***Meliola mannavenansis*** Hosag. et al.	Meliolaceae	*Meliola*	*Litsea angustima Litsea* sp. (Lauraceae)	Kerala
665	"	***Meliola manoharachari*** Hosag. & Riju	Meliolaceae	*Meliola*	Myristica *sp.* (Myristicaceae)	Kerala
666	Thomas et al. (2013)	***Meliola marthomaensis*** Jacob Thomas, Teena Elizabth and Riya	Meliolaceae	*Meliola*	*Hymenodictyon obovatum* Wallich. (Rubiaceae)	Kerala
667	Hosagoudar (2013a)	***Meliola mayapeae*** Stev.	Meliolaceae	*Meliola*	*Chionanthus ramiflora* Roxb. (*Linociera intermedia* Wight) (Oleaceae), Ligustrum perrottetti *DC.*	Kerala
668	Hosagoudar (2013a)	***Meliola mayapiicola*** Stev. var. ***indica*** Hosag.	Meliolaceae	*Meliola*	*Chionanthus mala-elengi* (Dennst.) Green (*Linociera malabarica* Wall. ex G. Don)(Oleaceae)	Kerala
669	Hosagoudar (2013a)	***Meliola megalocarpa*** Syd. var. ***microspora*** Hosag.	Meliolaceae	*Meliola*	*Diospyros buxifolia* (Blume) Hiern. (Ebenaceae)	Maharashtra
670	Hosagoudar and Thomas (2013)	***Meliola melicopegena*** V.B. Hosagoudar & Sony Thomas	Meliolaceae	*Meliola*	*Euodia lunuankenda* (Gaertn.) T.G. Hartley (Rutaceae),	Kerala
671	Hosagoudar (2013a)	***Meliola memecyli*** Syd. & Syd.	Meliolaceae	*Meliola*	*Memecylon depressum* Benth. ex Triana, *M. umbellatum* Burm.f. *Memecylon edule* Roxb. (Melastomataceae)	Kerala
672	Hosagoudar (2013a)	***Meliola memecyli*** Syd. var. ***microspora*** Hansf.	Meliolaceae	*Meliola*	*M. umbellatum* Burm.f. *Memecylon edule* Roxb. (Melastomataceae)	Kerala
673	Hosagoudar (2013a)	***Meliola memecylicola*** Hansf. var. ***longiseta*** Hosag.	Meliolaceae	*Meliola*	*Memecylon edule* Roxb. (Melastomataceae)	Kerala

674	Hosagoudar (2013a)	***Meliola memecylicola*** Hansf. var. ***indica*** Hosag.	Meliolaceae	*Meliola*	*Memecylon depressum*Benth. ex Triana (Melastomataceae)	Kerala
675	Hosagoudar (2013a)	***Meliola mesuae*** Hosag. et al.	Meliolaceae	*Meliola*	*Mesua ferrea* L. (Clusiaceae)	Kerala
676	Hosagoudar and Fathima (2013)	***Meliola microtropidis*** Hosag. & Fathima	Meliolaceae	*Meliola*	*Microtropis* sp. (Celastraceae),	Kerala
677	Hosagoudar (2013a)	***Meliola mitrephorae*** Hosag. & Rajendran	Meliolaceae	*Meliola*	*Mitrephora heyneana* (Hook.f. & Thoms.) Thw (Annonaceae)	Kerala
678	Hosagoudar (2013a)	***Meliola melanoxylonis*** Hosag. & Pillai	Meliolaceae	*Meliola*	*Acacia sinuate* (Lour.) Merr. (Mimosaceae), *Acacia melanoxylon* R. Br.	Kerala
679	Hosagoudar (2013a)	***Meliola millettiae-chrysophyllae*** Deight. var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Millettia splendens* Wight & Arn. (Fabaceae)	Tamil Nadu
680	Hosagoudar (2013a)	***Meliola mitragynae*** Syd.	Meliolaceae	*Meliola*	*Mitragyna* (Rubiaceae) *parviflora* Korth	Kerala
681	Hosagoudar (2013a)	***Meliola mitragynae-tubulosae*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Mitragyna tubulosa* (Arn.) Hav. (Rubiaceae)	Kerala
682	Hosagoudar (2013a)	***Meliola mucunae*** Hansf. var. ***hirsutae*** Hosag.	Meliolaceae	*Meliola*	*Mucuna hirsuta* Wight & Arn. (Fabaceae)	Kerala
683	Hosagoudar (2013a)	***Meliola mucunae-acuminatae*** Hansf. var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Mucuna pruriens* (L.) DC. (Fabaceae)	Maharashtra
684	Hosagoudar (2013a)	***Irenopsis murrayae*** Hosag. & Rajkumar	Meliolaceae	*Meliola*	*Murraya koenigii* (L.) Spreng. (Rutaceae)	Kerala
685	"	***Meliola myristicae*** Hosag. & Raghu (Bedd.) Sinclair	Meliolaceae	*Meliola*	*Myristica fatua* Houtt var. *magnifica*(Beddome) Sinclair	Karnataka
686	"	***Meliola nairii*** Hosag.	Meliolaceae	*Meliola*	*Trichilia connaroides* (Wight & Arn.) Bentvelzen (Meliaceae)	Kerala
687	Hosagoudar (2013a)	***Meliola neolitseae*** Yamam.	Meliolaceae	*Meliola*	*Neolitsea scrobiculata* (Meisner) Gamble(Lauraceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
688	Hosagoudar (2013a)	***Meliola neanotidis*** Hosag. et al.	Meliolaceae	*Meliola*	*Neanotis monosperma* (Wall. ex Wight & Arn.) Lewis (Rubiaceae)	Kerala
689	Hosagoudar and Divya (2013)	***Meliola neelikalluensis*** Hosag. & Divya	Meliolaceae	*Meliola*	Celastrarceae member	Kerala
690	Hosagoudar (2013a)	***Meliola nephelii*** Sacc. var. ***singalensis*** Hansf.	Meliolaceae	*Meliola*	*Allophylus serrulatus* Radlk. (Sapindaceae)	Kerala
691	Hosagoudar (2013a)	***Meliola odoratissimae*** (Kapoor) Hosag.	Meliolaceae	*Meliola*	*Albizzia odoratissima* (Mimosaceae)	Kerala
692	"	***Meliola olacicola*** Hosag.	Meliolaceae	*Meliola*	*Olax wightiana* Wall. ex Wight & Arn. (Olacaceae)	"
693	Hosagoudar (2013a)	***Meliola oldenlandiae*** Hansf. & Stev.	Meliolaceae	*Meliola*	*Hedyotis* sp. (Rubiaceae)	Kerala
694	"	***Meliola oldenlandiae*** Hansf. & Stev. var. ***indica*** Hosag. et al.	Meliolaceae	*Meliola*	*Hedyotis* sp. (Rubiaceae)	Kerala
695	Hosagoudar (2013a)	***Meliola oleacearum*** Hosag.	Meliolaceae	*Meliola*	*Olea dioica* Roxb. (Oleaceae)	Kerala
696	Hosagoudar (2013a)	***Meliola oligomera*** Syd.	Meliolaceae	*Meliola*	*Loecneriella obtusifolia* (Roxb.) A.C. Smith (Hippocrateaceae)	Maharashtra
697	Hosagoudar (2013a)	***Meliola opiliae*** Syd.	Meliolaceae	*Meliola*	*Opilia amentacea* Roxb.	Kerala
698	Hosagoudar (2013a)	***Meliola opiliae*** Syd. var. ***singalensis*** Hansf.	Meliolaceae	*Meliola*	*Lepionurus sylvestris* DC. (Opiliaceae), *Cansjera rheedi* Gmel.	Kerala
699	Hosagoudar (2013a)	***Meliola osyridicola*** Hansf.	Meliolaceae	*Meliola*	*Osyris arborea* Wall. (Santalaceae)	Tamil Nadu
700	Hosagoudar (2013a)	***Meliola osyridicola*** Hansf. var. ***indica*** Hosag.	Meliolaceae	*Meliola*	*Osyris quadriseptata* Salz. ex Decne (Santalaceae)	Maharashtra

701	Hosagoudar (2013a)	***Meliola otonephellii*** Hosag.	Meliolaceae	*Meliola*	*Otonephelium stipulaceum* (Bedd.) Radlk. (*Nephelium stipulaceum* Bedd.)(Sapindaceae)	Kerala
702	Hosagoudar (2013a)	***Meliola otophorae*** Yates var. ***indica*** Hosag. & Ravikumar	Meliolaceae	*Meliola*	*Lepisanthes senegalensis* (Juss. ex Poit.) Leenh.	Kerala
703	"	***Meliola ovatipoda*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Ficus* sp.(Moraceae)	Karnataka
704	Hosagoudar and Fathima (2013)	***Meliola padmanabhapurica*** Hosag. & Fathima	Meliolaceae	*Meliola*	*Semecarpus travancorica* Bedd. (Anacardeaceae),	Kerala
705	Hosagoudar (2013a)	***Meliola palmicola*** Wint. var. ***africana*** Hansf.	Meliolaceae	*Meliola*	*Phoenix sylvesteris* (L.) Roxb. (Arecaceae)	Tamil Nadu
706	"	***Meliola palawanensis*** Syd.	Meliolaceae	*Meliola*	*Morinda umbellata* L. (Rubiaceae)	Kerala
707	Hosagoudar (2013a)	***Meliola pandanacearum*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Pandanus* sp. (Pandanaceae)	Kerala
708	"	***Meliola panici*** Earle	Meliolaceae	*Meliola*	*Lophotherum gracile* Brongn, *Ischaemum zeylanicum* Bor, *Centotheca lappacea* (L.) Desr. (Poaceae)	Kerala
709	Hosagoudar (2013a)	***Meliola panici*** Earle var. ***macropodia*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Cyrtococcum longipes* (Wight & Arn. Hook.f.) Camus(Poaceae)	Kerala
710	Hosagoudar (2013a)	***Meliola panici*** Earle var. ***vetiveriicola*** Gawande et al.	Meliolaceae	*Meliola*	*Vetiveria zizanoides* (L.) Nash	Kerala
711	Hosagoudar (2013a)	***Meliola paramignyae*** Hosag.	Meliolaceae	*Meliola*	*Paramignya monophylla* (Rutaceae), *Paramignya armata* (Thw.) Oliver	Kerala
712	"	***Meliola parvula*** Syd.	Meliolaceae	*Meliola*	*Trichilia connaroides* (Wight & Arn.) Bentvelzen (Meliaceae)	Kerala
713	Hosagoudar (2013a)	***Meliola patileana*** Hosag.	Meliolaceae	*Meliola*	*Cryptocarya bourdillonii* Gamble (Lauraceae)	Maharashtra

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
714	Hosagoudar (2013a)	***Meliola pepparaensis*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Tabernaemontana heyneana* Wall. Apocynaceae	Kerala
715	Hosagoudar (2013a)	***Meliola pequensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Millettia pequensis* Ali (Fabaceae)	Kerala
716	Hosagoudar (2013a)	***Meliola peringamalaensis*** Hosag. & Kamar.	Meliolaceae	*Meliola*	*Alstonia scholaris* (L.) R. Br. Apocynaceae	Kerala
717	Hosagoudar and Divya (2013)	***Meliola periyakanalensis*** Hosag. & Divya	Meliolaceae	*Meliola*	*Cryptocarya* sp. (Lauraceae)	Tamil Nadu
718	Hosagoudar (2013a)	***Meliola petchii*** Hansf.	Meliolaceae	*Meliola*	*Strychnos nux-vomica* L. (Loganiaceae)	Karnataka
719	Hosagoudar (2013a)	***Meliola petrakii*** Stev. & Rold. ex Hosag.	Meliolaceae	*Meliola*	*Trichilia connaroides* (Wight & Arn.) Bentvelzen (Meliaceae)	Kerala
720	Hosagoudar (2013a)	***Meliola phaseoli*** A. N. Thite ex Hosag.	Meliolaceae	*Meliola*	*Vigna khandalensis* (Sant.) Raghu & Wadhwa *(Phaseolus khandalensis* Sant.)(Fabaceae)	Maharashtra
721	Hosagoudar (2013b)	***Meliola phyllanthigena*** Hosag.	Meliolaceae	*Meliola*	*Phyllanthus* sp. (Euphorbiaceae)	Kerala
722	Hosagoudar (2013a)	***Meliola phyllostachydis*** Yamam.	Meliolaceae	*Meliola*	*Bambusa* sp., (Poaceae)	Kerala
723	Hosagoudar (2013a)	***Meliola phyllostachydis*** Yamam. var. ***microspora*** Hosag. et al.	Meliolaceae	*Meliola*	*Bambusa* sp., *Sinarundinaria microphylla* (Munro) C.S. Chao & Renvoize (Poaceae)	Kerala
724	"	***Meliola plectroniae*** Hansf.	Meliolaceae	*Meliola*	*Canthium rheedii* DC.(Rubiaceae), *Canthium dicoccum* (Gaertn.) Teys & Benn	Kerala

725	Hosagoudar (2013a)	***Meliola pogostemonis*** Hansf.	Meliolaceae	*Meliola*	*Pogostemon pubescens* Benth. *(*Lamiaceae*)*	Kerala
726	Hosagoudar (2013a)	***Meliola polygoni*** Srinivasulu	Meliolaceae	*Meliola*	*Polygonum chinense* L. (Polygonaceae)	Kerala
727	"	***Meliola polygonicola*** Hosag.	Meliolaceae	*Meliola*	*Polygonum chinense* L. (Polygonaceae)	Kerala
728	Hosagoudar (2013a)	***Meliola polytricha*** Kalch. & Cooke	Meliolaceae	*Meliola*	*Pittosporum dasycaulon* Miq. (Pittosporaceae)	Karnataka
729	Hosagoudar (2013a)	***Meliola pongamiae*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Pongamia pinnata* (L.) Pierre (Fabaceae)	Kerala
730	Hosagoudar (2013a)	***Meliola prataprajii*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Dendropthoe falcata* (L.f.) Etting (Loranthaceae)	Kerala
731	Hosagoudar (2013a)	***Meliola premnicola*** Hosag.	Meliolaceae	*Meliola*	*Premna glaberrima* Wight. (verbenaceae)	Kerala
732	Hosagoudar (2013a)	***Meliola premnigena*** Hosag. et Riju	Meliolaceae	*Meliola*	*Premna glaberrima* Wight (Verbenaceae),	Kerala
733	Hosagoudar (2013a)	***Meliola pseudarthriae*** Hosag. & Manoj.	Meliolaceae	*Meliola*	*Pseudarthria viscida* (L.) wight & Arn.(Fabaceae)	Kerala
734	"	***Meliola pseudarthriae*** var. ***indica*** *Hosag*. et al.	Meliolaceae	*Meliola*	*Pseudarthria viscida* (L.) wight & Arn.(Fabaceae)	Kerala
735	Hosagoudar (2013a)	***Meliola psophocarpi*** Hosag. & Riju	Meliolaceae	*Meliola*	*Psophocarpus tetragonolobus* L. (Fabaceae)	Kerala
736	Hosagoudar (2013a)	***Meliola psychotriae*** Earle	Meliolaceae	*Meliola*	*Pavetta indica* L.	Kerala
737	Hosagoudar (2013a)	***Meliola psychotriae-nudiflorae*** Hosag.	Meliolaceae	*Meliola*	*Psychotria nudiflora* Wight & Arn. (Rubiaceae), *Ixora coccinea* L.	Kerala
738	"	***Meliola pterigotae*** Hosag. et al.	Meliolaceae	*Meliola*	*Pterygota alata* (Roxb.) R. Br. (Sterculiaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
739	"	***Meliola pterocarpi*** Yates	Meliolaceae	*Meliola*	*Pterocarpus santalinus* L.f. (Ceasalpiniaceae)	Kerala
740	Hosagoudar (2013a)	***Meliola pterospermis*** Stev. var. ***microspora*** Hosag. & Raghu	Meliolaceae	*Meliola*	*Pterospermum reticulatum* Wight & Arn.(Sterculiaceae)	Karnataka,
741	Hosagoudar (2013a)	***Meliola pudukadensis*** Hosag.	Meliolaceae	*Meliola*	*Litsea* sp.(Lauraceae)	Tamil Nadu
742	Hosagoudar (2013a)	***Meliola pulchella*** Speg. var. ***syzygii*** Hosag.	Meliolaceae	*Meliola*	*Syzygium laetum* (Buch.-Ham.) Gandhi (Myrtaceae)	Tamil Nadu
743	Hosagoudar (2013a)	***Meliola pushpangadanii*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Cryptocarya bourdillonii* Gamble (Lauraceae)	Kerala
744	"	***Meliola pycnosporae*** Hosag. & Archana	Meliolaceae	*Meliola*	*Pycnospora lutescens* (Poir) (Fabaceae)	Kerala
745	Hosagoudar (2013b)	***Meliola pygeicola*** Hosag.	Meliolaceae	*Meliola*	*Pygeum* sp. (Rosaceae),	Kerala
746	Hosagoudar (2013a)	***Meliola radhanagariensis*** Hosag.	Meliolaceae	*Meliola*	Euphorbiaceae member	Maharashtra
747	"	***Meliola rachammae*** Hosag. & Riju	Meliolaceae	*Meliola*	*Symplocos macrocarpa* ssp. *kanarana* (Talbot) Nooteb. (Symplocaceae	Kerala
748	Hosagoudar (2013a)	***Meliola rajamalaensis*** Hosag. et al.	Meliolaceae	*Meliola*	*Litsea* sp. (Lauraceae)	Kerala
749	Hosagoudar (2013a)	***Meliola ramacharii*** Hosag.	Meliolaceae	*Meliola*	*Persea macrantha* (Nees) Kosterm. (Lauraceae)	Tamil Nadu
750	"	***Meliola randiicola*** Hansf.	Meliolaceae	*Meliola*	*Ixora coccinea* L. (Rubiaceae), *Ixora brachiata* Roxb. ex DC	Kerala
751	Hosagoudar (2013a)	***Meliola ranganathii*** Hansf.	Meliolaceae	*Meliola*	*Eugenia* sp.	Karnataka

752	“	***Meliola rapaneae*** Syd. var. ***microspora*** Hosag. & . Ganesan	Meliolaceae	*Meliola*	*Rapanea wightiana* (Wall. ex DC.) Mez (Myrsinaceae)	Tamil Nadu
753	“	***Meliola ravii*** Hosag. et al.	Meliolaceae	*Meliola*	*Ixora elongata* Heyne ex G. Don. (Rubiaceae)	Kerala
754	Hosagoudar and Divya (2013)	***Meliola reinwartiodendricola*** Hosag. & Divya	Meliolaceae	*Meliola*	*Reinwartiodendron* sp. (Linaceae)	Karnataka,
755	Hosagoudar (2013a)	***Meliola reinwardtiodendri*** Hosag.	Meliolaceae	*Meliola*	*Reinwardtiodendron anamallayanum* (Bedd.) Saldana (Linaceae)	Tamil Nadu
756	Hosagoudar (2013a)	***Meliola rickiana*** Hansf. var. ***zanthoxyli*** Hosag.	Meliolaceae	*Meliola*	*Zanthoxylum ovata* Wight (Rutaceae), *Zanthoxylum ovalifolium* Wight	Kerala
757	Hosagoudar (2013a)	***Meliola rubi*** Stev. & Rold. ex Hansf. var. ***garhwalensis*** (Srivastava & Topal) Hosag. & Balakr.	Meliolaceae	*Meliola*	*Pyracantha crenulata* Roem. (Rosaceae)	Kerala
758	“	***Meliola rubiella*** Hansf.	Meliolaceae	*Meliola*	*Rubus* sp., *Rubus ellipticus,* (Rosaceae)	Kerala
759	Hosagoudar (2013a)	***Meliola rubiella*** Hansf. var**.** ***indica*** Hosag.	Meliolaceae	*Meliola*	*Rubus niveus* Thunb. (Rosacea)	Kerala
760	Hosagoudar (2013a)	***Meliola sacchari*** Syd.	Meliolaceae	*Meliola*	*Saccharum spontaneum* L. (Poacae)	Maharashtra
761	Hosagoudar (2013a)	***Meliola salaciae*** Hansf.	Meliolaceae	*Meliola*	*Salacia* sp. (Hippocrateaceae)	Karnataka
762	Hosagoudar (2013a)	***Meliola salleana*** Hansf. **var. *smilacis*** Hosag.	Meliolaceae	*Meliola*	*Smilax zeylanica* L.. (Smilacaceae)	Kerala
763	Hosagoudar (2013a)	***Meliola samaderae*** Hosag. et al.	Meliolaceae	*Meliola*	*Samadera indica* Gaertn. (*Quassia indica* (Gaertn.) Nooteboon) (Simaroubaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
764	Hosagoudar (2013a)	***Meliola sanjappae*** Hosag. et al.	Meliolaceae	*Meliola*	*Semecarpus travancorica* Bedd. (Anacardiaceae)	Kerala
765	Hosagoudar (2013a)	***Meliola sarcostigmaticola*** Hosag. et al.	Meliolaceae	*Meliola*	*Sarcostigma kleinii* Wight & Arn. (Icacinaceae)	Kerala
766	Hosagoudar (2013a)	***Meliola sarcostigmatis*** Hosag.	Meliolaceae	*Meliola*	*Sarcostigma kleinii* Wight & Arn. (Icacinaceae)	Kerala
767	Hosagoudar (2013a)	***Meliola scleropyri*** Hosag.	Meliolaceae	*Meliola*	*Scleropyrum pentandrum* (Dennst.) Mabberley (Santalaceae)	Kerala
768	Hosagoudar and Sabeena (2013)	***Meliola Scleropyricola*** Hosag. & Sabeena	Meliolaceae	*Meliola*	*Scleropyrum pentandrum* (Dennst) Mabb. (Santalaceae),	Kerala
769	Hosagoudar (2013a)	***Meliola scolopiae*** Doidge var. ***indica*** Hosag.	Meliolaceae	*Meliola*	*Scolopia crenata* (Wight & Arn.) Clos (Flacourtiaceae)	Tamil Nadu
770	Hosagoudar (2013a)	***Meliola serjaniae*** Stev. var. ***major*** Hansf.	Meliolaceae	*Meliola*	*Sapindus laurifolia* Vahl (Sapindaceae)	Kerala
771	"	***Meliola setariae*** Hansf. & Deight. var. ***indica*** Patil & Mahamu.	Meliolaceae	*Meliola*	*Setaria* sp. (Poaceae)	Maharashtra
772	Hosagoudar (2013a)	***Meliola shettyi*** Hosag. et al.	Meliolaceae	*Meliola*	*Actinodaphne* sp.(Lauraceae)	Kerala
773	"	***Meliola silentvalleyensis*** Hosag.	Meliolaceae	*Meliola*	Meliaceae member	Kerala
774	Hosagoudar (2013a)	***Meliola sirandhriana*** Hosag. & Archana	Meliolaceae	*Meliola*	*Aglaia minutiflora* Bedd.(Meliaceae)	Kerala
775	Hosagoudar (2013a)	***Meliola smilacacearum*** Hosag.	Meliolaceae	*Meliola*	*Smilax* sp.(Smilacaceae)	Kerala
776	Hosagoudar (2013a)	***Meliola smilacis*** Stev.	Meliolaceae	*Meliola*	*Smilax* sp.(Smilacaceae)	Kerala

777	Hosagoudar (2013a)	***Meliola spatholobii*** Hosag. et al.	Meliolaceae	*Meliola*	*Spatholobus parviflorus* (Roxb. ex DC.) Kuntze (Fabaceae), *S. roxburghii* Benth., *Spatholobus* sp.	
778	Hosagoudar (2013a)	***Meliola spigeliae*** Hansf.	Meliolaceae	*Meliola*	*Strychnos nux-vomica* L. (Loganiaceae)	Karnataka
779	"	***Meliola staphyleacearum*** Hosag.	Meliolaceae	*Meliola*	*Turpinia* sp. *Staphyleaceae)*	Tamil Nadu
780	Hosagoudar (2013a)	***Meliola stenospora*** Wint.	Meliolaceae	*Meliola*	*Piper* sp. (Piperaceae), *Piper trichostachyon* (Miq.) DC.	Kerala
781	"	***Meliola stenospora*** Wint. var. ***major*** Hansf.	Meliolaceae	*Meliola*	*Piper nigrum* L. (Piperaceae)	Kerala
782	Hosagoudar (2013a)	***Meliola sterculiacearum*** Hosag. & Kamar	Meliolaceae	*Meliola*	*Sterculia* sp. (Sterculiaceae)	Kerala
783	Hosagoudar (2013a)	***Meliola sterculicola*** Hosag. & Robin	Meliolaceae	*Meliola*	*Sterculia* sp. (Sterculiaceae)	Kerala
784	"	***Meliola strebli*** Hosag. & Archana	Meliolaceae	*Meliola*	*Streblus taxoides* (Heyne ex Roth) Kurz(Moraceae)	Kerala
785	Hosagoudar (2013a)	***Meliola stemonuri*** Hosag.	Meliolaceae	*Meliola*	*Stemonurus tetrandrus* Wall. ex Roxb. (Icacinaceae)	Kerala
786	Hosagoudar (2013a)	***Meliola stephaniae*** Hansf.	Meliolaceae	*Meliola*	*Stephania japonica* (Thunb.) Miers (Menispermaceae)	Kerala
787	Hosagoudar (2013a)	***Meliola strombosiae*** Hosag. et al.	Meliolaceae	*Meliola*	*Strombosia ceylonica* Gard. (Olacaceae)	Karnataka
788	Hosagoudar (2013a)	***Meliola strombosiigena*** Hosag. & Riju	Meliolaceae	*Meliola*	*Strombosia* sp.(Olacaceae)	Kerala
789	"	***Meliola strophanthicola*** Hansf. var. ***indica*** Hosag. & Jacob Thomas	Meliolaceae	*Meliola*	*Strophathus wightianus* Wallich ex Wight	Kerala
790	Hosagoudar (2013a)	***Meliola strychnacearum*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Strychnos* sp. (Strychnaceae)	Kerala

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
791	"	***Meliola strychnigena*** Hosag. & Manoj	Meliolaceae	*Meliola*	*Strychnos* sp. (Strychnaceae)	Kerala
792	Hosagoudar (2013a)	***Meliola subramanyaensis*** Hosag.	Meliolaceae	*Meliola*	*Cyclea peltata* Cooke (Menispermaceae)	Kerala
793	Hosagoudar (2013a)	***Meliola suttonii*** Hosag. et al.	Meliolaceae	*Meliola*	*Litsea* sp.(Lauraceae)	Kerala
794	"	***Meliola swieteniicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Swietenia mahagoni* (L.) Jacq. *(*Mliaceae*)*	Kerala
795	Hosagoudar (2013a)	***Meliola symphorematicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Symphorema involucratum* Roxb. (Symphoremataceae)	Kerala
796		***Meliola symplocicola*** Yamam.	Meliolaceae	*Meliola*	*Symplocos cochinchinensis* (Lour)-Moore ssp. *laurina* (Retz.) Nooteboom (Symplocaceae)	Kerala
797	Hosagoudar (2013a)	***Meliola syzygigena*** Hosag. & Kamar.	Meliolaceae	*Meliola*	*Syzygium* sp.(Myrtaceae)	Kerala
798	Hosagoudar (2013a)	***Meliola syzygii-benthamianii*** Hosag. **& Abraham**	Meliolaceae	*Meliola*	*Syzygium benthamianum* (Wight ex Duthie) Gamble	Kerala
799	Hosagoudar (2013a)	***Meliola tibigirica*** Hosag. & Abraham	Meliolaceae	*Meliola*	*Rhynchospora corymbosa* (L.) Britt. Cyperaceae	Kerala
800	"	***Meliola tecleae*** Hansf. var. ***toddaliae-asiaticae*** Hansf.	Meliolaceae	*Meliola*	*Toddalia asiatica* (L.) Lam. (Rutaceae)	Kerala
801	Hosagoudar (2013a)	***Meliola tenella*** Pat.	Meliolaceae	*Meliola*	*Murraya paniculata* (L.) Jack (M. exotica L.) (Rutaceae)	Kerala
802	Hosagoudar (2013a)	***Meliola tenella*** Pat. var. ***atalantiae*** (Pat.) Hansf.	Meliolaceae	*Meliola*	*Atalantia monophylla* (L.) Correa (Rutaceae	Kerala
803	"	***Meliola tenella*** Pat. var. ***atalantiicola*** Hosag.	Meliolaceae	*Meliola*	*Atalantia monophylla* (L.) Correa (Rutaceae)	Kerala

804	Hosagoudar (2013a)	***Meliola teramni*** Syd. var. ***millettiae*** Hosag.	Meliolaceae	*Meliola*	*Millettia rubiginosa* Wight & Arn.	Tamil Nadu
805	Hosagoudar (2013a)	***Meliola teramnicola*** Hosag.	Meliolaceae	*Meliola*	*Teramnus labialis* (L.f.) Spreng. (Fabaceae)	Kerala
806	Hosagoudar (2013a)	***Meliola tetradeniae*** (Berk.) Theiss. & Syd.	Meliolaceae	*Meliola*	*Neolitsea* sp. (Lauraceae)	Kerala
807	Hosagoudar (2013a)	***Meliola themedae*** Stev. & Rold. ex Hansf. var. ***indica*** Hosag.	Meliolaceae	*Meliola*	*Themeda cymbaria* Hack. (Poyaceae)	Kerala
808	Hosagoudar (2013a)	***Meliola themedicola*** Hosag. et al.	Meliolaceae	*Meliola*	*Themeda triandra* Forssk(Poyaceae)	Kerala
809	Hosagoudar (2013a)	***Meliola thetei*** Hosag.	Meliolaceae	*Meliola*	*Piper nigrum* L. (Piperaceae)	Kerala
810	"	***Meliola thirumalacharii*** Hosag. & Rajendran	Meliolaceae	*Meliola*	*Microcos paniculata* L. (*Grewia microcos* L.) (Tiliaceae)	Tamil Nadu
811	Hosagoudar (2013a)	***Meliola thiruvananthapurica*** Hosag. & Abraham	Meliolaceae	*Meliola*	Apocynaceae *member*	Kerala
812	Hosagoudar (2013a)	***Meliola thiteana*** Hosag.	Meliolaceae	*Meliola*	*Glochidion* sp. (Euphorbiaceae)	Maharashtra
813	Hosagoudar (2013a)	***Meliola thiyagesannii*** Hosag. et al.	Meliolaceae	*Meliola*	*Polygalla arillata* Buch. Ham. Ex D. Don.	Tamil Nadu
814	"	***Meliola toddaliae*** Doidge	Meliolaceae	*Meliola*	*Pamburus missionis* (Wight) Swingle(Rutaceae)	Kerala
815	Hosagoudar (2013a)	***Meliola toddaliicola*** Hansf. var. ***indica*** Hansf. & Thirum.	Meliolaceae	*Meliola*	*Toddalia asiatica* (L.) Lam. (*T. aculeata* Pers.). (Rutaceae)	Kerala
816	"	***Meliola toonae*** Hosag. & Sabu	Meliolaceae	*Meliola*	*Toona ciliata* M. Roem. (Meliaceae)	Kerala
817	Hosagoudar (2013a)	***Meliola toreniae*** Hosag.	Meliolaceae	*Meliola*	*Torenia travancorica* Gamble (Scrophulariaceae)	Kerala
818	Hosagoudar (2013b)	***Meliola tragiae*** Hosag. & Jagath Timmaih	Meliolaceae	*Meliola*	*Tragia* sp. (Euphorbiaceae)	Karnataka,

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
819	Hosagoudar (2013a)	***Meliola transvaalensis*** Doidge	Meliolaceae	*Meliola*	*Myrsine africana* L. (Myrsinaceae)	Kerala
820	Hosagoudar (2013a)	***Meliola trichostroma*** (Kunze) Toro	Meliolaceae	*Meliola*	*Psidium guajava* L. (Myrtaceae)	Maharashtra
821	Hosagoudar (2013a)	***Meliola trewiae*** Hosag.	Meliolaceae	*Meliola*	*Trewia polycarpa* Benth. ex Hook. (Euphorbiaceae)	Tamil Nadu
822	Hosagoudar (2013a)	***Meliola twaitesiana*** Hansf.	Meliolaceae	*Meliola*	*Ixora coccinea* L. (Rubiaceae), *Ixora arborea* Roxb. ex Smith	Kerala
823	Hosagoudar (2013a)	***Meliola urariae*** Hosag.	Meliolaceae	*Meliola*	*Uraria rufescens* (DC.) Schindl. (Fabaceae)	Kerala
824	"	***Meliola vatsavayai*** Hosag. & Riju	Meliolaceae	*Meliola*	*Zanthoxylum rhetsa* (Roxb) DC. (Rutaceae)	Kerala
825	"	***Meliola vazhachalensis*** Hosag. & Jacob Thomas	Meliolaceae	*Meliola*	*Aglaia* sp.	Kerala
826	Hosagoudar (2013a)	***Meliola vitis*** Hansf.	Meliolaceae	*Meliola*	*Vitis* sp. (Vitaceae)	Kerala
827	Hosagoudar (2013a)	***Meliola vittalii*** Hosag. et al.	Meliolaceae	*Meliola*	*Symplocos* sp. *Symplocos cochinchinensis* (Lour)-Moore ssp. *laurina* (Retz.) Nooteboom (Symplocaceae)	Kerala
828	Hosagoudar (2013a)	***Meliola vivekananthanii*** Hosag. et al.	Meliolaceae	*Meliola*	*Antidesma zeylanica* Laun. (*Antidesma alexiteria* L.) (Stilaginaceae)	Kerala
829	Hosagoudar (2013a)	***Meliola vepridis*** Hosag. et al.	Meliolaceae	*Meliola*	*Vepris bilocularis* (Wight & Arn.) Engl. (Rutaceae)	Kerala

830	Hosagoudar (2013a)	***Meliola weberae*** Kapoor	Meliolaceae	*Meliola*	*Webera corymbosa* Willd. (Rubiaceae), *Tarenna asiatica* (L.) Sant. & Merch. *Meliola weberae* Kapoor (*Chomelia asiatica* O. Kze.),	Kerala
831	Hosagoudar (2013a)	***Meliola wendlandiae*** Hosag.	Meliolaceae	*Meliola*	Wendlandia tomentosa (Rubiaceae), *Wendlandia notoniana* Wall.	Kerala
832	Hosagoudar (2013a)	***Meliola woodfordiae*** Srinivausulu	Meliolaceae	*Meliola*	*Woodfordia fruticosa* Kurz. (Lythraceae)	Maharashtra
833	Hosagoudar (2013a)	***Meliola zanthoxyli*** Hansf.	Meliolaceae	*Meliola*	*Zanthoxylum ovalifolium* Wight, *Zanthoxylum* tetraspermum Wight & Arn. (Rutaceae)	Kerala
834	Hosagoudar (2013a)	***Meliola zanthoxyli-ovalifolii*** Hosag.	Meliolaceae	*Meliola*	*Zanthoxylum ovalifolium* Wight	Kerala
835	Hosagoudar (2013a)	***Meliola ziziphi*** Hansf. & Thirum.	Meliolaceae	*Meliola*	(*Z. trinervia* Roxb.) *Z. rugosa* Lamk. *Z. xylopyrus* (Retz.) Willd.. *Ziziphus glabrata* Heyne ex Roth	Kerala
836	Hosagoudar (2013a)	***Meliolaster aporusae*** Hosag. et al.	Asterinaceae	*Meliolaster*	*Aporusa lindleyana* (Wight) Baill. (Euphorbiaceae),	Kerala
837	Hosagoudar and Divya (2013)	***Palawaniella jasmini*** (Doidge) Arx & Müller	Asterinaceae	*Palawaniella*	*Ligustrum perrottetti* DC. (Oleaceae),	Kerala
838	Hosagoudar (2013a)	***Phyllachor gymneme*** Hosag. et al.	Phyllachoraceae	*Phyllachora*	*Gymnema sylvestre* R. Br. (Asclepiadaceae)	Kerala
839	"	***Phyllachora hugoniae*** Theiss. & Syd.	Phyllachoraceae	*Phyllachora*	*Hugonia mystax* L. (Linaceae)	Tamil Nadu

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
840	Hosagoudar (2013a)	***Phyllachora millettiae-rubiginosae*** Hosag. & Pande	Phyllachoraceae	*Phyllachora*	*Millettia rubiginosa* Wight & Arn.	Kerala
841	"	*Phyllachora travancorica* Ramakr., K.	Phyllachoraceae	*Phyllachora*	Polyalthia longifolia(Annonaceae)	Kerala
842	Lonkar et al. 2018	***Prillieuxina dichapetali*** Lonkar, Patil & Salunkhe	Asterinaceae	*Prillieuxina*	*Dichapetalum gelonioides* (Roxb.) Engl (Dichapetalaceae),	Maharashtra
843	Hosagoudar (2013a)	***Prataprajella rubi*** Hosag. et al.	Meliolaceae	*Prataprajella*	*Rubus ellipticus* Smith(Rosaceae), *Rubus* sp.	Kerala
844	Hosagoudar (2013a)	***Prataprajella turpiniicola*** (Hosag.) Hosag	Meliolaceae	*Prataprajella*	*Turpinia malabarica* Gamble (Staphyleaceae)	Kerala
845	Hosagoudar (2013a)	***Prillieuxina anamirtae*** (Syd. & Syd.) Ryan	Asterinaceae	*Prillieuxina*	*Anamirta cocculus* (L.) Wight & Arn. (Menispermaceae),	Kerala
846	Hosagoudar (2013a)	***Prillieuxina argyreiae*** (Hosag., Balakr. & Goos) Hosag.	Asterinaceae	*Prillieuxina*	*Argyreia* sp. (Convolvulaceae),	Tamil Nadu
847	"	***Prillieuxina aquifoliacearum*** Hosag. et al.	Asterinaceae	*Prillieuxina*	*Ilex denticulata* Wall. *ex* Wight (Aquifoliaceae),	Tamil Nadu
848	Hosagoudar (2013a)	***Prillieuxina elaegni*** Hosag. & C.K. Biju	Asterinaceae	*Prillieuxina*	*Elaegnus kologa* Schlecht. (Elaegnaceae)	Kerala
849	Hosagoudar (2013a)	***Prillieuxina garciniae*** Hosag.	Asterinaceae	*Prillieuxina*	*Garcinia imberti* Bourd. (Clusiaceae),	Kerala
850	Hosagoudar et al. (2013a)	***Prillieuxina humboltiae*** Hosag., Jagath Thimmaiah & Archana	Asterinaceae	*Prillieuxina*	*Humboltia* sp. (Fabaceae),	Karnataka
851	Hosagoudar (2013a)	***Prillieuxina ixorigena*** Hosag. & Chandr.	Asterinaceae	*Prillieuxina*	*Ixora coccinea* L. (Rubiaceae),	Kerala
852	Hosagoudar (2013b)	***Prillieuxina loranthi*** (Syd. & P. Syd.) Syd	Asterinaceae	*Prillieuxina*	*Loranthus* sp. (Loranthaceae)	Kerala

853	Hosagoudar (2013a)	***Prillieuxina diospyri*** Hosag. & Chandr.	Asterinaceae	*Prillieuxina*	*Diospyros malabaricus* (Desr.) Kostel. (Ebenaceae),	Kerala
854	"	***Prillieuxina jasmini*** (Hosag. & Abraham) Hosag.	Asterinaceae	*Prillieuxina*	*Jasminum flexile* Vahl (Oleaceae),	Kerala
855	Hosagoudar and Divya (2013)	***Prillieuxina memecylonis*** Hosag. & Divya	Asterinaceae	*Prillieuxina*	*Memecylon* sp. (Melastomataceae)	Kerala
856	Hosagoudar and Sabeena (2013)	***Prillieuxina pavettae*** Hosag. & Sabeena	Asterinaceae	*Prillieuxina*	*Pavetta tomentosa* Roxb. ex Smith (Rubiaceae)	Kerala
857	Hosagoudar (2013a)	***Prillieuxina polyalthiae*** Hosag. & Abraham	Asterinaceae	*Prillieuxina*	*Polyalthia longifolia* (Sonn.) Thawaites. (Annonaceae),	Kerala
858	"	***Prillieuxina pterigotae*** Hosag. & Abraham	Asterinaceae	*Prillieuxina*	*Pterygota alata* (Roxb.) R. Br. (Sterculiaceae),	Kerala
859	Sabeena and Hosagoudar (2018a, b)	***Questieriella ardisiae*** Hosag. & Vijay	Schiffnerulaceae	*Questieriella*	*Ardisia Solanaceae* (Myrsinaceae)	Karnataka, Kerala
860	"	***Questieriella grewiae*** Hosag. & C.K. Biju	Schiffnerulaceae	*Questieriella*	*Grewia* sp. (Tilaceae)	Kerala
861	"	***Questieriella malloti*** Hosag. & C.K. Biju	Schiffnerulaceae	*Questieriella*	*Mallotus philippensis* (Euphorbiaceae)	Kerala
862	"	***Questieriella ophiorrhizae*** Hosag., Jagath Thimmaiah & Jayashankara	Schiffnerulaceae	*Questieriella*	*Ophiorrhiza* sp. (Rubiaceae),	Karnataka,
863	"	***Questieriella passiflorae*** Hosag. & C.K. Biju	Schiffnerulaceae	*Questieriella*	*Passiflora maculifolia* (Passifloraceae	Kerala
864	"	***Questieriella rhamni*** Hosag., Ravikumar & Archana,	Schiffnerulaceae	*Questieriella*	*Rhamnus wightii* (Rhamnaceae)	Tamil Nadu
865	"	***Questieriella sarcoccae*** Hosag., Manoj. & H. Biju	Schiffnerulaceae	*Questieriella*	*Sarcocca brevifolia* (Buxaceae); *S. coriacea*	Kerala Tamil Nadu

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
866	”	***Questieriella strychni*** Hosag	Schiffnerulaceae	*Questieriella*	*Strychnos nuxvomica* (Strychnaceae)	Karnataka Kerala
867	”	***Questieriella tephrosiae*** Hosag. & Agarwal	Schiffnerulaceae	*Questieriella*	*Tephrosia tinctoria* (Fabaceae)	Kerala
868	”	***Questieriella toddaliae*** Hosag., Madhavan, Dhivaharan & Sangeetha	Schiffnerulaceae	*Questieriella*	*Toddalia asiatica* ((Rutaceae); *T. asicatica* var. *floribunda*	Tamil Nadu
869	”	***Questieriella zanthoxyli*** Hosag., Jacob. & Robin	Schiffnerulaceae	*Questieriella*	*Zanthoxylum khasianum* (Rutaceae); *Zanthoxylum* sp.	Karnataka
870	”	***Sarcinella allophyli*** Hosag	Schiffnerulaceae	*Sarcinella*	*Allophylus cobbe* (Sapindaceae)	Karnataka
871	”	***Sarcinella atalantiae*** Hosag., Riju	Schiffnerulaceae	*Sarcinella*	*Atalantia* sp. (Rutaceae)	Kerala
872	Hosagoudar et al. (2013a)	***Sarcinella bischofiae*** Hosag	Schiffnerulaceae	*Sarcinella*	*Bischofia javanica* (Euphorbiaceae)	Karnataka
873	Sabeena and Hosagoudar (2018a, b)	***Sarcinella caralliae*** Hosag., Jagath Thimmaiah & Jayashankara	Schiffnerulaceae	*Sarcinella*	*Carallia brachiata* (Rhizophoraceae)	Karnataka
874	”	***Sarcinella cassiae*** Butler ex Munjal & Kapoor	Schiffnerulaceae	*Sarcinella*	*Cassia tora* (Caesalpiniaceae); *C. occidentalis*	Karnataka
875	”	***Sarcinella cassiae-fistulae*** Hosag. & Shajivaz	Schiffnerulaceae	*Sarcinella*	*Cassia fistula* (Caesalpiniaceae)	Kerala
876	”	***Sarcinella cipadessae*** Hosag. & Jacob Thomas	Schiffnerulaceae	*Sarcinella*	*Cipadessa baccifera* (Meliaceae)	Kerala
877	”	***Sarcinella dalbergiae*** Hosag. & Agarwal	Schiffnerulaceae	*Sarcinella*	*Dalbergia* sp. (Fabaceae)	Kerala
878	”	***Sarcinella embeliae*** Hosag., & Sabeena	Schiffnerulaceae	*Sarcinella*	*Embelia tsjeriam- cottam* (Myrsinaceae)	Maharashtra

879	”	***Sarcinella gmelinae*** Hosag., Archana, Harish, Riju & Agarwal	Schiffnerulaceae	*Sarcinella*	*Gmelina arborea* (Verbenaceae)	Kerala
880	”	***Sarcinella gymnosporiae*** Subhedar & Rao ex Hosag.,	Schiffnerulaceae	*Sarcinella*	*Gymnosporia rothiana* (Celastraceae)	Maharashtra
881	”	***Sarcinella hippocrateae*** Srivastava, Chandra & Gupta	Schiffnerulaceae	*Sarcinella*	*Hippocratea arborea* (Hippocrataceae); *Hippocratea* sp.	Karnataka
882	”	***Sarcinella hughesii*** Hosag. & Venkanna	Schiffnerulaceae	*Sarcinella*	*Nothopodytes foetida* (Icacinaceae); *Nothopodytes* sp.	Kerala, Maharashtra
883	”	***Sarcinella hugoniae*** Hosag. & Kamar	Schiffnerulaceae	*Sarcinella*	*Hugonia mystax* (Linaceae)	Kerala,
884	”	***Sarcinella kamalii*** Singh & Singh	Schiffnerulaceae	*Sarcinella*	*Syzygium cumini* (Myrtaceae)	Karnataka
885	Bhise et al. 2014	***Sarcinella ligustri*** Bhise and Patil	Schiffnerulaceae	*Sarcinella*	*Ligustrum perrotetti* (Oleaceae)	Maharashtra
886	Sabeena and Hosagoudar (2018a, b)	***Sarcinella limoniae*** Hosag., Sabeena & Riju	Schiffnerulaceae	*Sarcinella*	*Limonia altissima* (Rutaceae)	Kerala
887	”	***Sarcinella loranthacearum*** Hosag., Jacob Thomas & Agarwal,	Schiffnerulaceae	*Sarcinella*	*Loranthus* sp. (Loranthaceae)	Kerala
888	“	***Sarcinella oreocnidecola*** Hosag	Schiffnerulaceae	*Sarcinella*	*Oreocnide integrifolia* (Urticaceae)	Kerala
889	”	***Sarcinella oreophila*** H. Sydow	Schiffnerulaceae	*Sarcinella*	*Carissa carandas* (Apocyanaceae)	Kerala
890	Hosagoudar et al. (2013a)	***Sarcinella pogostemonis*** Hosag., C. Jagath Thimmaiah & Sabeena	Schiffnerulaceae	*Sarcinella*	*Pogostemon benghalensis* (Lamiaceae)	Karnataka
891	Sabeena and Hosagoudar (2018a, b)	***Sarcinella pouzolziae*** Hosag	Schiffnerulaceae	*Sarcinella*	*Pouzolzia* sp. (Urticaceae)	Karnataka
892	“	***Sarcinella quesqualidis*** Hosag. & Jacob Thomas	Schiffnerulaceae	*Sarcinella*	*Quisqualis indica* (Combretaceae)	Kerala
893	”	***Sarcinella raimundi*** Sacc.	Schiffnerulaceae	*Sarcinella*	*Solanum* sp. (Solanaceae)	Kerala

(continued)

Table 1 (continued)

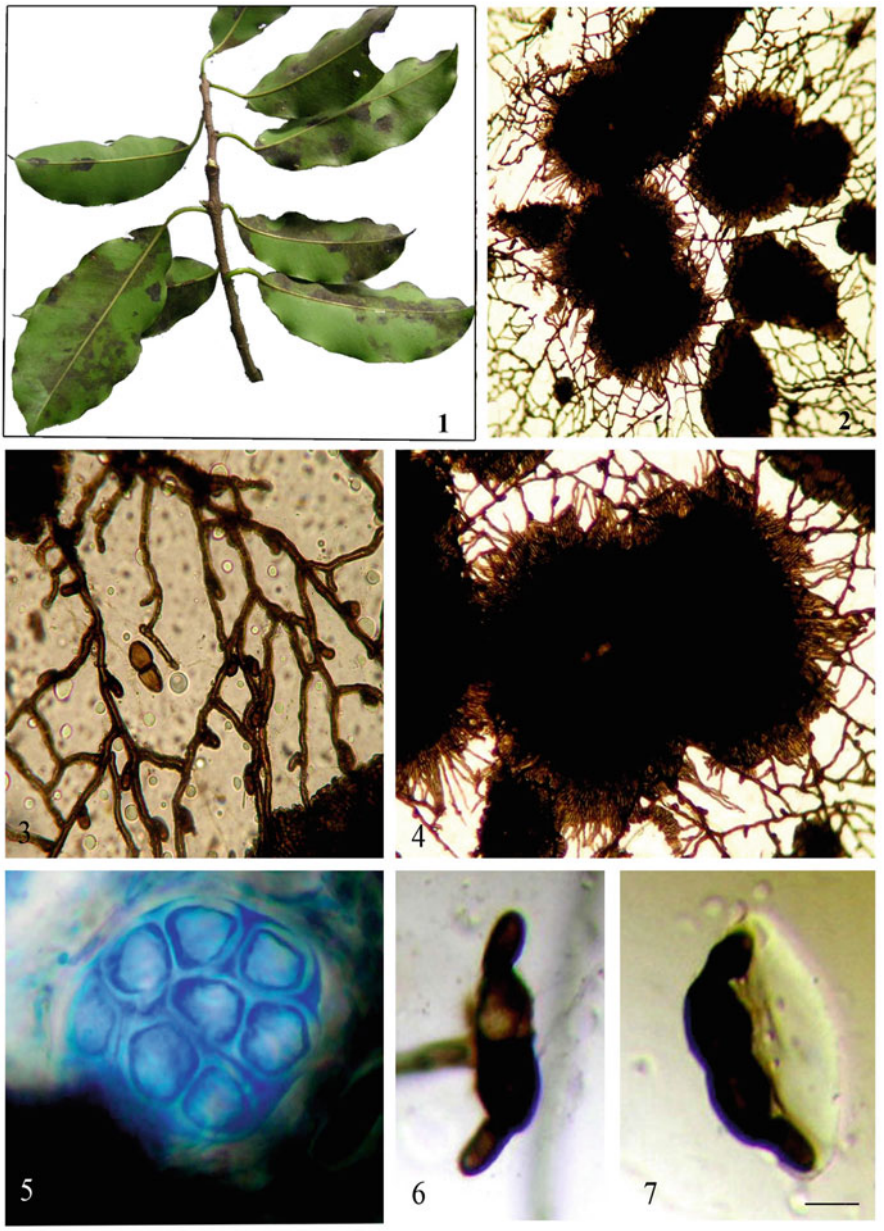
No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
894	Hosagoudar et al. (2013a)	***Sarcinella securinegae*** Hosag., C. Jagath Thimmaiah & Sabeena	Schiffnerulaceae	*Sarcinella*	*Securingea leucopyrus* (Euphorbiaceae	Karnataka
895	Sabeena and Hosagoudar (2018a, b)	***Sarcinella tamarindi*** Hosag. & Riju	Schiffnerulaceae	*Sarcinella*	*Tamarindus indica* (Caesalpiniaceae)	Kerala
896	”	***Sarcinella theae*** Hosag	Schiffnerulaceae	*Sarcinella*	*Thea sinensis* (Theaceae)	Karnataka
897	”	***Sarcinella wrightiae*** Hosag., Archana, & Agarwal	Schiffnerulaceae	*Sarcinella*	*Wrightia sp. (*Apocynaceae*)*	Kerala
898	”	***Schiffnerula actinodaphnes***Hosag., Archana, Harish, Riju & Agarwal	Schiffnerulaceae	Schiffnerula	*Actinodaphne (*Lauraceae*)*	Kerala
899	”	***Schiffnerula aristolochiae*** Hosag., Jagath Thimmaiah & Jayashankara	Schiffnerulaceae	Schiffnerula	*Aristolochia tagala (*Aristolochiaceae*),*	Karnataka
900	”	***Schiffnerula azadirachtae*** Hosag. & Sabeena	Schiffnerulaceae	Schiffnerula	*Azadiracta indica (*Meliaceae*)*	Kerala
901	”	***Schiffnerula braunii*** Hosag. & Sabeena	Schiffnerulaceae	Schiffnerula	*Morinda pubescence (*Rubiaceae*)*	Kerala
902	”	***Schiffnerula brideliae*** Hansf	Schiffnerulaceae	Schiffnerula	*Bridelia sp. (*Euphorbiaceae*); Bridelia macrantha*	Kerala
903	”	***Schiffnerula camelliae*** (Sydow, Sydow & But-ler) Hughes	Schiffnerulaceae	Schiffnerula	*Thea sinensis (*Theaceae*)*	Kerala
904	”	***Schiffnerula canthii*** Hosag. & Archana	Schiffnerulaceae	Schiffnerula	*Canthium sp. (*Rubiaceae*)*	Kerala
905	”	***Schiffnerula catharanthi*** Hosag. & Archana	Schiffnerulaceae	Schiffnerula	*Catharanthus roseus (*Apocynaceae*)*	Kerala

906	”	***Schiffnerula celastri*** Hosag., Riju & Sabeena	Schiffnerulaceae	Schiffnerula	*Celastrus paniculatus Willd.* (Celastraceae),	Kerala Karnataka Maharashtra
907	”	***Schiffnerula cryptolepidis*** (Patil & Thite) Hughes	Schiffnerulaceae	Schiffnerula	*Cryptolepis buchanani* (Periplocaceae)	Maharashtra
908	Sabeena and Hosagoudar (2018a, b)	***Schiffnerula cryptostegiae*** Hosag. & JacobThomas in Hosagoudar	Schiffnerulaceae	Schiffnerula	*Cryptostegia sp.* (Asclepiadaceae)	Kerala
909	Nair et al. (2015)	***Schiffnerula dioscoriae*** Lini K. Mathew, Neeta N. Nair & S. Swapna	Schiffnerulaceae	Schiffnerula	*Dioscorea wallichii* (Dioscoreaceae)	Kerala
910	Sabeena and Hosagoudar (2018a, b)	***Schiffnerula ecliptae*** Hosag., Sabeena & Riju	Schiffnerulaceae	Schiffnerula	*Eclipta alba* (Asteraceae)	Kerala
911	Sabeena and Hosagoudar (2018a, b)	***Schiffnerula flacourtiae*** Hosag. & JacobThomas	Schiffnerulaceae	Schiffnerula	*Flacourtia sp.* (Flacourtiaceae)	Kerala
912	Sabeena and Hosagoudar (2018a, b)	***Schiffnerula girijae*** Hosag. & Archana	Schiffnerulaceae	Schiffnerula	*Aegle marmelos* (Rutaceae)	Kerala
913	”	***Schiffnerula glochidii*** Hosag	Schiffnerulaceae	Schiffnerula	*Glochidion sp.* (Euphorbiaceae)	Kerala Karnataka
914	”	***Schiffnerula hoddurensis*** Hosag., Jagath Thimmaiah & Jayashankara	Schiffnerulaceae	Schiffnerula	*Vitex negundo* (Verbenaceae)	Karnataka
915	”	***Schiffnerula hughesii*** Hosag	Schiffnerulaceae	Schiffnerula	*Trema orientalis* (Ulmaceae)	Kerala
916	”	***Schiffnerula lagerstroemiae*** Hosag. & Riju	Schiffnerulaceae	Schiffnerula	*Lagerstroemia microcarpa* (Lythraceae)	Kerala
917	”	***Schiffnerula meliosmatis*** Hosag., Jacob Thomas & Agarwal	Schiffnerulaceae	Schiffnerula	*Meliosma simplicifolia ssp. pungens*(Sabiaceae)	Kerala
918	”	***Schiffnerula mirabilis*** Hohn.	Schiffnerulaceae	Schiffnerula	*Passiflora foetida* (Passifloraceae); *Passiflora edulis*	Karnataka

(continued)

Table 1 (continued)

No	Ref.No	Fungus	Family	Genera	Host Plants	Locality
919	”	***Schiffnerula palodensis*** Hosag. & Riju	Schiffnerulaceae	Schiffnerula	*Solanum sp.* (Solanaceae)	Kerala
920	”	***Schiffnerula pulchra*** (Sacc.) Petrak	Schiffnerulaceae	Schiffnerula	*Ligustrum sp.* (Oleaceae)	Karnataka
921	”	***Schiffnerula ricini*** Hansf	Schiffnerulaceae	Schiffnerula	*Ricinus communis* (Euphorbiaceae	Karnataka Kerala
922	”	***Schiffnerula spilanthi*** Hosag., Sabeena & Riju	Schiffnerulaceae	Schiffnerula	*Spilanthes radicans* (Asteraceae)	Kerala
923	”	***Schiffnerula tectonae*** (Thite & Patil) Hosag	Schiffnerulaceae	Schiffnerula	*Tectona grandis* (Verbenaceae)	Karnataka Kerala
924	”	***Schiffnerula terminaliae*** Hosag. & Riju	Schiffnerulaceae	Schiffnerula	*Terminalia catappa* (Combretaceae)	Kerala
925	”	***Schiffnerula theissenii*** Hughes	Schiffnerulaceae	Schiffnerula	*Solanum* sp. (Solanaceae)	Maharashtra
926	”	***Schiffnerula triumfetticola*** (Patil & patil) Pande	Schiffnerulaceae	Schiffnerula	*Triumfetta rotundifolia* (Tiliaceae)	Maharashtra
927	”	***Schiffnerula vernoniae*** Hosag., Sabeena & Riju	Schiffnerulaceae	Schiffnerula	*Vernonia anthelmintica* (Asteraceae); *Vernonia peninsularis*	Kerala
928	Nair et al. 2015	***Schiffnerula wedeliae*** Hosag., Sabeena & Riju	Schiffnerulaceae	Schiffnerula	*Wedelia chinensis* (Asteraceae)	Kerala
929	Hosagoudar (2013a)	***Trichasterina goniothalami*** Hosag. & Goos	Asterinaceae	*Trichasterina*	*Goniothalmus wighti* Hook. f. & Thoms. (Annonaceae),	Tamil Nadu Kerala
930	“	***Symphaster mimusopsidis*** Hosag. et al.	Asterinaceae	*Symphaster*	*Mimusops elenji* L. (Sapotaceae),	Kerala
931	Hosagoudar et al. (2013b)	***Viegasia cissampeli*** (Hansf.) Bat	Asterinaceae	*Viegasia*	*Cissampelos pareira* L. (Menispermaceae),	Kerala
932	Hosagoudar (2013a)	***Vishnumyces otonephelii*** Hosag. & Harish	Asterinaceae	*Vishnumyces*	*Otonephelium stipulaceum* (Bedd.) Radlk. (Sapindaceae),	Kerala



1. Infected leaves of *Mimusops elengi*; 2. Colony with thyriothecia; 3. Appressoriate mycelium; 4. Matured thyriothecia; 5. Ascus; 6 & 7 Germinating ascospores

Fig. 3 *Asterina mimusopsidicola* Hosag. et al. on *Mimusops elengi*

on the host surface, superficial thyriothecia, globose asci, and 1–3-multi-septate ascospores. The genus mainly adopts obligate biotrophic lifestyle on living leaves and develops different heterogeneous infection strategies like expanded hypostromata, intercellular hyphae, or penetration of the host stomata (Gautam and Avasthi 2016). Cirsosia species are characterized by superficial hyphae with intercalary appressoria, the lirelliform or V–Y-shaped ascomata opening by a longitudinal fissure, and 2-celled ascospores (Batista & Maia 1960, Bezerra 2004, Hosagoudar 2010, 2012).

Schiffnerulaceous fungi flourish well in the tropics and have extended their distribution to subtropical to temperate regions (Fig. 4). The connection between teleomorph and synanamorphs is well established (Hughes 1987). The genus *Schiffnerula* includes four synanamorphs, namely, *Questieriella, Mitteriella, Digitosarcinella*, and *Sarcinella*. These are characterized by black colonies formed on the leaf surface, mycelium septate, brown, superficial, appressoriate; appressoria unicellular, formed laterally; thyriothecia orbicular, cells on the upper surface are radiating, dissolve at the center during maturity; asci globose, bitunicate, 8-spored; ascospores conglobate, brown, uniseptate, constricted at the septum. Schiffnerulaceous fungi are represented by 96 species in India belonging to the anamorphs: *Mitteriella* (1), *Questieriella* (11), *Sarcinella* (49), and the teleomorph *Schiffnerula* (35) (Sabeena and Hosagoudar, 2018).

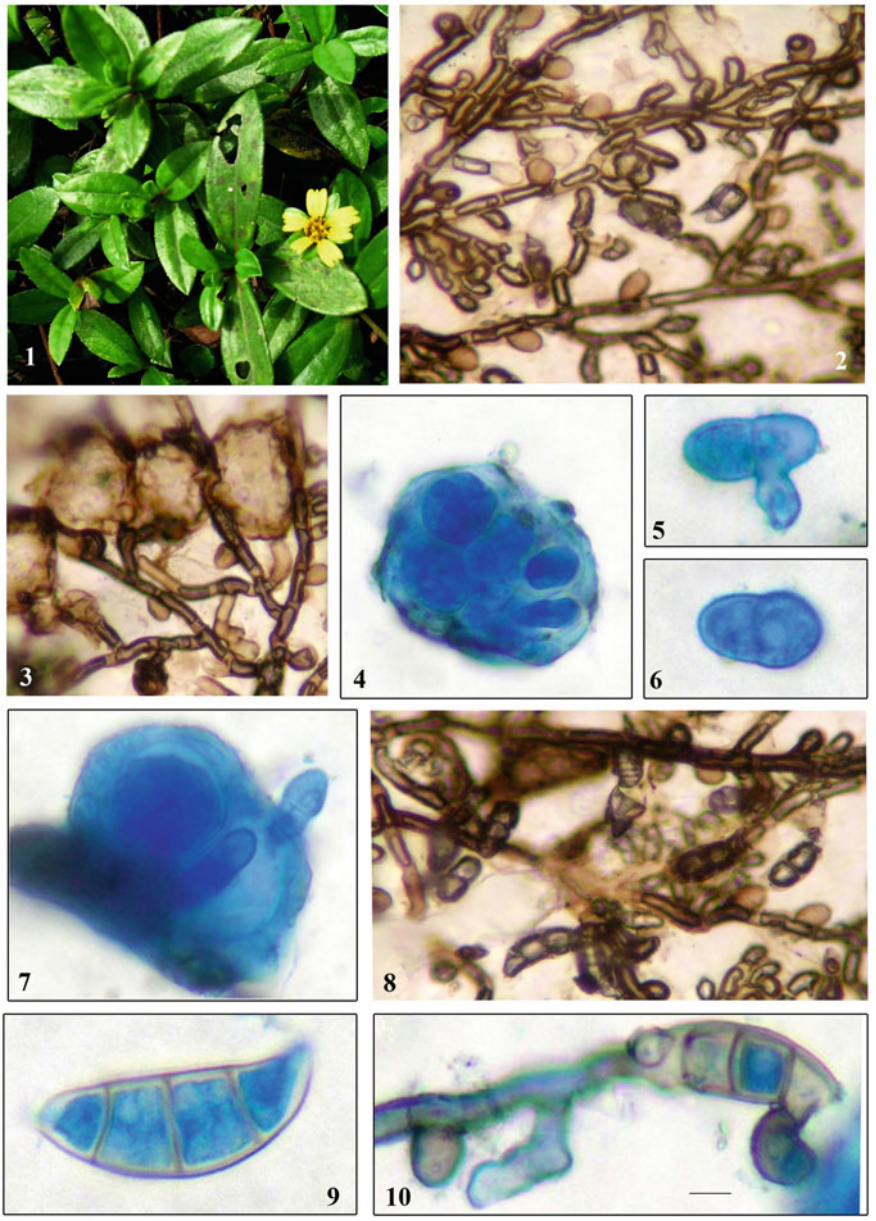
When we examine the wooly colonies or over mature colonies, actual parasites are dominated by the hyperparasites and jeopardize the black mildew's identity. Hansford (1946), Katumoto (1977, 1983), Ellis (1971, 1976), Deighton (1969), and Deighton and Pirozynski (1972) have dealt these fungi in detail.

This work includes the fungi from the Western Ghats. It comprises an account of 1500 fungal taxa belonging to 80 genera.

10 Ecological Significance Phylloplane Fungi

The value of fungi to humankind is immeasurable. Certain types of fungi, including several types of mold, have proven extremely valuable in synthesizing antibiotics and hormones used in medicine and enzymes used in specific manufacturing processes. Some fungi, such as mushrooms and truffles, are considered tasty delicacies that enhance various recipes. Not all fungi are beneficial-some release poisonous toxins in food. Sound knowledge of the taxonomy and biology of tropical fungi has immediate relevance to controlling harmful interactions and harnessing useful fungal activities for human welfare (Subramanian, 1982).

The phylloplane (leaf surface) in a forest canopy is regularly exposed to vivid sunlight, dynamic winds, and heavy rain. They are defined specialized nutritional associations found on the surface of living plant parts, particularly on leaves, including saprobes, plant parasites, fungal parasites, and lichens. Many fungal epiphytes are obligate parasites that can damage the host plants by penetrating host cells to intake nutrients. Simultaneously, some species are saprobes and cause



1. Infected leaves of *Wedelia chinensis*; 2. Appressoriate mycelium; 3. Colony with centrally dissolved thyriothecia; 4. Translucent thyriothecia reveal arrangement of asci; 5 & 6 Ascospores; 7. Release of ascospores from ascus; 8. Germinating and colony formed *Questieriella* conidia; 9. *Questieriella* conidium; 10. Germinating *Questieriella* conidium.

Fig. 4 *Schiffnerula wedeliae* Hosag. et al. on *Wedelia chinensis*

commercial problems due to the black hyphae coating plants' surface, especially economic fruits. Furthermore, they reduce the photosynthetic ability of plants through the hyphal cover; they can also cause chlorosis under the hyphae and can cause plant-stunting disease and lower yield.

Indicator species respond to the critical factors of ecological continuity, such as microclimate (temperature, humidity, light, and altitude), diversity of microhabitats for colonization, and successful dispersal and establishment possibilities (Nordén & Appelqvist 2001). Little is known about the capability of climate warming on phylloplane fungi, despite their substantial impact on plant communities' dynamics and diversity. The structure of phyllosphere fungal assemblages along elevation gradients may provide information about this capability because elevation gradients correspond to temperature gradients over short geographic distances. The analysis of these fungal diversity and distribution holds great promise for improving our understanding of the ecology and anticipating their global warming response. Global warming is ongoing and has already caused species distribution transfer and extinctions, and changes to community composition and ecosystem functioning.

The ideal temperature for fungal spore germination is 25-35 °C. The temperature and rainfall play a vital role in distributing black mildew fungi. The number of species collected from the different climatic times revealed that black mildew fungal collection negatively correlated with rainfall and temperature. During the highest rainfall time, these species' collection was comparatively meager than the remaining time. From January to March, the species collection was moderate, and it was slightly decreasing after April–August. The number of the collection was slightly increased in September to October. The number of collections was more in November and December than the remaining months. The highest temperature affects the distribution of Black Mildew fungi.

Species of Meliolales order produce appressoria that penetrate the leaf surface to gain nutrients from host plants and results in a reduction of chlorophyll, starch, sugar, protein, and amino acid, however without causing pathogenic damage (Hosagoudar et al. 1997, Old et al. 2003, Rodríguez Justavino & Piepenbring 2007). They could reduce photosynthesis by covering the host surface, and increase the temperature and respiration in those areas (Hosagoudar et al. 1997, Hosagoudar & Riju 2013, Hongsanan et al. 2015). However, generally, plants can photocompensate for the light lost to epiphyllous cover by increasing chlorophyll (Anthony et al. 2002, Gilbert et al. 2007). Species of Meliolales can cause significant effects on crops, such as reducing yield and quality of fruits of *Citrus* (e.g., *Meliola butleri*, *M. camelliae*, and *M. citricola*), but has not been seriously studied (Rao 1969, Wellman 1972).

11 Future Perspectives and Conclusion

Biodiversity is the greatest treasure of a country, and taxonomy is the base of understanding biodiversity. The more we learn about biodiversity, the more we realize how much the world reliant on it. There is an urgent need to protect biodiversity since whole species of plants, animals, fungi, and microscopic organisms are being vanished at alarming rates.

Leaves and other green parts of plants are the principal food manufacturing units (mini sugar factories). Leaves indicate the health of the plants. Any abnormality or ill health of the leaves directly affects the growth of the plant and its produce, which is directly proportional to the country's economy. Hence, an explanation of the plants' friends and foes are to be brought to light by surveying them systematically. Black mildew fungi are the leaf dweller or leaf infecting parasitic microfungi. Exploratory studies help to know: Diversity richness, which is the indication of the region's wealth, the study of the individual taxon of the Region/Nation leads to an understanding of the biodiversity of the region and host-parasite-environment relation.

Since the start of the study of fungi in India, both macro and micro fungi have been collected from all the Western Ghats ecosystems. However, none of the works are comprehensive in stating the exact number of fungi from any region of Western Ghats. New taxa and teleomorphs of imperfect fungi are frequently adding from different parts of the world, and mycologists are still revising the fungal classification. A new system of classification based on all available information and molecular data may be expected. Studying fungal diversity is essential because the estimated number of fungi is far from the currently known species. According to Hawskworth and Rossman (1997), the undescribed fungi will be in tropical forests, new habitats, and lost or unknown species. It is pertinent to consider progress in each of these categories (Hawksworth 2004). New molecular techniques like "species bar code" developed by Paul Hebert and DNA sequencing would accelerate the identification of fungi and other organisms to describe the remaining life forms on our earth before their extinction. Determining the existence and role of the undiscovered without damaging the population of their natural habitat will change the present biodiversity concept's dimension.

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Three Actinobacterial Isolates from Western Ghats of Kerala, India: Genome Mining for Their Bioative Potential

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Abstract The biocatalysts have gotten more attention, as they act as substitute in chemical processes and enabling the use of green technology. The demand for enzyme and their robust source of production is fast growing and the global market projected to $14.7 Billion by 2025. Compared to other sources, microorganisms play an important role the production sector as its easiness of handling, versatile to genetic manipulations, cost effectiveness etc., currently being employed industrial production of various enzymes. Actinobacteria are the major part of soil microbiota taken part in most of the biogeohemial proesses by producing a wide array of extracellular enzymes. Most of these enymes have high industrial signifiane as they have substrate specificity than that of other sources. Previous studies conducted by our research group led to the isolation and characterization of a few Actinomycetes, producing enzymes of robust characteristics. These include an α-amylase from *Streptomyces griseus* TBG19NRA1, chitinase from *S. californicus* TBG-201, and *Streptosporangium nondiastaticum* TBG75A20. The α-amylase was highly thermostable without any stabilizer and had an advantage over the commercially available α-amylase from *Bacillus licheniformis.* Characterization of the chitinases revealed its potential antifungal property. These chitinase can degrade the cell wall of many phytopathogenic fungi, hence having potential application in agriculture as the bio-control agent. The draft genome analysis of these actinobacteria was already been completed using the Illumina-HiSeq 2500 platform using the paired-end

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technology. The genome-based analysis of the bio-efficiency of these isolates is discussed in this chapter.

Keywords Western Ghats forests · Biodiversity · Actinobacteria · *Streptomyces* · *Streptosporangium*

1 Introduction

The Western Ghats in Indian subcontinent are globally recognized as a region of conceding global importance for its biological diversity and endemism. This mountain range is otherwise called as Sahyadri hills, having a length of 1600 km, situated parell to the Arabian sea in the western side of Indian subcontinent. It start from Songath town, south to Tapti River in the Gujarat state and end at Marunthuvazh Malai of Kanyakumari Distrit of Tamilnadu state. The Western Ghats is regarded as one of the hotspots of biodiversity as it have high level of biodiversity, and endemism, The earlier studies has shown that the area is also a storehouse of high microbial diversity and gene pool, whih offers a good source of discovery of bioactive secondary metabolites (Madhavan et al. 2013). The Agasthyamala region is situated in the south most part of Western Ghats, in the Neyyar Wildlife sanctuary and is considered as one of the centres of high endemism and biodiversity. Studies have shown that this area is a reservoir of potential actinobacteria (Shiburaj and Abraham 1998).

Actinobacteria or actinomycetes are the myceliate, gram-positive bacteria with fungal-type morphology. They also charaterised by the ability to produce spores at any stage of their growth and with high guanine-plus-cytosine (>70 mol% of G + C) content in their genomic DNA (Ghai et al. 2012). The phylum Actinomycetales (Barka et al. 2016) has five different classes, has19 orders and 50 families omprising of 221 genera (Nouioui et al. 2018; Amin et al. 2020). Many Actinomycetales genera, such as *Frankia, Mycobacterium, Corynebacterium, Streptomyces, Streptosporangium, Micromonospora*, etc., can produce a broad range of bioactive metabolites, which find applications in various industrial sectors (Bérdy 2012). *Streptomyces* spp. is regarded as the most prolific producer of antimicrobial compounds, responsible for producing over two-thirds of molecules in clinical practice (Fernández-Martínez 2019).

The rapid development of advanced genome sequencing technologies has profusely improved the chances of discovering of novel bio-active molecules from microorganisms. It has overcome many phenotypic screening limitations and metabolite isolation (Cantillo et al. 2018). Modern bioinformatics tools have elaborated on the possibilities of genome mining of microorganisms and identifying novel biosynthetic gene clusters (BGCs) (Tracanna et al. 2017). The first ever reported genome of an actinomycete was that of *Streptomyces coelicolor* (Bentley et al. 2002), and encoding a vast chemical diversity. Previously, it was known to produce only four compounds, like actinorhodin, CDA (calcium-dependent antibiotic), methylenomycin, and undecylprodigiosin, (Rebets et al. 2014). The updated

genomic information on previously studied microorganisms expanded the chances of discovering novel bio-activities and offered the possibility to express silent gene clusters in heterologous hosts.

The availability of more powerful *in-silico* tools, big-data management facilities, and the emergence of Mass spectrum-guided genome mining strategies has significantly contributed to developing novel ways to screen for biologically active compounds. This has been evidenced by the increasing number of available genomes of Actinobacteria and the transformation of genomic data into secondary metabolites.

Our research group has isolated and characterized a few Actinobacteria from the Western Ghats including *Streptomyces griseus* TBG19NRA1, *S. californicus* TBG-201, and *Streptosporangium nondiastaticum* TBG75A20. These were screened for their bioactive metabolite potential (Shiburaj 2011; Shiburaj and Preethi 2012; Divya et al. 2015) and completed their Genome sequening. With the rapid advancement of genome mining methods and subsequent bioinformatics analysis tools revealed the gene clusters responsible for the bioactive compounds.

The *Streptomyces griseus* TBG19NRA1 is known for producing thermostable α-amylase, *S. californicus* TBG-201, and *Streptosporangium nondiastaticum* TBG75A20 are capable of producing extracellular chitinase with antifungal activity, presumed to have bio-control properties against many phytopathogenic fungi. In this chapter, the authors compare the above actinobacteria's genomes and discuss the further potential of traits.

2 Hydrolases from Native Actinobacteria for Commercial Exploitation

Green technologies are promising substitute to many chemical processes, where chemial catalists are replaced by bio-catalysis. The global enyme market has predicted to have a compound annual growth rate (CAGR) of 6.6% and will attain 9.1 billion USD by 2026 (Research and Markets 2022). The key source of industrial enzymes is microorganisms, mainly due to their diversified nature, unique characteristics, versatile biochemical properties and ease for genetic manipulation. Though many microbes has been used to produce various commerialy important enzymes, a significant fraction (more than 50%) are derived from bacteria due to their ease of handling at bioprocessing level. Pristine soils have many native miroorganisms, being one of the vital component of this, members of Actinobacteria have actively partiipting in all the biogeochmical cycles producing extracellular enzymes that have ability decompose various complex organic molecules. The increased substrate specificity, and compatively higher stability than thoe from other sources making these enymes more attractive from industrial point of view. Industries always welcome robust enzymes, which offer a high turnover, cost effetivenes and availability in the market. Biocatalyst research is always channelized for the novelty and

robustness of enzymes, novel produccers with some advantage over known ones, or even existing enzymes, those improved/altered by genetic or protein engineering.

India is one the largest consumer of industrial enzymes and imports 70% of its demand. This indicates the need for development of novel robust producing trains, technologies and promot indigenous manufacturers. The essential enzymes in requirement in pharmaceutical sector represent more than 50% of the total enzyme utilization. While the detergent and textile industries require 20% each and the rest comprises food enzymes (http://forbesindia.com, 2012). There is an immense scope for developing novel and robust enymes, either by employing high through sccreening methods or by genome mining of day-by-day increasing genome data bases.

Different varieties of starh degrading enymes especially α and β-amylases are used in many industrial applications ranging from starch hydrolysis to biofuel applications. Amylase enzymes are essential in hydrolysis and saccharificcation in the production of ethanol and high fructose syrup etc. Applications in dish and laundry detergents, textile de-sizing, production of modified starches, hydrolysis of oil-field drilling fluids, paper recycling, baking and beverage industries, etc. The α-amylase from mesophilic soil bacterium, *Bacillus licheniformis* has been widely using for these appliations since 1980, which offers a thermostability upto 90 °C. But the α-amylase soucesd from this bacteria requires pH 6 for its optimum activity and calcium (Ca^{2+}) ions for its thermostability. These conditions are not suitable or eonomially viable for all industrial appliations. This disparity in industrial requirements drives the search for alternative and novel sources thermostable α-amylases. Employing recombinant tehnologies will improve the productivity of this enzyme, as the yield of α-amylase from wild-type microorganisms are comparatively less, and the process optimization often fails to improve the production rate. An α-amylase enyme with robust haraters like thermostability and calcium-independence from a mesophilic strain of *Streptomyces griseus* TBG19NRA1 was reported from the southern part of the Western Ghats (Divya et al. 2014).

The Chitinases are of glycosyl hydrolases that breaks the 1–4 β-glycoside bond of *N*-acetyl-d-glucosamine units in chitin, the second-largest biopolymer globally. Chitin is the major components of the cell walls of filamentous fungi, shells of crustaceans, exoskeletons of insects, etc. (Bhattacharya et al. 2007). The depolymerization of chitin leads to the production of monomeric and oligomeric carbohydrate units, that find various sectors of applications like protoplast preparation from fungi, biocontrol agents against plant pathogenic fungi and nematodes, etc. Recently chitin oligomers find appliations in biomedical products, drug delivery and in agriulture. Family 19 chitinases can act on fungal cell wall chitin and are found only in higher plants and certain bacteria like actinomycetes. Our previous studies demonstrated the antifungal and chitinase activities of two native isolates, *Streptomyces californicus* TBG-201 (Renjukrishna 2012) and *Streptosporangium nondiastaticum* TBG75A20 (Shiburaj 2011; Gayathri and Shiburaj 2018).

Four primier research institutions in India namely Jawaharlal Nehru Tropical Botanic Garden and Research Institute (KSCSTE-JNTBGRI), Palode, Thiruvanathapuram; National Institute for Interdisciplinary Science and Technology

(CSIR-NIIST),Thiruvananthapuram, Kerala; Department of Biotechnology-Alagappa University, Karaikudi, Tamil Nadu; and Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu, India started a programme on 'microbial bioprospecting' in 2015. This was with the available leads on hydrolases enzymes produced by native actinobacterial isolates to develop processes or products for industrial utility with financial support from Department of Biotechnology of Ministry of Science and Technology, Government of India. The study focused on Genome analysis, heterologous expression, improved production, process development, industrial utility evaluation, and validation of the enzymes on which JNTBGRI has a lead.

3 The α-Amylase Producing *Streptomyces griseus* TBG19NRA1

The amylases (1, 4-α-D-glucan glucanohydrolases) are the hydrolyse enymes (GH13 family) act on 1–4 α glucosidic bonds in the amylose and amylopectin of starch granules, making them them to water soluble simple low molecular weight carbohydrates. This has a wide range of distribution in almost all organisms. Amylases dominate the world enzyme market, with nearly one-fourth of the annual revenue (Sindhu et al. 2017). Based on their catalytic activities, and structure, these enymes grouped into α-amylases, β-amylases, and glucoamylases.

The starch liquefaction industries demand thermostable α-amylase as they perform at elevated temperatures ranging from 70–100 °C (Mobini-Dehkordi and Javan 2012). The strain of *S. griseus* TBG19NRA1 (Fig. 1) was isolated from forest soils collected in Neyyar Wild Life Sanctuary of Western Ghats of Kerala, India (Shiburaj and Abraham 1998), capable to produce a highly divergent α-amylase with calcium-

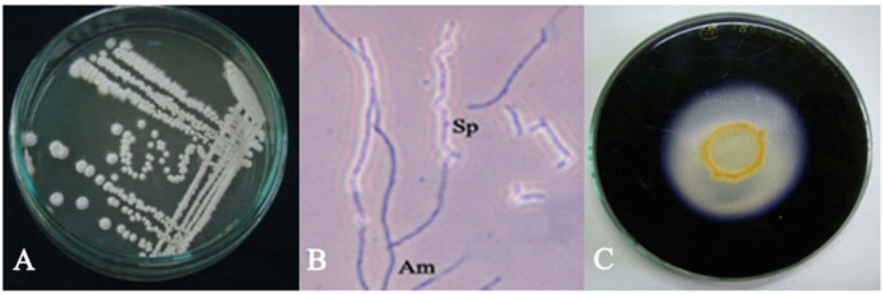

Fig. 1 (**a**) Colonies of *Streptomyces griseus* TBG19NRA1 on ISP2 Medium, (**b**) Microphotograph showing Aerial mycelia (Am) with spore chain (Sp), (**c**) Amylase activity with a zone of clearance seen on Starch Casein Agar on 4th day and stained Gram's Iodine

independent and thermostable charateristis (Divya et al. 2014; Lakshmi et al. 2020a, b).

4 Chitinase from *Streptomyces californicus* TBG-201

Chitin is the second most abundant biopolymer present in nature and is a beneficial chelating agent as it contains 6.89% nitrogen. α-chitin and β-chitin are the two allomorphic forms of chitin. Chitinase enymes belong to is glycosyl hydrolase families, with molecular size ranges from 20 to 90 kDa. The catabolism of chitin is a two-step process. With the initial cleavage, chitinases convert chitin polymer into chitin oligosaccharides, further cleave to N-acetylglucosamine, and monosaccharides chitobiases. The application of chitinases ranges from preparing single-cell protein, preparing pharmaceutically important chitooligosaccharides and N-acetyl D-glucosamine, and pathogenic control fungi wastewater treatment, mosquito control, morphogenesis, etc. Many microorganisms can produce chitinase, including bacteria, fungus, Actinomycetes, etc. Based on their substrate speificity, presance of catalytic domains and structure, chitinases are grouped into families 18 and 19. The Actinomycetes strain *Streptomyces californicus* TBG-201 (Fig. 2) is a potential source for family19 (antifungal) chitinase and was isolated from the soil samples collected from sacred groves in South Kerala (Renjukrishna 2012).

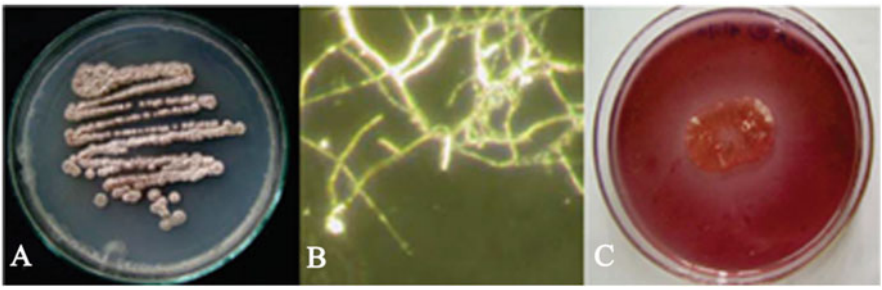

Fig. 2 (**a**) Colonies of *Streptomyces californicus* TBG-201 on ISP2 Medium, (**b**) Microphotograph showing Aerial mycelia with spore chain, (**c**) Chitinase activity with a zone of clearance seen on colloidal chitin agar on 3rd day and stained with congo red

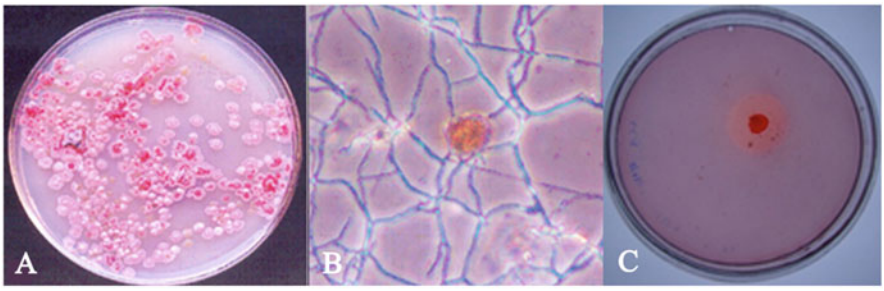

Fig. 3 (**a**) Colonies of *Streptosporangium nondiastaticum* TBG75A20 on Oat Meal Agar Medium, (**b**) Microphotograph showing Aerial mycelia with sporangium, (**c**) Chitinase activity with a zone of clearance of 3 cm diameter was seen on colloidal chitin agar on 3rd day and stained with congo red

Table 1 Summary of sequencing of genomes of three actinobacteria

Organism	*Streptomyces griseus* TBG19NRA1	*Streptomyces californicus* TBG-201	*Streptosporangium nondiastaticum* TBG75A20
Sequencing platform	Illumina HiSeq 2500	Illumina HiSeq 2500	Illumina HiSeq 2500
Library type	Paired end	Paired end	Paired end
Project type	De novo whole genome assembly	De novo whole genome assembly	De novo whole genome assembly
Calculated GC%	72.08	72.6	71.45
Number of predicted proteins	7079	7144	7228

5 Chitinase from *Streptosporangium nondiastaticum* TBG75A20

Family 19 chitinases can act on soluble and insoluble chitinous substrates more efficiently than family 18 chitinase and possess antifungal activity. The actinomycetes other than genus *Streptomyces* are grouped as rare actinobacteria; S*treptosporangium* is one among them, has attracted as a novel and vital source of different bioactive metabolites. The *S. nondiastaticum* TBG-75A20 was isolated from the Neyyar wildlife sanctuary of Kerala (Fig. 3) (Shiburaj 2011). It can produce family 19 chitinases. The native proteins were found to have antifungal activity, making this chitinase a promising biocontrol agent against fungal phytopathogens.

6 Genome Analysis

Many Streptomyces genome sequences have been reported after the publication of shotgun sequencing *Streptomyces coelicolor* A3 (Bentley et al. 2002) and *S. avermitilis* (Ikeda et al. 2003). Emerging next-generation sequencing (NGS) technologies have advanced genome analysis and witnessed a radical rise in the number of reported genomes for Streptomyces since 2013. A total of 4917 *Streptomyces* genomes are available in NCBI geneme Sequence database as of February 2022 (https://www.ncbi.nlm.nih.gov/datasets/genomes). We have also sequenced and analyzed the genomes of Actinobacteria with promising biocatalytic properties. Table 1 summarises the details of the same.

The genomic DNA of *Streptomyces griseus* TBG19NRA1, *S. californicus* TBG-201, and *Streptosporangium nondiastaticum* TBG75A20 were isolated and almost complete nucleotide sequence was determined by Next Generation Sequencing (NGS) strategy, using the Illumina-HiSeq 2500 platform and paired-end technology (outsourced to M/s Scigenom, Kochi). The quality of the sequences obtained was checked by various parameters like base quality score distributions, average base content per reading, and GC distribution in the reads. It is clear from the analysis that the average base quality is above Q30 for 84.16% of bases.

7 *De Novo* Assembly

De novo whole-genome sequencing involves assembling a genome without using a genomic reference and is often used to sequence novel microbial genomes. The obtained (Fastq files) were processed before *de novo* genome assembly. The Illumina, adapter sequences were trimmed and filtered out reads shown low quality score of less than 30. *De novo* assembly was performed using MaSuRCA after fetching unique reads using FastUniq. (Zimin et al. 2013), SPades (Bankevich et al. 2012), and SOAPdenovo2 (Luo et al. 2012) with default options. After the assembly generation, conserved genes in the assembled contigs were checked using BUSCO v2 (Simao et al. 2015). The Spades assembly was used for further downstream analysis since it has better statistics than all other packs generated, and the complete assembly statistics were made using QUAST 4.0 (Gurevich et al. 2013).

8 Gene Prediction and Annotation

We have predicted CDSs from the ABySS or SPades assembled contigs using Glimmer software. The distribution of each library's fragment sizes were calculated empirically by aligning paired reads to the contigs produced by the single-end assembler. The N50 of the single-end assembly was well over the fragment size,

Table 2 Summary of predicted and annotated proteins

Genomes	% of predicted proteins with significant BLASTX match	Gene ontology terms identified in each category		
		Biological Processes	Molecular Functions	Cellular Components
S. griseus TBG19NRA1	96.63	515	884	60
S. californicus TBG-201	96.09	501	873	64
S. Nondiastaticum TBG75A20	91.63	1017	1744	349

which shows an accurate empirical distribution in all the genomes studied. The number of predicted proteins in *Streptomyces griseus* TBG19NRA1 was 7079, *S. californicus* TBG-201 7144, and *Streptosporangium nondiastaticum* TBG75A20 7228. Number of predicted proteins with significant BLASTX match were 6841 (E-value <=1e-3 and Similarity score >=40%), 6865 (E-value <=1e-3 and Similarity score > =40%) and 6623 (E-value <=1e-5 and Similarity score > =40%) for *S. griseus*, *S. californicus* and *S. nondiastaticum* respectively.

The predicted genes were annotated using a standard pipeline with the steps; (a) Matching with the UniProt database using the BLASTX program, (b) Organism annotation, (c) Assigning annotation for predicted genes, and (d) Gene Ontology (G.O.) annotation. The predicted proteins were compared with the UniProt protein database (Bacteria) using the BLASTX program. The details are given in Table 2.

9 Phylogenetic Analysis of Amino Acid Sequence of the Enzyme of Interest

The nucleotide sequences encoding α-amylase from *Streptomyces griseus* TBG19NRA1 and chitinase genes of *S. californicus* TBG-201 and *Streptosporangium nondiastaticum* TBG75A20 were retrieved from the genome data. The ExPASy translation tool (https://web.expasy.org/translate/) was used to translate these to amino acid sequences and utilized for phylogenic analysis. Homology search analysis was done using NCBI/Blast analysis. After multiple sequene alignment of similar sequenes retrived from NBI data base, phylogenetic analysis were conducted using MEGA6 software and phlogeneti trees were constructed (Tamura et al. 2013).

The results of phylogenetic analysis and evolutionary trees are shown in Fig. 4. *S. griseus* TBG19NRA1 α-amylase formed a cluster with *S. violaceus, S. griseus* IMRU3570, and *S. limosus* α-amylases. While the α-amylase from *S. scrofa* and *H. sapiens* formed a group with *R. norvegicus* and *M. musuclus* α-amylase under the series mammals. The α-amylase of *T. molitor* and *T. castaneum* clustered with

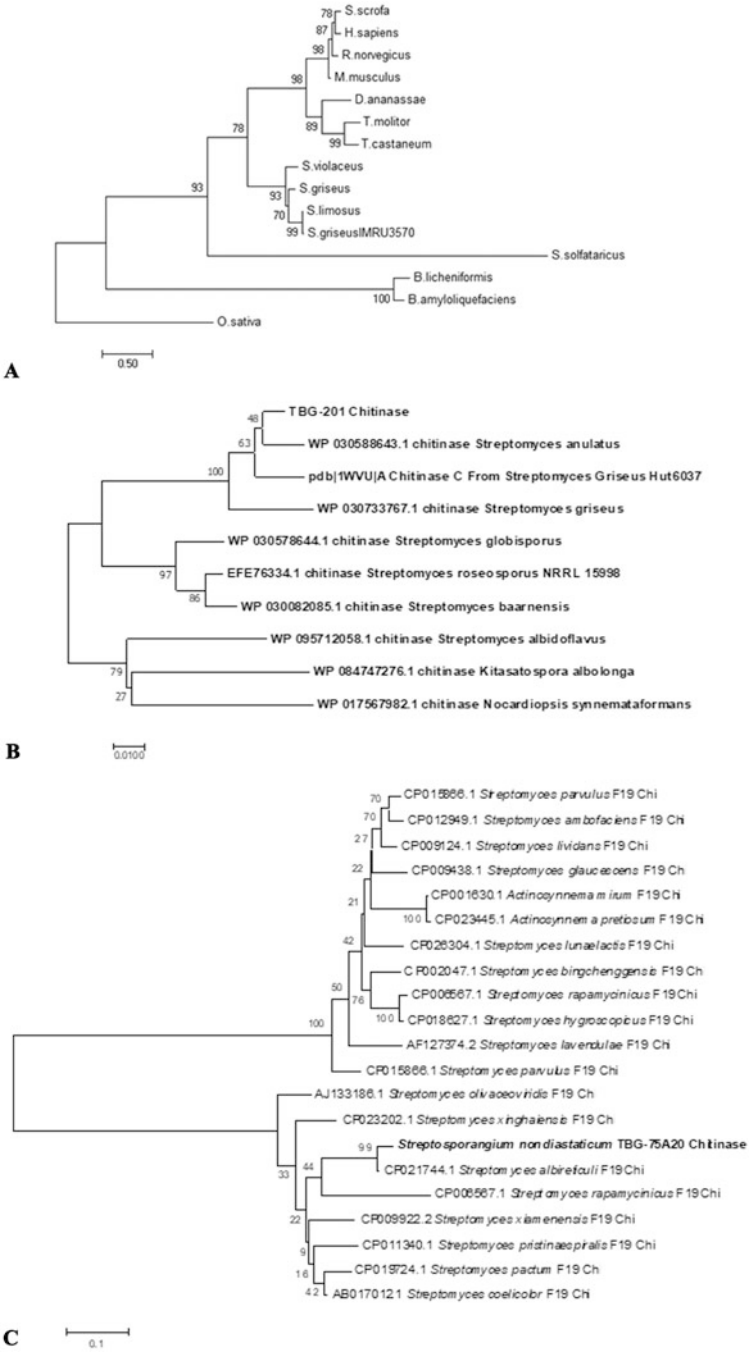

Fig. 4 Phylogenetic trees of Amino acid sequences of Amylase Gene of *Streptomyces griseus* TBG19NRA1 (**a**), Chitinase genes of *S. californicus* TBG-201 (**b**) and *Streptosporangium nondiastaticum* TBG75A20. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the

D. ananassae α-amylase under the series arthropods. The amylase from *S. solfataricus* of the archaea series is positioned distantly, whereas *Bacillus* formed an exact branch outside the clusters. The α-amylase of *Oriza sativa* forms as an outgroup, created a separate branch outside the tree (Fig. 4a). Thus, the result suggests the tree's appropriateness concerning the recently derived α-amylases from *Streptomyces*, arthropods, mammals, and divergence α-amylases from *Bacillus*, archaea, and plants. The *S. californicus* TBG-201 Chitinase shows similarity to family 19 chitinases of *Streptomyces anulatus* and *S. griseus* (Fig. 4b). The chitinase of *S. nondiastatcum* TBG75A20 showed high similarity to family 19 chitinases from *Streptomyces albireticuli* (Fig. 4c).

10 Conclusions and Future Perspective

Actinobacteria, are treasure houses of bioactive metabolites of various applications. Many of them have proved applications in agriculture, industrial and therapeutic sectors. The Western Ghats region of South India is well known for its plant diversity. Naturally, the area's high floral diversity is favorable for the growth of a wide range of microorganisms either in the rhizosphere, endophytic, or soil microecosystems related to higher plants. These will serve as a vast reservoir of a wide array of chemical diversity and gene pools. The high throughput genome sequencing and bioinformatics analytic tools have been applied to mapping many actinobacterial genomes. The knowledge about phylogenetic relationships of different actinobacterial species, genetic diversity, and distribution patterns of biosynthetic gene clusters (BGCs) is critical in drug discovery programs, development of engineered biocatalysts, and other metabolites of novel bioactivity. The genome analysis of three representatives from the Western Ghats and related areas has demonstrated high biosynthetic potential. The application of genome mining and synthetic biology studies will offer more and more bioactive molecules from these candidates. Furthermore, this study also urges the need to focus on developing state-of-the-art facilities for genome analysis and facilitating the heterologous expression of potential BGCs.

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Fig. 4 (continued) bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 10 amino acid sequences. All positions containing gaps and missing data were eliminated

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Phylogenetic Analysis in Yeast Population Using Microsatellites and Simple Sequence Tandem Repeats

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Abstract Proper identification and classification of the microbiota in several environments has prompted the search for rapid response molecular tools. Microsatellites are among the most widely used markers because they are distributed along the genome, are highly variable, and the fragments of different lengths describe genetic diversity. Repeated intergenic sequences (ISSR) are other molecular markers that allow obtaining variations between individuals due to the high rate of mutation that they experience. Application of the two techniques, correlating both and developing data analysis platforms, is an approach to differentiate individuals of the same species. Dendrograms were constructed from the similarity matrix analyzing the existence or absence of regions amplified by the markers, allowing them to work with various types of genomic and/or phenotypic data. This strategy was applied to study the phylogenetic analysis of the yeast populations present in an alcoholic fermentation, which permitted to describe the genetic diversity of the species using microsatellite markers and repeated short sequences in a correlation matrix. The approach presented here is novel for the analysis of yeast populations and showed vast biodiversity and a high polymorphism in the isolates evaluated. This finding was possible because of the high sensitivity of the markers and the use of the entire genome.

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1 Introduction

Alcoholic fermentation is characterized by the presence of different genera and species of yeast, due to its industrial interest, its identification and classification is essential. Methods based on DNA polymorphism analysis have been used to differentiate oenological strains of *Saccharomyces cerevisiae* (Versavaud et al. 1995). Molecular characterization based on the PCR technique has been shown to be more suitable for studies of yeast diversity (Granchi et al. 1999), within which are the molecular markers used for the genetic classification and characterization of yeasts. (Querol et al. 1992). These techniques include repeating inter simple sequences (ISSR) (Zietkiewicz et al. 1994) and microsatellites. The combination of different markers provides a more authentic characterization of the yeasts instead of a single marker system (Vezinhet et al. 1990; Martinez et al. 1995; Baleiras-Couto et al. 1996 y Schuller et al. 2004). The advantages of ISSR primers are that they are fast, highly polymorphic, easy to use, the primers used are long (14 to 20 nucleotides), due to this the conditions required for the reaction are more stringent, producing more reproducible profiles (Gente et al. 2002; Larpin et al. 2006), amplify the specific region between two microsatellite motifs and no prior DNA sequence knowledge is required for primer design (Wang et al. 2005; Tripathi et al. 2012; Gu et al. 2014). Microsatellites or Simple Repeated Sequences (SSR) are short DNA sequences, formed from 1 to 10 base pairs, scattered throughout the genome and codominantly inherited, in yeasts have a high degree of variability (Pérez et al. 2001). The technique consists of the amplification of fragments with specific primers for repeated simple sequences present in the DNA, called microsatellites. It differs from the randomly amplified DNA polymorphism (RAPD) technique, in that the hybridization temperature is higher than 37 ° C, so they hybridize in specific areas of the genome, so their reproducibility is higher (Orberá Ratón 2004). Mercado et al. 2010, proposes the amplification of polymorphic loci of microsatellites as a tool for the differentiation of strains of *Saccharomyces cerevisiae*.

2 Materials and Methods

For this, the strains were isolated from the walls of the mash piles and from the fermentation piles at the beginning and end of it. From the samples taken, serial dilutions in physiological solution up to 10–6 were prepared, to seed an aliquot of 100 µL in PYF medium (20 g/L agar, 10 g/L fructose, 5 g/L peptone, 5 g/L yeast extract), from Handbook of Microbiological Media (Atlas 2004) added with0.2 g/L de ampicillin at pH 4.6. Incubated until the growth of the colonies. Harrison's disc technique was used (Harrigan 1998), whereby it is possible to obtain a random representative sample of the colonies in a Petri dish (Fig. 1), selecting only those colonies that are in certain quadrants of the box that had between 150 and 300 colonies.

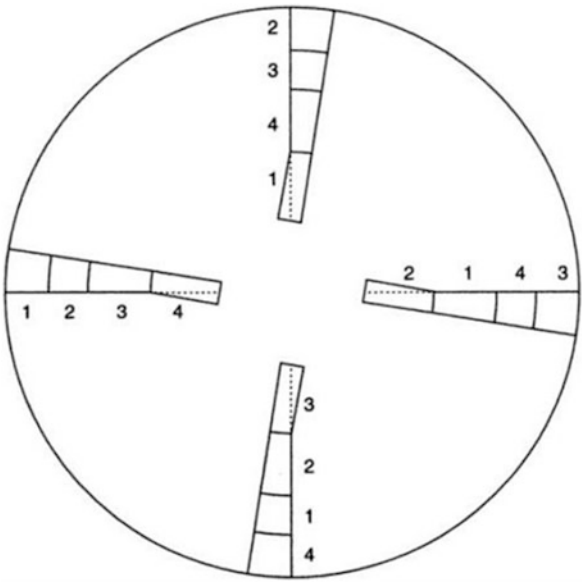

Fig. 1 Harrison's disc shows random sampling areas (Harrigan 1998)

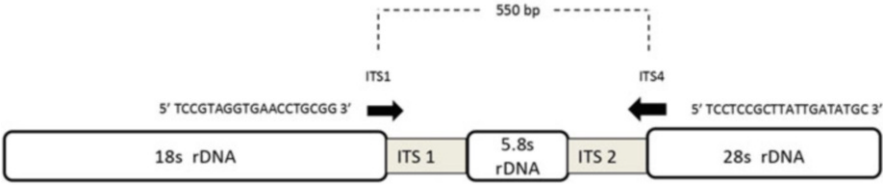

Fig. 2 Ribosomal DNA representation

The isolated colonies were growth in Petri dishes by cross streak until obtaining pure strains. The strains were preserved in 30% v / v glycerol at −20 ° C. For the identification of the isolated strains, the intergenic region was amplified ITS1–5.8S-ITS2 (Fig. 2) using the primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3′) e ITS4 (5' TCCTCCGCTTATTGATATGC 3′) (White et al. 1990). Amplifications were performed with a denaturation temperature at 95 ° C for 15 mins, 40 cycles consisting of initial denaturation at 95 ° C for 1 min, hybridization at 55.5 ° C for 2 mins and extension at 72 ° C for 2 mins, followed of a final extension of 72 ° C for 10 mins. The amplified products were digested for 6–12 h at 37 ° C with restriction enzymes *Cfo*I, *Hae*III y *Hinf*I, at final volume 25 μL (Esteve-Zarzoso et al. 1999; Guillamón et al. 1998; Belloch et al. 2002). La identificación de los fragmentos de restricción se realizó empleando la base de datos en http://www.yeast-id.com/, developed by the collaboration of the Higher Council for Scientific Research (IATA), the Spanish Collection of Type Crops and the University of Valencia.

To carry out ISSR and microsatellite amplification, genomic DNA was extracted from yeast strains according to the following description. The strains preserved in

Table 1 Primers ISSR used for amplification of DNA from isolates

Primer	Secuence (5′-3′)	Alignment temperature (°C)	Reference
ISSR 1	GAGAGAGAGAGAGAGATC	47.7	Escobar-Saucedo et al. 2018
ISSR 2	AGAGAGAGAGAGAGAGTC	50.4	Escobar-Saucedo et al. 2018
ISSR 3	CTCCTCCTCCTCCTCCTC	55.1	Escobar-Saucedo et al. 2018
ISSR 4	GTGTGTGTGTGTGTGTA	48.4	Escobar-Saucedo et al. 2018
ISSR 5	AGCAGCAGCAGCAGCGA	49.3	Escobar-Saucedo et al. 2018
ISSR 6	ACACACACACACACACA	47.0	Fracaro and Echeverrigaray 2006
ISSR 7	GTGTGTGTGTGTGTGTTC	47.2	Escobar-Saucedo et al. 2018

glycerol under freezing conditions were reactivated by water bath at 37 ° C and replanted in GPY broth (20 g/L glucose, 20 g/L casein peptone and 10 g/L yeast extract), incubating at 30 °C for 24 hours. Then the activated strains were seeded in GPY medium (20 g/L glucose, 20 g/L casein peptone, 10 g/L yeast extract and 20 g/L agar) (Páez-Lerma 2009). Total genomic DNA was extracted using a 12 h culture of the strain, which was centrifuged for 3 mins at 12000 rpm. For cell lysis, the pellet was resuspended in 500 μL of solution 1, consisting of 0.9 M sorbitol and 0.1 M EDTA added with 30 μL of zimoliase (1 mg/mL). The mixture was incubated for 20 mins at 37 °C. Subsequently, it was centrifuged at 12000 rpm for 3 mins to remove the supernatant. The obtained pellet was resuspended in 500 μL of solution 2 (50 mM Tris-HCl, 20 mM EDTA). 13 μL of 10% SDS were added and incubated at 65 °C for 5 mins, to then add 200 μL of 5 M potassium acetate. The sample was gently shaken and placed on ice for 10 mins, then centrifuged at 14000 rpm for 15 mins at 4 °C. The supernatant was transferred to a new tube with 700 μL of isopropanol, gently shaken and allowed to stand for 10 mins. The supernatant was decanted and the pellet washed with 500 μL of 70% ethanol to then centrifuge the sample for 5 mins at 12000 rpm. The supernatant was removed and the samples were dried at 37 °C. Finally, the pellet was resuspended in 20 μL of sterile milliQ water to later freeze the DNA obtained (Querol et al. 1994). Seven ISSR primers (Table 1) were tested at different temperatures under standard conditions to obtain clear and polymorphic bands. Amplification for ISSR was performed by PCR in a total volume of 23 μL, composed of 8 μL of ddH_2O, 2.17X of 10X buffer added with MgCl2, 0.21 mM of dNTPs, 2.6 μM of primer, 1 U of PaqDNA polymerase and 300 ng of DNA.

Amplifications were performed with a denaturation temperature of 94 ° C for 1 mins, 45 cycles composed of initial denaturation at 94 ° C for 1 mins, alignment temperature of each primer for 45 s (Table 1) and extension at 72 °C for 2 mins,

followed by a final extension of 72 °C for 7 mins. Amplification products were visualized on 1.5% agarose gel with 1X SB buffer (5 mM sodium borate, pH 8.0) and stained with ethidium bromide. With the help of a 100 bp marker (PROMEGA, WI, U.S.A). Amplification of loci containing microsatellites was performed with the following primers: $(GTG)_5$ and $(GAC)_5$, with a total reaction volume of 52 μL, containing 40.1 μL of ddH2O, 0.96X of 10X buffer added with $MgCl_2$, 0.8 mM of dNTPs, 0.2 μM of primer, 1 U of PaqDNA polymerase and 300 ng of DNA. The amplifications were carried out in an Axygen System with an initial denaturation temperature of 94 °C for 5 mins, 30 cycles composed of initial denaturation at 94 °C for 30 s, alignment temperature of 52 °C for 30 s and extension at 72 °C for 1 mins, followed by a final extension of 72 °C for 10 mins. The amplification products were visualized on 2% agarose gel with 1X SB buffer and stained with ethidium bromide. With the help of a 100 bp marker (PROMEGA, WI, U.S.A). The DNA fragments amplified with microsatellites and ISSR were registered visually, from the reading of the gels a binary matrix of presence (1) and absence (0) of bands was constructed, considering those of the same size as similar. The data of each one of the species will be grouped in a matrix and will be analyzed separately. With the data generated by the microsatellite and ISSR molecular markers, the percentage of polymorphic bands, the number of monomorphic bands and the number of total bands were calculated for each species. Dendrograms will be constructed from the similarity matrix with the help of the Info-Gen software. Info-Gen is software for statistical analysis of genetic data that implements a variety of analysis techniques in an integrated environment capable of processing large volumes of data. The software is oriented to the statistical analysis of data produced by new biotechnologies, such as genetic markers, and its application for the use, management and conservation of genetic diversity in ecosystems, as well as for genetic improvement. (Balzarini et al. 2010).

3 Results and Discussion

A total of 23 strains of *Saccharomyces cerevisiae*, 26 strains of *Torulaspora delbrueckii* and 25 strains of *Pichia fermentans* were isolated. DNA extraction was confirmed by 1% agarose gel electrophoresis. Figure 3 shows one of the gels in which DNA is observed in the upper band of each lane, above the molecular weight marker in the middle lane and on the right bank. It was possible to obtain a high concentration of DNA from each of the selected strains.

Of the seven ISSR primers evaluated, only one generated reproducible, polymorphic band patterns in the isolates of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* y *Pichia fermentans*. Analysis of the amplified products showed that the ISSR7 primer generated polymorphic band patterns. As can be seen in Fig. 4, a comparison of the band patterns between ISSR7 that generated polymorphic bands unlike ISSR6. The ISSR5 (Fig. 5) was the one that showed very similar bands between the strains, making it difficult to clearly define the band patterns; the ISSR1,

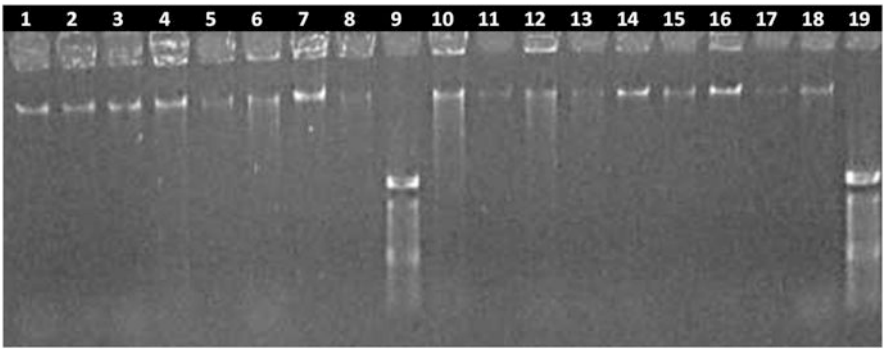

Fig. 3 Electrophoresis 1% agarose gel to confirm DNA extraction from strains of *Saccharomyces cerevisiae* (1, 2, 3, 8, 16), *Pichia fermentans* (4, 5, 6, 11, 12, 14) y *Torulaspora delbrueckii* (7, 10, 13, 15), molecular weight 100 pb (9,19)

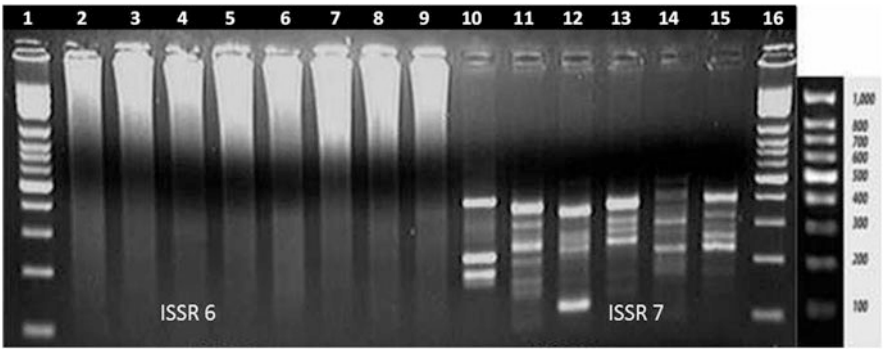

Fig. 4 Evaluation of ISSR6 (2–9) and ISSR7 (10–15) primers in agarose gel at 1.5% *Pichia fermentans* (3, 11), *Saccharomyces cerevisiae* (4, 12), *Torulaspora delbrueckii* (5, 13), other species not include in this chapter *(2, 6, 7, 8, 9, 10, 14, 15)* Molecular weight 100 bp *(1, 16)*

ISSR2, ISSR3 and ISSR4 primers generated very weak bands and had a lot of background (Fig. 6). Therefore, the ISSR7 primer was selected to analyze the genetic variability in isolates of the afore mentioned species.

Once the ISSR primer to be used was selected, the rest of the isolated strains were amplified to obtain the banding patterns and to perform the analysis of results. The analysis for microsatellites was carried out from the DNA extracted from the strains described in the methodology. The amplifications of the microsatellite regions were carried out following the parameters described in the methodology. To confirm amplification and obtaining standards, 2% agarose gel electrophoresis was performed, as shown in Fig. 7. Amplified products were observed in all samples. Agarose gels were used to establish the sizes of the alleles in each of the amplified strains. To then continue with the analysis of results.

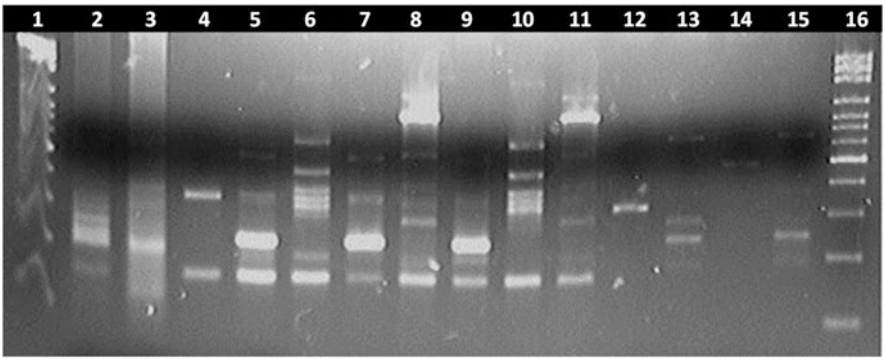

Fig. 5 Evaluation of ISSR5 (2–15) primer in agarose gel at 1.5% *Pichia fermentans* (6), *Saccharomyces cerevisiae* (7), *Torulaspora delbrueckii* (8), other species not include in this chapter *(1–5, 9–5)* Molecular weight 100 bp *(1, 16)*

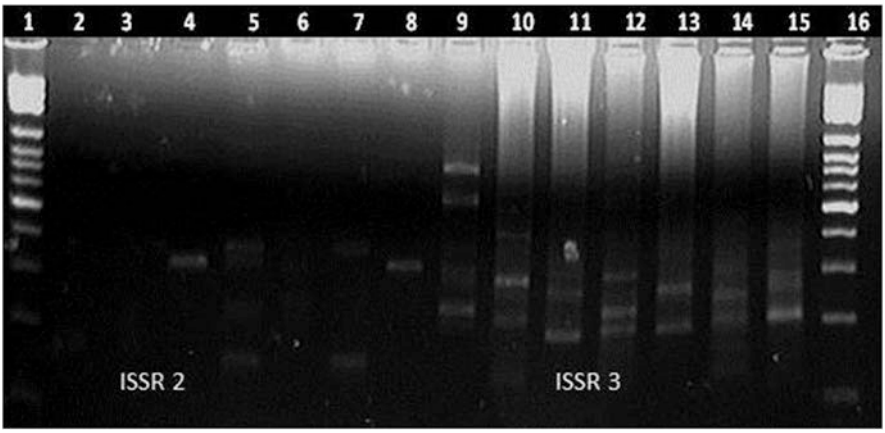

Fig. 6 Evaluation of ISSR2 (2–8) and ISSR3 (9–15) primer in agarose gel at 1.5% *Pichia fermentans* (4, 12), *Saccharomyces cerevisiae* (5, 13), *Torulaspora delbrueckii* (6, 14), other species not include in this chapter *(2, 3, 7–11, 15),* Molecular weight *(1, 16)*

From the microsatellite markers $(GTG)_5$ y $(GAC)_5$ and ISSR $(GT)_8TC$ were obtained reproducible reading patterns, so the phylogenetic analysis was continued with the help of the Info-Gen software for each of the species.

4 Philogenetic Analysis For *Saccharomyces cerevisiae*

For the 23 isolates of *S. cerevisiae*, the selected molecular markers obtained a total of 75 bands, 70.67% of which were polymorphic bands: finding two duplicate samples and 22 monomorphic bands. The markers turned out to be informative for

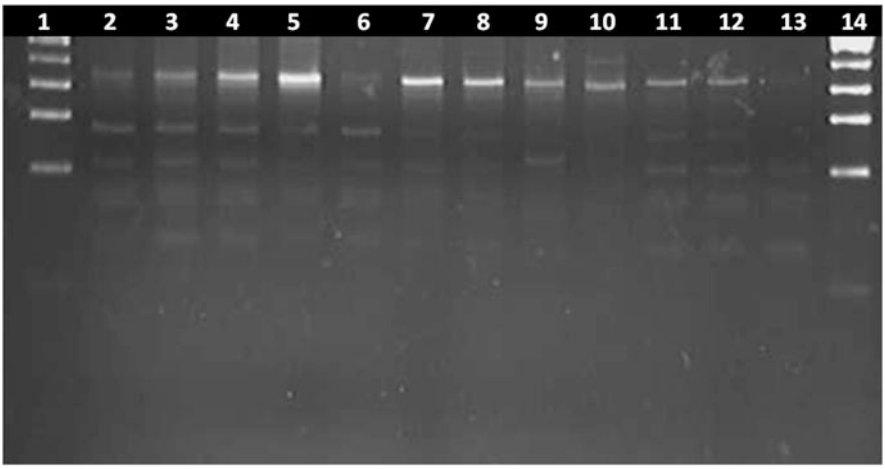

Fig. 7 Electrophoresis 2% agarose gel electrophoresis to confirm microsatellite amplification, primer $(GTG)_5$. *Saccharomyces cerevisiae.* Molecular weight 100 bp (1, 16)

Table 2 Measures of genetic diversity for *S. cerevisiae*

Summary statistical information	
Polymorphic loci (95)	0.74
Genetic diversity	0.24
Nei's unbiased heterozygosity	0.24
# allele average	2.00
# allele effectiveness	1.36
Number of alleles	106.00

S. cerevisiae since the value of the content of polymorphic information (PIC) was above 50% of the theoretical range of the content of polymorphic information (0.01 to 0.50) (Balzarini et al. 2010). Therefore, useful for subsequent characterization studies. The measures of genetic diversity (Table 2) indicate a total of 0.74 for polymorphic loci and 0.24 for genetic diversity. What generates this difference may be linked to the mutation present in the strains of *S. cerevisiae*. Polymorphic loci indicate the number of polymorphic loci present in a population. Genetic diversity is the variation in DNA sequences that may or may not be reflected in differences in the morphological characteristics of individuals of the same species (Escobar-Saucedo 2012).

Heterozygosity represents a better measure of genetic variation, as it is precise and not arbitrary (Aranguren-Méndez et al. 2005). The software returned an unbiased Nei heterozygosity of 0.24. Nei's unbiased heterozygosity is a good estimator of variability, since it applies to any species, regardless of its reproductive or genetic structure. (Balzarini et al. 2010). The average number of alleles per locus corresponds to the average of the different alleles identified for each locus in each species, in the case of the current analysis. Effective number of alleles determines the number of prevalent alleles (Balzarini et al. 2010). That is, 2 alleles were present in all

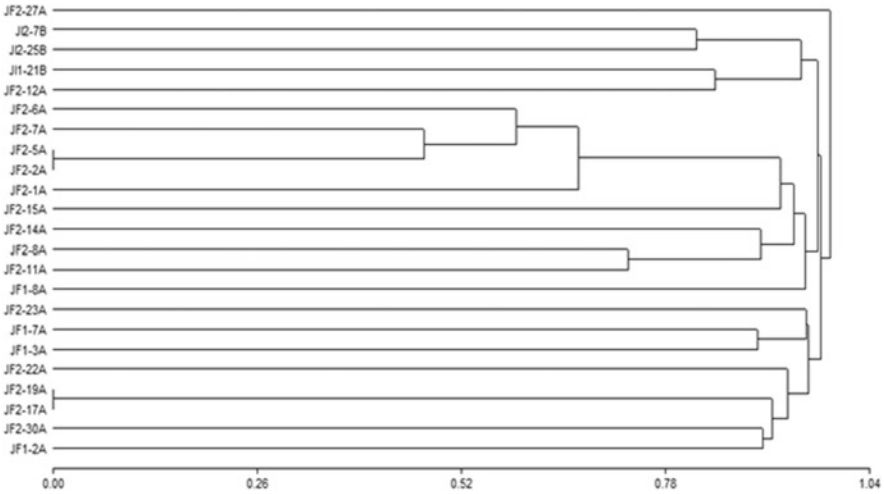

Fig. 8 Cluster analysis of *S. cerevisiae* strains based on the molecular markers of the study

individuals. And an average of 1.36 dominant alleles. Having a total number of 106 alleles.

Figure 8 shows the cluster analysis for the 23 *S. cerevisiae* samples, at an approximate distance of 0.99 all the individuals are grouped. Likewise, it indicates the formation of two main groups, the first one a completely separate genotype from the others, the JF2-27A strain and the second group showing two highly variable groups, which is shown in the main coordinate analysis (Fig. 9). Based on the clusters, two duplicate samples can be observed, the strains JF2-5A and JF2-2A, the second standard the strains JF2-19A and JF2-17A. For the group of individuals JF2-6A, JF2-7A. JF2-5A and JF2-2A, present average genetic distances less than 0.08, which is indicating a narrow genetic base between these groups of genotypes.

In Fig. 9 the two-dimensional space obtained from the principal coordinate analysis is shown. The first two dimensions explain 24.2% of the total variability and allow the formation of three groups of genotypes using the molecular markers microsatellites and ISSR. This graphic representation of multivariate data displays the information obtained in the dendrogram. Often the graphical representation of data reveals characteristics such as individual groupings. The results indicate a high genetic similarity within each of the graphed groups. Representations in principal coordinates do not always present exactly the relationships that truly exist between the elements in multidimensional space since they are only planar projections of them. The technique known as minimum path tree can help to improve the interpretations since it allows to identify this type of deformations (Arroyo et al. 2005).

In Fig. 10 the minimum path tree between the different strains of Saccharomyces cerevisiae is observed, in which it can be seen that the strains JF2-23A, JF1-7A, JF1-3A are phylogenetically close. As shown the furthest strain is JF2-27A. The relationship between the three groups formed in main coordinates is observed, where these are highly related to the JF2-12A strain.

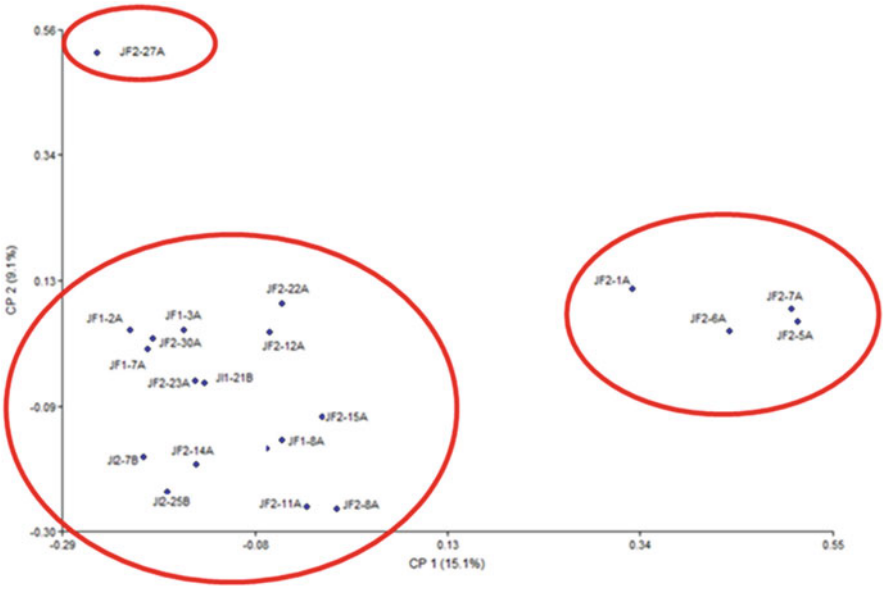

Fig. 9 Principal coordinate analysis for *S.cerevisiae*

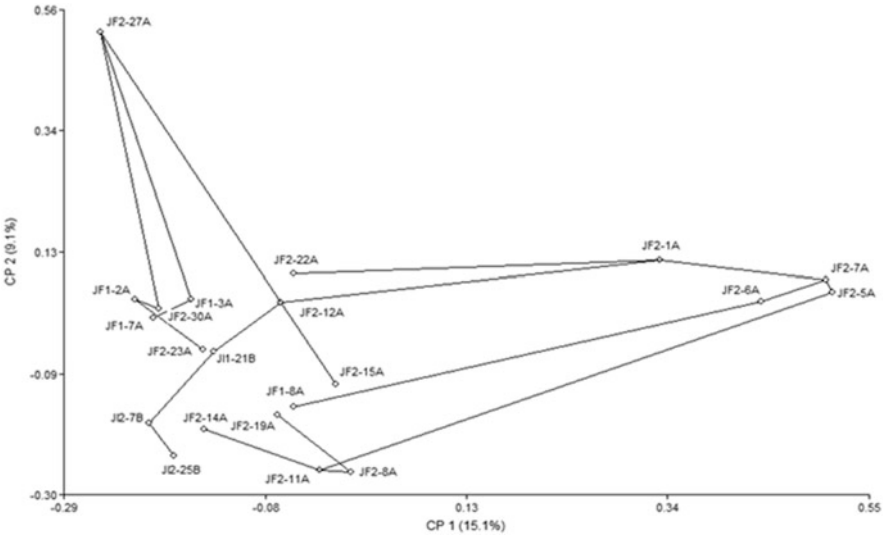

Fig. 10 Minimum path tree calculated with the Anderberg formula, for the strains of *S. cerevisiae*

The minimum sample size was calculated to calculate some measure of genetic variability (Fig. 11). This shows the sample size necessary to estimate genetic diversity with confidence and precision. (Balzarini et al. 2010). Its value is plotted based on the given sample size. As can be seen from the inflection of the graph, the minimum sample size for *Saccharomyces cerevisiae.*

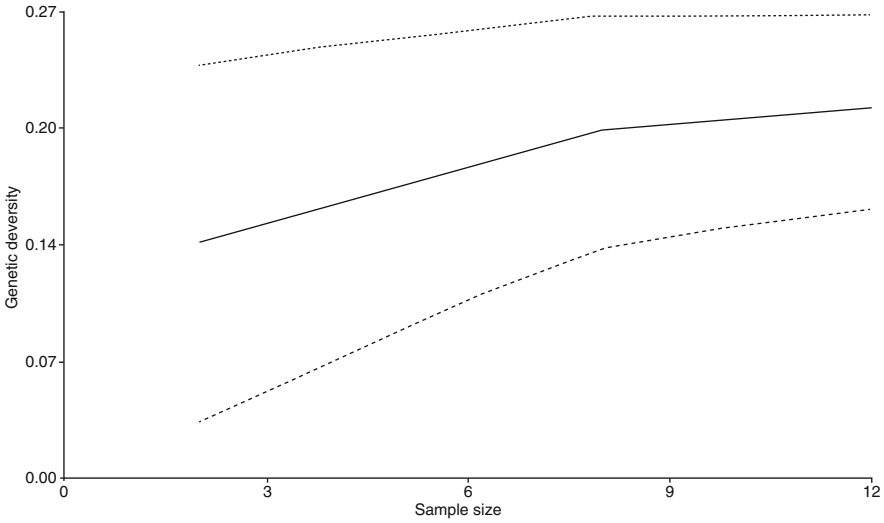

Fig. 11 Minimum sample size chart for genetic diversity

It is approximately 8 samples. The result shows that the analysis of genetic diversity is overrun in sample size, therefore it is representative for *S. cerevisiae*.

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Some results suggested that traditional agave fermentation for mezcal production has a low diversity of *S. cerevisiae*, compared to other reported alcoholic fermentations, such as Páez-Lerma et al. 2013 reported four polymorphic groups with the combination of mtDNA and δ elements, from S. cerevisiae strains isolated from the agave fermentation process for the production of mezcal. Querol et al. 1994 reported 41 different patterns analyzing mtDNA of S. cerevisiae strains isolated from wine fermentation, this with the analysis of a large number of samples from this work, therefore it is important to mention that with the use of molecular markers it is possible to perform a diversity analysis with a small sample size compared to that used by Querol et al. 1994. Cluster analysis of results generated from molecular markers, microsatellites, and ISSRs show increased genetic diversity because the entire genome is used for analysis in addition to being highly sensitive to genetic variation in populations.

5 Philogenetic Analysis For *Torulaspora delbrueckii*

For *T. delbrueckii* a total of 75 bands were obtained, 37.33% of them were polymorphic bands, four duplicate samples and 47 monomorphic bands. Genetic diversity measures indicate a total of 0.71 polymorphic loci and 0.21 for genetic diversity (Table 3).

Table 3 Measures of genetic diversity fort *T. delbrueckii*

Summary statistical information	
Polymorphic loci (95)	0.71
Genetic diversity	0.21
Nei's unbiased heterozygosity	0.21
# allele average	2.00
# allele effectiveness	1.30
Number of alleles	56.00

The heterozygosity lower than the polymorphic loci may be due to the high relationship between the strains and the level of homozygosity. The cluster analysis of *T. delbrueckii* shows a cluster at 0.93 (Fig. 12). The analysis indicates the formation of two main groups, the I3-7A strain, different phylogenetically from the rest of the isolates, as can be seen in the main coordinate analysis (Fig. 13) and the minimum path tree (Fig. 14); the second group in turn shows two highly variable subgroups.

In Fig. 13 the principal coordinate analysis is shown, the first two components explain 42.5% of the total variability and allow the formation of a compact group of four dispersed strains. The relationship between the strain I3-7A and the strains J12-18B and I3-29A is observed in the tree of minimum path (Fig. 14). The minimal path tree (Fig. 15) shows how the isolates segregate from JI1-13B, which suggests that there is a common relationship with this isolate.

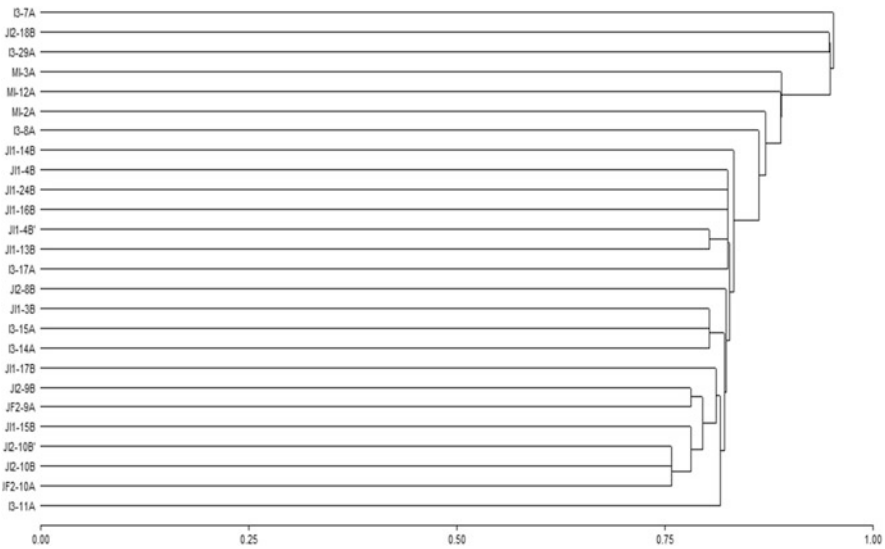

Fig. 12 Cluster analysis of strains of *Torulaspora delbrueckii*, based on the study molecular markers

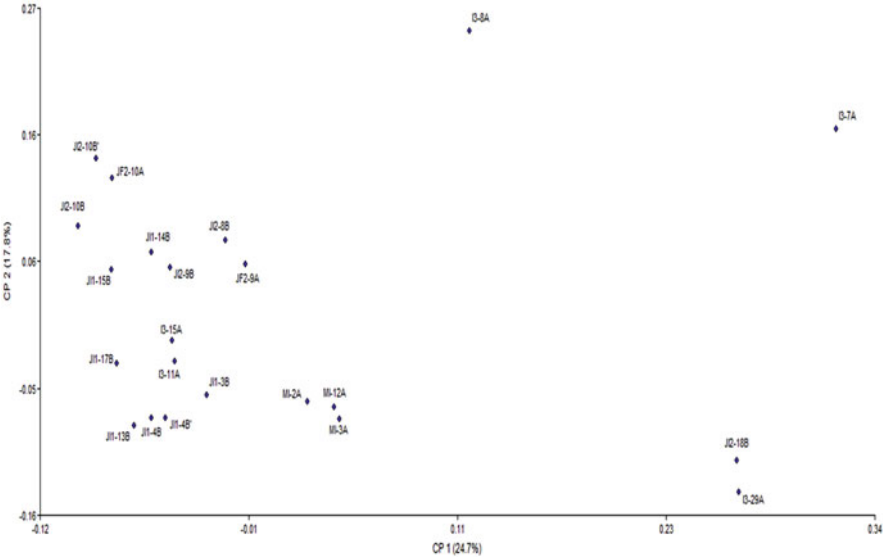

Fig. 13 Principal coordinate analysis for *T. delbrueckii*

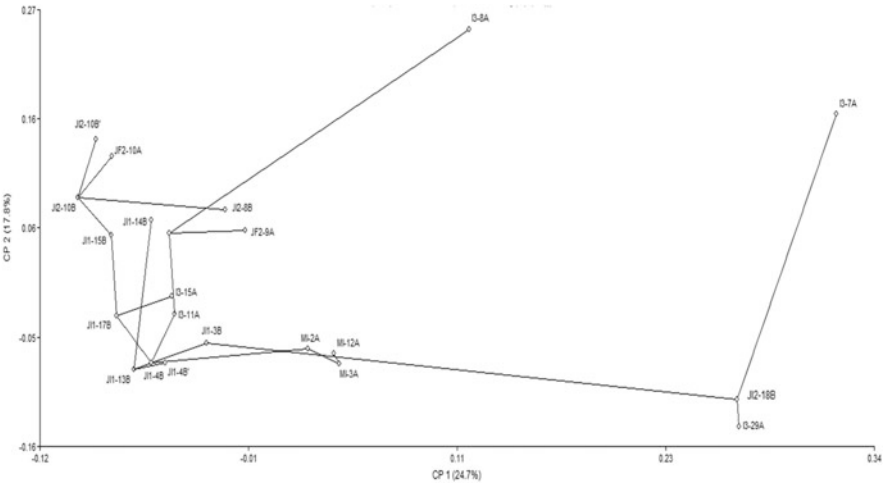

Fig. 14 Minimum path tree calculated with the Positive matching, for *T. delbrueckii* strains

The minimum sample size was obtained to calculate some measure of genetic variability (Fig. 15), showing an inflection in approximately 8.16 samples. Hence, the analysis of genetic variability performed with molecular markers for 26 samples is highly decisive and informative.

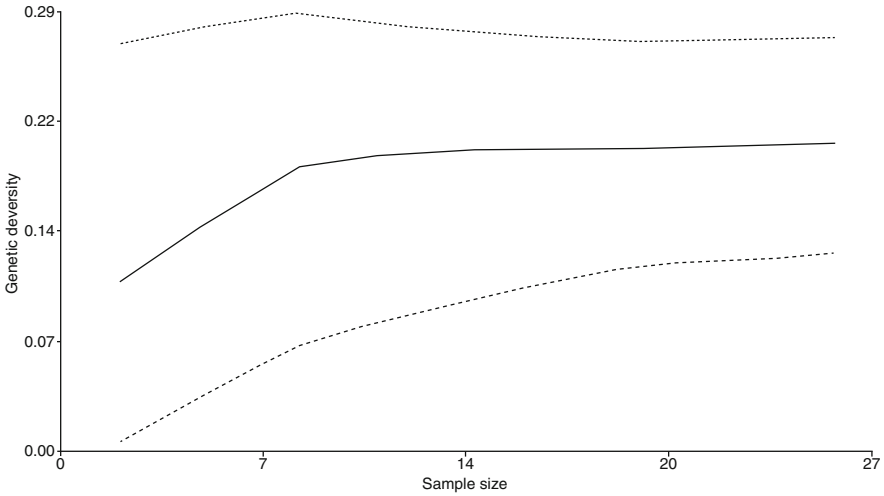

Fig. 15 Minimum sample size chart for genetic diversity of *T. delbrueckii*

Table 4 Measures of genetic diversity for *P. fermentans*

Summary statistical information	
Polymorphic loci (95)	0.74
Genetic diversity	0.27
Nei's unbiased heterozygosity	0.27
# allele average	2.00
# allele effectiveness	1.43
Number of alleles	78.00

6 Philogenetic Analysis For *Pichia fermentans*

Analysis of amplified products from *P. fermentans*, yielded a total of 75 bands, of which 52% were polymorphic and there were no duplicate bands. The selected molecular markers were informative for *P. fermentans* because the value of the polymorphic information content (PIC) was above 50%. (Balzarini et al. 2010). As for *S. cerevisiae* and *T. delbrueckii*, the markers are useful for detecting polymorphisms in *P. fermentans* in subsequent studies. The measures of genetic diversity (Table 4) obtained from the analysis indicate a total of 0.74 for polymorphic loci, those present in a population, and 0.27 for genetic diversity, which is the variation in DNA sequences that may or may not be reflected in differences in the morphological characteristics of individuals of the same species (Escobar-Saucedo 2012). This difference may be linked to the mutations present in the isolate strains.

Nei's unbiased heterozygosity yielded a value of 0.27, heterozygosity represents a better measure of genetic variation, since it is precise and not arbitrary (Aranguren-Méndez et al. 2005). Nei's unbiased heterozygosity is a good estimator of variability (Balzarini et al. 2010). Two alleles were found present in all individuals, 1.43

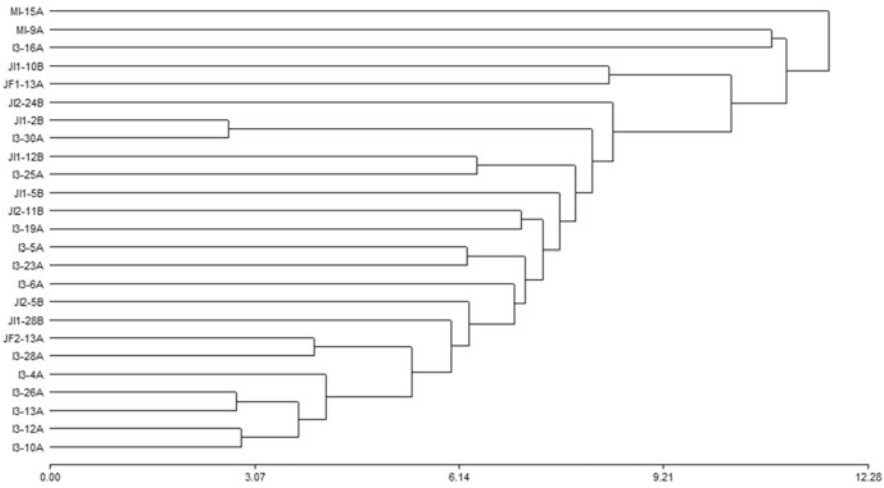

Fig. 16 Cluster analysis of strains of *Pichia fermentans*, based on the study molecular markers

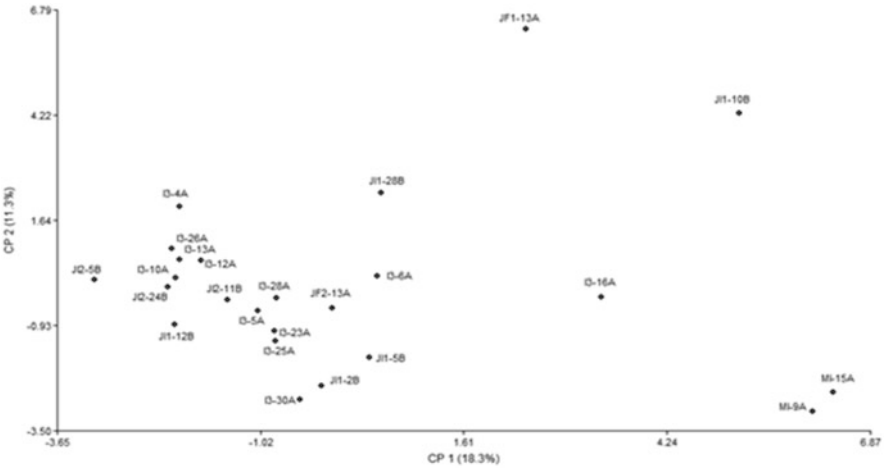

Fig. 17 Principal coordinate analysis for *P. fermentans*

effective alleles with a total of 78 alleles. Cluster analysis (Fig. 16) shows that the 25 *P. fermentans* isolates are grouped into two main groups, the first with the M1-15A strain completely out of group. Duplicate isolates are not shown.

The principal coordinate analysis (Fig. 17) shows the two-dimensional space explaining 29.6% of the total variability and allows the formation of a group of 20 phylogenetically close strains, leaving five strains far from the main group. In the minimal path tree (Fig. 18) it is observed how these five strains are related to the main group, showing the closeness that exists between the JI1-10B and JF1-13A

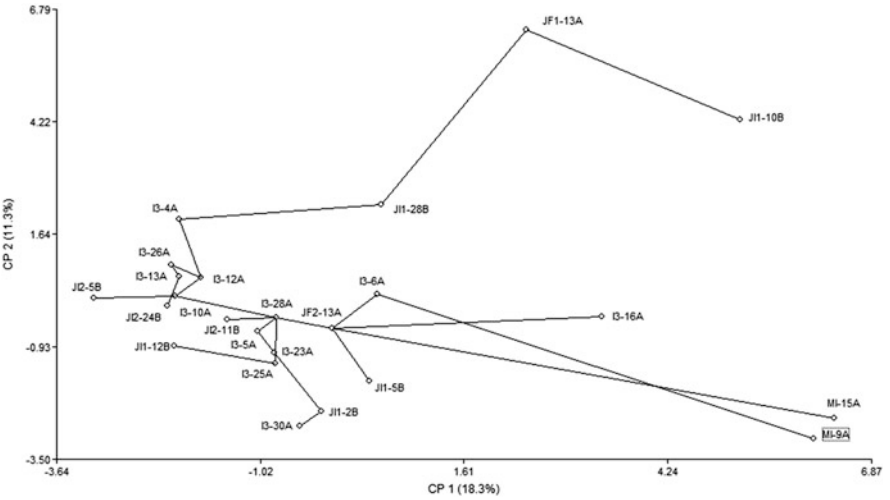

Fig. 18 Minimum path tree calculated with the Euclidea equation para *P. fermentans*

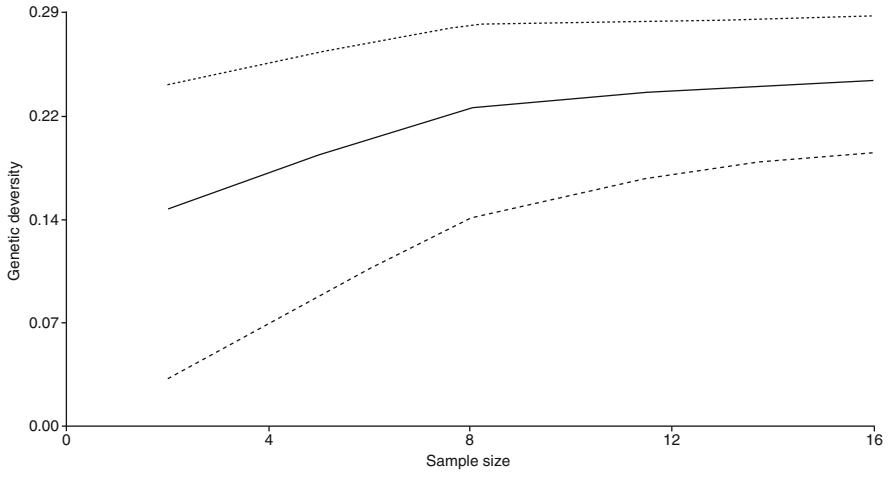

Fig. 19 Minimum sample size chart for genetic diversity calculated with the Euclidea equation for *P. fermentans*

strains. The strains seem to segregate from I3-28A, likewise, it is shown that the less phylogenetically close strains are M1-15A and M1-9A.

The minimum sample size value to calculate some measure of genetic variability was graphed showing inflection in eight samples (Fig. 19). Therefore, the diversity analysis carried out for *P. fermentan*s is highly decisive.

7 Conclusions

The joint use of microsatellites and ISSR was novel for the analysis of yeasts, exhibiting a high polymorphism in the isolates evaluated due to the high variation they detect, since the entire genome is used for the analysis, in addition to being markers highly sensitive to genetic variation in populations.

The selected molecular markers allowed detecting intraspecific variability between the isolates of each of the species, and therefore determining the population structure.

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Polydnaviruses: Evolution and Applications

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Abstract Polydnaviruses ("poly" referring to the poly-dispersed DNA segments) (PDVs) are the unique viruses, which are obligatory symbionts with parasitoid wasps (the primary host). Polydnaviridae was formally recognized as a family of viruses in 1991. Corresponding to the PDV-carrying wasp families, Braconidae and Ichneumonidae, PDVs are subdivided into two genera, Bracovirus (BV) and Ichnovirus (IV). The PDV genome is integrated into the wasp's chromosome as a provirus and vertically transmitted through wasp germ lines. PDV virions only replicate in the calyx cells of female wasps, which are injected into caterpillar hosts (the secondary host). PDV genes are expressed in the secondary host, which suppress the host's immune system, prevent encapsulation, and regulate the host's physiology to facilitate parasitism; this results in the death of the secondary host. A breakthrough study on the wasp transcriptome showed that BVs evolved from a nudivirus, while IVs originate from a different virus ancestor that belongs to a new virus family. Due to PDV gene function, PDV-associated gene products are also used for pest control in crops. In this chapter, the evolution, life cycle, functional genes and applications of PDVs will be reviewed.

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1 Introduction

Polydnaviruses (PDVs), i.e. double-stranded DNA viruses with segmented genomes, are unique insect viruses that exist in obligate association with certain parasitic wasps in mutualistic symbioses. They have a unique life cycle between their primary host (parasitoid wasps) and secondary host (lepidopteran insects). PDVs occur as proviruses integrated in the wasp genome. During oviposition, the female wasp produces virion particles and injects them into the wasp's lepidopteran host, with wasp eggs. The PDV genes expressed in the secondary host to lead to parasitoid survival. PDVs replicate exclusively in parasitoid wasp ovaries, and the gene products in the secondary host can regulate host physiology and suppress the host's immune system to facilitate parasitism. This also ensures the vertical transmission of the PDV genome to the next wasp generation (Béliveau et al. 2015).

According to the parasitoid wasp species families Ichneumonidae and Braconidae, PDVs are divided into two genera, bracoviruses (BVs) and ichnoviruses (IVs), respectively. BVs are associated with wasp species comprised of six subfamilies, and are estimated to contain >50,000 species (Strand and Burke 2019). IVs have so far been observed associated with only two subfamilies, Campopleginae and Banchinae, which contain >13,000 species (Gundersen-Rindal et al. 2013). Also, a virus group associated with ichneumonid wasps of banchinae subfamily has been proposed as a third taxonomic genus of Polydnaviridae, based on the study of PDVs from the wasp *Glypta fumiferanae* (Lapointe et al. 2007). This PDV is different from Campoplegine IVs with different virion morphology, genes coding for protein tyrosine phosphatases shared with BVs, and different wasp lineages (Lapointe et al. 2007).

The main function of PDV genes is to prevent wasp egg encapsulation by the host's immune system. PDV-mediated suppression of the host's immune system was first reported by Edson, who showed that *Campoletis sonorensis* ichnovirus (CsIV) gene expression is essential for preventing *C. sonorensis* egg encapsulation (Edson et al. 1981). Subsequently, a number of PDVs genes that interact with the parasitoid's immune system have been studied. Some of them are formed into families such as the Glc family (Johnson et al. 2010), protein tyrosine phosphatase (PTP) family (Pruijssers and Strand 2007), Anks family (Beck and Strand 2007) and Egf family (Lu et al. 2008). Other genes exist as a single copy, such as CrV1 (Asgari et al. 1997), CrV2 (Cooper et al. 2011) and CCV1 (Labropoulou et al. 2008). Parasitoids are able to disrupt host insect development and result in the mortality of the host. Functional genes of PDVs open new ways to pest biological control in agriculture.

2 Where Did the PDVs Come from?

The study of PDVs started in the late 1960s (Salt 1965). In the early 1970s, these particles were identified in the ovaries of certain wasps through electron microscopy (Volkoff et al. 2010). Polydnaviridae was formally recognized as a family of viruses in 1991 (Francki et al. 2012). Early morphological research showed that BV and IV virions are similar to viruses from the family Baculoviridae and Ascoviridae, respectively (Bigot et al. 2008; Federici and Bigot 2003; Stoltz et al. 1976). However, further research noted that PDVs neither share any significant homologous genes with baculovirus or ascovirus nor share homology with other viral genes that have predicted function in DNA replication, transcription or virion formation (Strand and Burke 2012). Therefore, two models have been suggested to answer questions related to PDV evolution. One is that PDVs evolved from a wasp ancestor, and during prolonged evolution obtained the ability to produce and package circular DNA that contains wasp genes encoding proteins that withstand the immune responses of the insect host (Espagne et al. 2004; Schmidt et al. 2005; Whitfield and Asgari 2003). Another model proposes that PDVs evolved from insect DNA viruses associated with the ancestor of campopleginae and microgastroid wasps and developed a beneficial association. The PDVs integrated into the wasp genome and lost viral replication and structural genes and acquired virulence genes from the wasp. PDVs are not viruses, but rather wasp organelles that evolved from a virus (Federici and Bigot 2003). PDVs have virus-like features, such as viral particle assembly and infectibility in wasp hosts. However, PDVs are also not like other known viruses due to their unique attributes, such their large segmented circular dsDNA genomes, their ability to act as beneficial symbionts in one host (wasps) and pathogens in another (the hosts of wasps), and genome replication and gene expression in different hosts (Strand and Burke 2019).

The advanced demonstration of the origin of these particles was confirmed with developments in DNA sequencing technologies and mass spectrometry, which have allowed the analysis of PDV genomes and protein components (Béliveau et al. 2015; Volkoff et al. 2010). Currently, nine PDV genomes have been sequenced: *Cotesia congregata* BV (CcBV), *Microplitis demolitor* BV (MdBV), *Cotesia plutellae* BV (CpBV), *Glyptapanteles flavicoxis* BV (GfBV), *Glyptaanteles indiensis* BV (GiBV), CsIV, *Tranosema rostrale* IV (TrIV), *Hyposoter fugitivus* IV (HfIV) and *Glypta fumiferanae* IV (GfIV) (Lapointe et al. 2007; Espagne et al. 2004; Webb et al. 2006; Desjardins et al. 2008; Tanaka et al. 2007; Chen et al. 2011). All genome data of PDV viruses share common features, such as large genomes (from 189 to 606 kb), low coding densities (17–33%), many genes organized into gene families, and numerous genes that share homology with the genes of wasps or other eukaryotes. BVs and IVs rarely share genes with each other, which indicates that their evolution was independent.

2.1 BVs Evolved from a Betanudivirus

Within Braconidae, six subfamilies form the microgastroid complex, which diverged approximately 100 million years ago (Mya) from other braconid subfamilies without BVs. Based on this phylogeny, it suggested that all BV-carrying species originate from a common ancestor (Strand and Burke 2019). The first breakthrough came from a study on the transcriptome in ovary DNA libraries of two braconid wasps (*C. congregate* and *Chelonus inanitus*) (Bézier et al. 2009). Complementary genomic and proteomic analysis showed that several genes typical of nudiviruses were expressed in ovaries in bracovirus-associated species during replication (Wang and Jehle 2009).

Nudiviruses form a sister taxon to the Baculoviridae, which is an extensively studied insect family used for biological control of lepidopteran pests, such as *Autographa californica multinucleopolyhedrosis* virus (AcMNPV) (Rohrmann 2014). The divergence of nudiviruses and baculoviruses dates back to 300 Mya, while nudiviruses and bracoviruses diverged approximately 100 Mya (Strand and Burke 2012; Thézé et al. 2011). Mass spectrometry analysis (LC MS-MS) of purified particles showed that the nudivirus-like genes that are expressed in BV-carrying wasps encode viral particle components (Bézier et al. 2009). The involvement of nudivirus genes in virus particle production was also confirmed by functional analyses with RNAi knockdown methods (Burke et al. 2013). Moreover, homologs of nudiviral genes were isolated successfully by PCR with primers designed for two nudiviral genes (HzNVorf9-like1 andHzNVorf128-like), well-conserved in *C. congregate* and *C. inanitus*, in species from a series of BV-associated wasps. These results strongly suggest that they were inherited from a common ancestor (Bézier et al. 2009). This ancestral wasp lived approximately 100 million years ago based on phylogenetic analysis (Murphy et al. 2008). The ancestor virus could belong to the genus Betanudivirus according to the phylogenetic analysis (Thézé et al. 2011).

2.2 IVs Evolved from Viruses that Have Not Yet Been Described

The transcripts of the ichneumonid wasp *Hyposoter didymator*, gene organization within the wasp genome, and proteins associated with IV virus particles were analyzed. Unlike braconids, no nudivirus or baculovirus-like genes have been identified (Volkoff et al. 2010). Some domains of the genomes which contain the genes coding for structural proteins in IV virions were found. These genes have virus-like features and form clusters in the genome of the wasp, which indicate that IVs originated from a virus, whereas these regions share no homologous genes with other virus families. The genomic regions encoding genes involved in IV virus particle production, which exist in the gene-rich regions of wasp chromosomes,

were named ichnovirus structural protein encoding regions (IVSPER). Until now, three IVSPERs have been identified for IVs, and two are located near a sequence segment corresponding to virus assembly. The gene content of the three IVSPERs belongs to seven gene families. Homologs of IVSPER genes have been found in *T. rostrale* by blast searches of an expressed sequence tag (EST) database (Volkoff et al. 2010) and were identified in an ovarian cDNA library derived from *T. rostrale*. IVSPERs have a high density of genes lacking introns, suggesting an endogenized virus origin. Most IVSPER genes lack similarities with any other currently known viral genes and thus cannot be used for identification of the IV ancestor, which suggests that IVs evolved from a virus ancestor that has not yet been characterized (Volkoff et al. 2010).

IVs from subfamily Banchinae are different from those in Campopleginae wasps in their particle morphology and gene content (Lapointe et al. 2007), which raises the question as to whether these two IV groups had a common virus ancestor or not. Several analyses have shown that IV particles from both subfamilies are produced by IVSPER genes that are conserved between Banchinae and Campoplegine wasps (Béliveau et al. 2015; Volkoff et al. 2010). Two hypotheses have been proposed to explain this. First, IV may have originated from a single viral ancestor followed by virus gene loss; second, IVs may have originated from two independent viruses of the same family that occurred in both wasp subfamilies (Béliveau et al. 2015).

3 The Life Cycle of PDVs

PDVs have an obligate association with thousands of species of parasitic wasps in the families Braconidae and Ichnomonidae as they persist as an integrated provirus in the germline and somatic cells of associated parasitoid Hymenopteran wasps. The PDV life cycle occurs in two hosts, in which PDV replicates and transmits in the wasp host and replication specifically occurs in the calyx cells of pupal and adult stage female wasp ovaries (Gundersen-Rindal et al. 2013) (see Fig. 1). Following excision of the proviral DNA from wasp chromosomes, encapsidated virions accumulate at high concentrations in the lumen of late oviducts (Belle et al. 2002; Fleming and Summers 1991; Gruber et al. 1996; Xu and Stoltz 1991). The assembled virions contain multiple circular dsDNAs with large aggregate sizes, from 190 to 730 kb. The PDV pathogenic step occurs in the second host. At oviposition, the wasp injects one or more eggs into the lepidopteran host with a number of virions, ovarian proteins and venom secretions from the venom gland (Schmidt et al. 2001). PDV virions rapidly infect the host's hemocytes, fat body and other tissues, express virulence genes, and integrate their DNA segments into the genome of infected host cells (Strand and Burke 2014). Viral gene expression can be detected as early as 1 h after parasitism and continues throughout wasp larval development. These gene products have two main functions. First, they suppress the host's humoral and cellular immunity, which prevents egg encapsulation and mobilizes host protein stores to support parasitoid growth. Second, they regulate the host

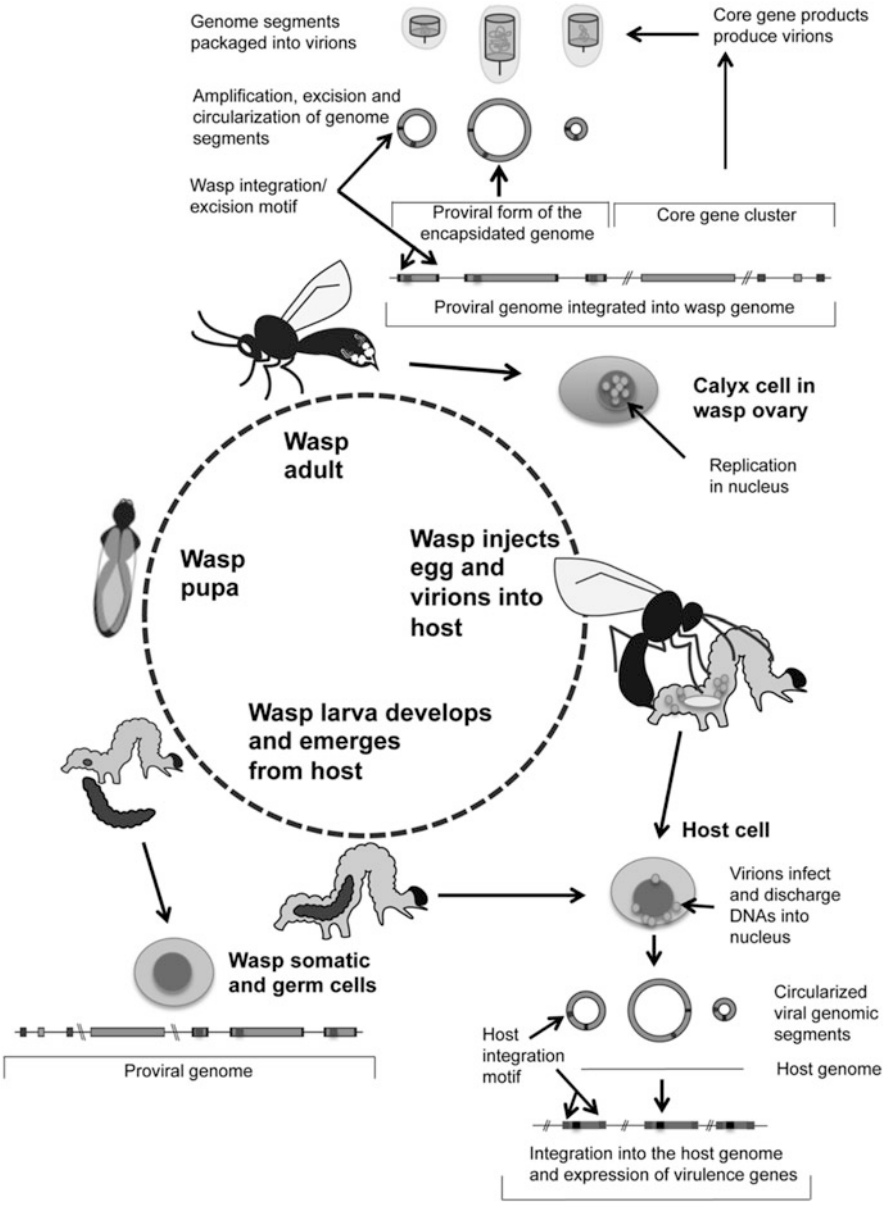

Fig. 1 Life cycle of PDV. (From Strand MR, Burke GR., PLoS Pathog 8(7): e1002757, 2012. With permission.)

physiology, which facilitates wasp offspring development, leads to parasitism success and results in the death of the host (Chevignon et al. 2014, 2015). The parasitoid wasp completes its life cycle inside the lepidopteran larva and emerges as an adult wasp while the host larva dies.

PDVs exist in two different forms, one of which is a provirus integrated into the parasitoid wasp's chromosome and the other exists as double-stranded circular DNA segments which are assembled into virions. Unlike viruses in any other family, PDVs cannot replicate in the wasp host because the genes for viral replication machinery and structural proteins that are required to produce virus particles are not assembled into virions, but are rather integrated into the chromosome of the parasitoid wasp. PDV are only vertically transmitted as integrated proviruses through the germline of wasps (Burke et al. 2013). During evolution, PDV proviral genomes have divided into two functional units. One includes genes (such as structural genes) with replication function, which are expressed but are not encapsidated into virions; the other includes multiple DNA domains including virulence genes, which are amplified and assembled into virions (Strand and Burke 2014).

The main function of PDV genes is to prevent wasp egg encapsulation by the host's immune system. The host insect's immune system includes cellular and humoral components. The cellular immune system refers to a response mediated by hemocytes, such as nodule formation, encapsulation and phagocytosis (Strand 2008). Hemocytes are composed of plasmatocytes, granulocytes, spherule cells and oenocytoids, which are classified by morphological, functional and molecular markers (Strand 2008; Nakahara et al. 2009). Granulocytes are responsible for non-self recognition and phagocytosis, while plasmatocytes form a capsule around foreign objects to induce encapsulation. Oenocytoids can express phenoloxidase and other components of the PO cascade to start melanization, while the function of spherule cells is not yet clear (Beck et al. 2011). Humoral defenses involve molecules produced by hemocytes or tissues with the ability to kill foreign objects (Kanost and Gorman 2008). Three classes of innate humoral responses have been defined, including defensive melanization, sentinel molecule binding and the induction of antimicrobial peptides (Gillespie et al. 1997).

The most important immune response of lepidopteran insects toward the eggs of parasitoids is encapsulation (Strand 2008). This response, involving the action of hemocytes, is mediated by two kinds of recognition receptors. The first kind is cell surface receptors, which are expressed on the surface of hemocytes, such as scavenger receptors, integrins and the nimrod superfamily (Kocks et al. 2005; Moita et al. 2005; Wertheim et al. 2005; Somogyi et al. 2008). The second kind is humoral pattern recognition receptors, such as lipopolysaccharide-binding protein, hemolin, soluble peptidoglycan recognition proteins, immunolectins, complement-like thioester-containing proteins and Gram-negative bacteria recognition proteins (Moita et al. 2005; Levashina et al. 2001; Irving et al. 2005; Dong et al. 2006; Ling and Yu 2006; Terenius et al. 2007). Encapsulation usually starts 2–6 h after parasitism and is accomplished by 48 h (Strand 2008).

4 PDV Genes that Interact with the Host Immune System

The key function of most PDV gene products is to disrupt host cellular and humoral immunity, including cytoskeleton degradation, adhesion disruption and hemocyte inactivation (Asgari and Schmidt 2004a). PDVs encode many genes, some of which form multimember families and while others exist as a single copy. Here, we have reviewed some important gene families and single genes in PDVs.

4.1 Cysteine (Cys) Motif Family

Cys-motif coding genes are detected in BVs and IVs. Each gene of this family has a variable cys-motif but shares a very conserved exon-intron structure, hydrophobic signal peptide and N-terminal glycosylation site (Dupas et al. 2003). The gene Mbcrp1 from *Microplitis bicoloratus* bracovirus (MbBV) contains a cysteine-rich trypsin inhibitor-like (TIL) domain coding sequence and the expression of recombinant MbCRP1 inhibits the expression actin in Hi5 cells, suggesting that expression of MbCRP is related to the disruption of the actin cytoskeleton (Luo and Pang 2006). Cys-motif proteins VHv1.1 and Vhv1.4 in CsIV are expressed in the fat boy and secreted into the hemolymph where they bind to the surface of hemocytes. VHv1.4 protein has been detected by immunoblot in the lepidopteran *Heliothis virescens* starting at 6 h post parasitization, and throughout the entire course of parasitism (Cui et al. 1997). In *H. virescens* larvae, VHv1.4 protein expressed from a recombinant baculovirus bound to granulocytes, which suggests that it has an important function in the suppression of the host encapsulation response (Cui et al. 1997). Another analysis of Cys-motif gene family gene transcripts from CsIV showed that WHv1.6 interacts with the cell membrane along with other organelles and prevents immunocytes from spreading or adhering to a foreign surface, which plays a major role in inhibiting the cellular encapsulation response by *H. virescens* (Gill and Webb 2013).

4.2 Glycosylated Central (Glc) Gene Family

The Glc gene family contains two identical genes (glc1.8) encoding for a cell surface mucin-like glycoprotein in MdBV. In parasitized hosts, the Glc1.8 protein localizes to the cell surface of MdBV-infected hemocytes and blocks both encapsulation and phagocytosis (Beck and Strand 2005). The expression of recombinant Glc1.8 in High Five cells as well as of S2 cells from *Drosophila melanogaster* greatly reduced phagocytosis ability, suggesting that this protein is involved in the suppression of the insect cellular immune response (Beck and Strand 2005). Similarly, knockdown of Glc1.8 by RNA silencing in MdBV infected cells reinstated normal adhesion and

phagocytosis ability (Beck and Strand 2003). Although Glc1.8 severely impaired adhesion and phagocytosis, it did not cause cell death (Beck and Strand 2003).

4.3 Epidermal Growth Factor (Egf) Family

The Egf family, which includes three members (egf1.0, egf1.5 and egf0.4), is encoded by MdBV. To date, only two PDV genes (ef1.0 and egf1.5) have been confirmed to inhibit the melanization of plasma from two permissive lepidopteran hosts (*Pseudoplusia includens* and *Helicoverpa zea*) and two non-permissive lepidopteran hosts (*Manduca sexta* and *Bombyx mori*) (Lu et al. 2010). Egf0.4 does not have anti-melanization activity (Beck and Strand 2007). The expression of egf1.0 and egf1.5 begins at about 2 h post parasitization and continues for over 7 days during the development of the parasitoid offspring. The MdBV Egf family encodes for small serine proteinase inhibitor (smapins) homologs that include a cysteine-rich trypsin inhibitor-like domain and function as competitive inhibitors of specific target enzymes (Zang and Maizels 2001). Further studies showed that egf1.0 inhibits the melanization of hemolymph in *M. sexta* by disabling the processing and catalytic activity of phenoloxidase activating proteinases1 (PAP1); the processing of pro-PAP1 and pro-PAP3 was inhibited (Lu et al. 2008). Efg1.5 shares an identical cys motif with efg1.0 but with an extended C-terminal repeat domain. Egf1.5 inhibited the processing and amidolytic activity of PAP1 and PAP3 in *M. sexta*, and moreover bound to PAP1, PAP3 and serine proteinase homolog 2 (SPH2) (Lu et al. 2010).

4.4 Ankyrin (Ank) Family

Ankyrin genes are present both in BVs and IVs, and have been named "IκB-like", "vankyrins", "cactus-like", "viral ankyrin" or "ank" genes (Huguet et al. 2012). A hypothesis has been proposed that PDV expression may disrupt the activities mediated by nuclear factor kappa-B (NF-κB) due to the presence of ankyrin genes in PDVs. NF-κB is a eukaryotic transcription factor that exists in the cytoplasm of cells in a dimeric inactive form bound to the inhibitor IκB. NF-κB transcription factors are key regulators of the immune responses of both insects and mammals. Further study showed that ankyrin proteins have the potential ability to disrupt NF-κB signaling in lepidopteran hosts, interfering with the regulation of apoptosis, elicitation of antiviral responses and antimicrobial peptide production (Fath-Goodin et al. 2009; Bae and Kim 2009; Shrestha et al. 2009; Falabella et al. 2007). Ank-H4 and Ank-N4 have been reported to disrupt Toll and imd signaling and antimicrobial peptide expression by binding to NF-κB (Thoetkiattikul et al. 2005). One member of the ank gene family encoded by TnBV has also been shown to interact with insect NF-κB and cause its retention in the cytoplasm, which may function to suppress the

immune response in parasitoid hosts (Falabella et al. 2007). Another ank gene interferes with cytoskeleton organization in *Drosophila* germline cells (Duchi et al. 2010). In Spodopteran hosts, an ank gene (Hd27-vank1) of *Hyposoter didymator* Ichnovirus (HdIV) with high expression suggests that Hd27-vank1 might have pleiotropic functions during the parasitism of these insect species (Clavijo et al. 2011). In *H. virescens*, ank family genes are expressed within 2–4 h post parasitization and reach peak levels by 3 days post parasitization (Kroemer and Webb 2005).

4.5 Cotesia rubecula *Polydnavirus Gene 1 (CrV1)*

CrV1, a gene product of *Cotesia rubecula* Bracovirus (CrBV), is the best characterized gene so far among the four gene products detected in *Pieris rapae* tissues. This gene was cloned and sequenced by Dr. Asgari (Asgari et al. 1996). The CrV1 gene product is a secreted glycoprotein expressed in the hemocytes and fat body cells of the secondary host *Pieris rapae*. This gene product has been implicated in the depolymerization of the actin cytoskeleton of host hemocytes and disruption of the capacity of hemocytes to spread onto foreign surfaces (Asgari et al. 1997). In *C. rubecula*, CrV1 is the only gene expressed in the host hemocyte. The expression of this gene lasts no more than 4–8 h after parasitization (Asgari and Schmidt 2002). Its function is transient and, after the appearance of the wasp, the cells return to normal function (Le et al. 2003). It has been shown that CrV1 is endocytosed or phagocytized by host hemocytes. Asgari and Schmidt found that a coiled-coil domain containing a putative zipper is required for CrV1 function and this domain affects the binding and uptake of the CrV1 protein by hemocytes (Asgari and Schmidt 2002). It is known that CrV1 binds to lipophorin, forming a complex with the lipid carrier (Asgari and Schmidt 2002; Glatz et al. 2004b). The CrV1 complex with lipophorin interacts with hemocytes, and is then taken up by lipophorin or scavenger receptors as part of a clearance reaction (Schmidt et al. 2005). Within the hemocyte, the complex interacts with membrane-anchored hemolin to inhibit its functions, including actin depolymerization and lipopolysaccharide binding and agglutination (Ye et al. 2018).

4.6 Protein Tyrosine Phosphatase (PTP)

PTP is a BV gene family that interrupts kinase/phosphatase cycles via the dephosphorylation of target proteins, thereby inactivating the signaling pathway (Eum et al. 2010; Serbielle et al. 2012). PTP transcripts were detected starting at 2 h post parasitization by quantitative polymerase chain reaction analysis (Q-PCR), for approximately 8 days, and the maximum expression occurred between 24 and 48 h post parasitization (Chen et al. 2003). PTP transcripts were detected in the

hemocytes, fat body and brain of parasitoid hosts (Chen et al. 2003). Some PTP genes encoded by MdBV were found to be expressed in prothoracic glands and the nervous system, which indicates that PTPs may be involved in disrupting the prothoracicotropic hormone signaling pathway (Pruijssers and Strand 2007). An alteration in the wasp host by PTP cannot be ruled out. Some PTP genes inactivate host hemocytes to suppress the immune system by regulating the actin cytoskeleton (Chen et al. 2003). Additionally, PTP-H2 of MdBV expressed in Sf21 cells causes apoptosis (Suderman et al. 2008), and further enzymatic analysis showed that PTP-H2 is a tyrosine phosphatase (Eum et al. 2010). Similarly, transient expression of CpBV-PTPs in *Spodoptera exigua* hemocytes caused reductions in cell spreading and encapsulation activities (Ibrahim and Kim 2008). Therefore, PTPs are involved in the suppression of multiple processes including development and immunity.

4.7 Cotesia congregate *Polydnavirus Gene 1 (CcV1)*

CcV1 gene is an orthologous gene of CrV1 and is expressed in the lepidopteran *M. sexta* parasitized by *C. congregata*. CcV1 transcripts are detected in the fat body and hemocytes within 2 and 4 h post parasitization, respectively, and the expression lasts for at least 48 h (Le et al. 2003). The expression of CcV1 has been observed from 24 h post parasitization in the hemocyte cytoplasm until the emergence of the wasp pupa (Amaya et al. 2005). Functional experiments showed that CcV1 interacts directly with hemolin and inhibits hemocyte function in the normal immune response (Labropoulou et al. 2008).

4.8 Cotesia rubecula *Polydnavirus Gene 2 (CrV2)*

The protein CrV2 is encoded by CrBV, which is expressed in the lepidopteran host *P. rapae* within 4–12 h post-parasitization. CrV2 with an ORF of 963 bp produces a glycoprotein of approximately 40 kDa and transcripts in hemocytes and fat body cells. CrV2 has a coiled-coil region, which may be involved in the formation of putative CrV2 trimers that are detected in the hemolymph of parasitized host *P. rapae* larvae (Glatz et al. 2004b). Further study showed that CrV2 protein specifically interacts with mammalian Gα subunits of heterotrimeric G-proteins using a time-resolved Förster resonance energy transfer (TR-FRET) assay. This result suggests that CrV1 may target Gα subunits to alter immune signaling pathways to regulate the insect hemocyte immune system, which may be developed for novel insect control strategies (Glatz et al. 2004b).

5 Polydnavirus Applications in Biological Control

As described above, PDV genes are used by parasitoids to develop themselves in their lepidopteran hosts. PDV genes play an important role in this process, by suppressing the immune system and altering feeding behavior, leading to the death of the lepidopteran host. The virulence genes, proteins and venom involved in the parasitization process represent a source of tools for pest control. Here, we have reviewed three methods using PDVs for pest control.

5.1 Parasitoids Used in the Biological Control

Since the interaction of parasitoids with their hosts using PDVs has been well-studied, parasitoids are widely used in biological control against crop pests (Clarke et al. 2019). They have been used as introduced biocontrol agents in classical biological control programs (Brewer et al. 2005). Parasitoids usually have a range of hosts and rarely only attack one host species (Barahoei et al. 2013, 2014). As an example, *Binodoxys communis* (Gahan) can attack several closely related aphid species (Raymond et al. 2016). The host range of parasitoids is not always the same in an entire species, as different races of parasitoids may be specific to different host species. Within the host rage, parasitoids have preferred hosts (Zepeda-Paulo et al. 2013; Henry et al. 2008). The first successful example of classical biological control used the predatory beetle *Rodolia cardinalis* (Mulsant) and the parasitic fly *Chryptochaetum iceryae* (Williston) to control cottony cushion scale, *Icerya purchasi Maskall* in California in 1888 (Heimpel and Cock 2018; Caltagirone and Doutt 1989). Since then, many successful classical biological control programs have been performed, such as the control of cassava mealybug (*Phenococcus manihoti*) by encyrtid parasitoids (*Epidinocarsis lopezi*) in sub-Sharan Africa and control of the diamondblack moth (*Plutella xylostella*) by the introduction of a parasitoid complex (Furlong and Zalucki 2017; Furlong et al. 2013; Alene et al. 2006). Biological control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) by the parasitoid *Encarsia formosa Gahan* (Hymenoptera: Aphelinidae) was established in the UK in the 1920s and stopped with the introduction of pesticides in 1940. In the 1970s, this practice was re-introduced to control whitefly due to increasing problems with insecticide resistance and the environmental and health effects of pesticides (De Clercq et al. 2011). Another most studied example of using parasitoids for biological control is *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), an endoparasitoid wasp with PDV, which has been used to against *Busseola fusca*, a significant pest of maize and sorghum (Gundersen-Rindal et al. 2013).

To successfully implement biological control, studying parasitoid diversity and understanding intra- and interspecific plasticity is necessary. Parasitoids complete development in other insects, leading to their death or sterility; this offers an

excellent mechanism for natural and sustainable pest control (Clarke et al. 2019). For classical and enhanced biological control, the positioning of target pests depends on the interaction between parasites, target pests and crops. Parasitoids can be trained to become more efficient at different stages of the host search and host acceptance process; using this strategy, the regulation of parasite olfaction can lead to "foraging efficacy gain" (Kruidhof et al. 2019). Parasitoids recognize host and non-host species using chemical compounds to locate and accept their hosts. Enzymes in host oral secretions play a key role in host acceptance and oviposition by parasitoids (Clarke et al. 2019).

The use of biological control has huge economic and ecological benefits, such as the reduction of damage by agricultural and forestry pests, protection of biodiversity in the natural system and providing valuable ecosystem services (De Clercq et al. 2011). However, biological control agents also incur potential risks to the environment, including changes in the abundance or distribution of native species, transferring harmful pathogens to native species, biodiversity loss, genetic dilution of native species and so on (De Clercq et al. 2011).

5.2 *PDV Genes Expressed in the Baculovirus Expression Vector System*

Baculoviruses are used as bio-insecticides in biological control programs against lepidopteran pests, which have a narrow host range. They are harmless to non-target organisms, are highly pathogenic and stable in the environment (Szewczyk et al. 2006). Several wild-type baculoviruses are used for pest control, such as *Helicoverpa armigera nucleopolyhedrovirus* (HaMNPV) used on cotton, *Spodoptera frugiperda* NPV (SfMNPV) on maize and *Anticarsia gemmatalis* NPV (AgMNPV) on soybean (Moscardi 1999; Srinivasa et al. 2008). However, the application of the baculoviruses has limits due to its low median lethal time. Genetically engineered baculoviruses have been developed to enhance the insecticidal activity of wild-type baculoviruses. Some PDV genes significantly enhance baculovirus protein expression and kill more efficiently than wild-type viruses.

Five members of the CsIV cys-motif gene family have been produced using the baculovirus expression system, and the recombinant proteins were injected or fed to *H. virescens* in the diet. rVHv1.1 caused a significant reduction in the growth of *H. virescens* and *S. exigua* larvae. This protein also caused delayed development, reductions in pupation and increased mortality (Fath-Goodin et al. 2006). The cys-motif expressed by the recombinant baculovirus system affected lepidopteran physiology in terms of both immunity and development (Gundersen-Rindal et al. 2013).

In our lab, we constructed one recombinant baculovirus with the PDV gene CrV1. The CrV1 secreted protein of CrBV is responsible for actin depolymerization in hemocytes and the suppression of immune functions such as phagocytosis and cell

spreading, thus allowing the successful embryonic development of the parasitoid wasp. The recombinant baculovirus was tested against the insect pests *S. exigua* and *P. rapae*. The recombinant virus expressing CrV1 protein showed significantly lower lethal concentration 50 and lethal time 50 as compared with the wild-type virus, which indicated that recombinant baculoviruses expressing only the CrV1 gene have improved virulence (Wei et al. 2016a, b).

Another novel recombinant baculovirus, NeuroBactrus, was also constructed to use for biological control. In this construction, the *Bacillus thuringiensis* crystal protein gene (here termed cry1-5) and an insect-specific neurotoxin gene, AaIT, from *Androctonus australis* were introduced into the AcMNPV genome under different promoters. NeuroBactrus showed high insecticidal activity against *Plutella xylostella* larvae and a significant reduction in the median lethal time against *S. exigua* larvae compared to those of wild-type AcMNPV (Shim et al. 2013).

Two enhancing factors from *Agrotis segetum* granulovirus and *Cydia pomonella* granulovirus were constructed into AcMNPV. The recombinant viruses were tested against the second and fourth instars of *S. exigua* larvae, and the recombinant viruses showed four- to sevenfold lower median lethal doses compared to those of the wild-type virus. Further bioassays showed that the recombinant viruses were incapable of infecting the second instar larvae of *Spodoptera litura*, *H. armigera* and *Pyrausta nubilalis*, which were not sensitive to wild-type AcMNPV (Lei et al. 2020).

As described, PDV genes combined with baculovirus could be a good strategy for effective and environmentally friendly pesticide development.

5.3 Transgenic Plants Expressing PDV Genes

For insect control, transgenic approaches have also been used, by introducing insecticidal genes into plants, such as resistance genes, lectin genes, inhibitors of digestive enzymes and *Bacillus thuringiensis* (Bt) toxins (Zhao et al. 2003; Cavalieri et al. 1996; Dowd et al. 1998; Huesing et al. 1991). The first development of transgenic plants expressing a parasitoid gene was done in 2003, which introduced a single teratocyte secretory protein (TSP) 14 into tobacco. Teratocytes suppress host insect growth and development and cause immunosuppressive conditions in the host (Dahlman and Vinson 1993). Teratocytes produce TPSs in host hemolymph, and this protein expressed in vitro injected into host larvae produced similar responses to parasitization (Schepers et al. 1998). TSP14 has a cysteine-rich motif and shares significant sequence similarity with proteins of the Cys-motif gene family, which are encoded by the ichnovirus associated with CsIV. The transgenic plants were tested against *H. virescens*. The results showed that the growth and/or development in *H. virescens*, as well as plant damage, were significantly reduced and the mortality rates were higher compared with controls (Maiti et al. 2003).

Another viral gene from CpBV, CpBV-CST1, was also introduced into tobacco. Bioassay results showed that young larvae of *S. exigua* exhibited high mortality after

feeding on transgenic tobacco, which suggests the prospective possibility of using PDV genes in a transgenic approach to pest control (Kim et al. 2016).

A virulence factor encoded by *Toxineuron nigriceps* bracovirus (TnBV), a member of the Ank protein family, TnBVANK1, was genetically modified into tobacco plants. This gene is involved in both immunosuppression and endocrine alterations in the host. Transgenic tobacco plants showed insecticidal activity and caused developmental delay in *Spodoptera littoralis* larvae feeding on them (Di Lelio et al. 2014).

Transgenic plants with parasitoid-derived genes open a new door to insect control. Future work must be done to evaluate any effect on non-target organisms, the environment and humans.

6 Conclusion

Regarding PDV, many questions still need to be answered, such as the ancestor of IVs and the mechanism of PDV gene interactions with the immune defenses of the host. Further scientific research is encouraged to answer these questions. With the further development of sequencing technology, more species of PDV and/or more PDV genes involved in the parasitization process will be identified. PDV genes are a rich source of viral genes that could be applied to pest control. Many approaches to using PDV genes could be further studied for novel control methods against pest insect species.

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Broad Spectrum Amino Acid Racemases (Bsrs): A Potential Target in Microbial Research

Lija L. Raju and Ajith M. Thomas

Abstract Broad spectrum amino acid racemases (Bsrs) aid bacteria in producing non-canonical D-amino acids (NCDAA), which play significant functions in the modification of cell walls and biofilm breakdown. Bsr reversibly racemizes 10 of the 19 naturally occurring chiral amino acids (AAs), like both non-branching aliphatic AAs and positively charged amino acids, contrary to monospecific racemases. Additionally, it helps in racemization reactions involving AAs which are not characteristically incorporated into proteins. The substrate range of Bsr is wide-ranging in comparison to other known amino-acid racemases suggesting its tremendous potential for biotechnological and industrial applications. Furthermore, since it was discovered that many taxonomically unrelated bacteria can discharge massive amounts of D-amino acids (D-AAs) into the environment, D-AAs has become a popular area in microbial research. The obtainability of D-AAs might influence microbial evolution, altering the diversity and longevity of organisms in niches. The finding of this family of Bsr has further made researchers focus on the impact of discovering the presence of new multi-specific enzymes in nature. This chapter explores the properties of Bsr that makes it a potential candidate for research because of its immense potential in biotechnology. This chapter follows *in silico* tools to unravel the significance of Bsr in *Vibrio cholerae* (BsrV).

Keywords BSR protein · BsrV gene · Alr2 · NCDAA and Protein-protein interaction

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1 Introduction

The D-AAs are considered vital effectors of bacterial physiology. The latest evidence has provided insights on D-amino acids' ever-expanding involvement in biofilms and growth phase transition of microbes. Moreover, D-AAs are abundant in microbe-rich materials like fermented foods and soils, where they are supposed to be derived from microbial activity (Radkov and Moe 2018). The vulnerability of microbes to Non-canonical D-amino acids (NCDAA) is more prominent than the production of NCDAA, raising the likelihood that some bacteria may synthesize and release NCDAA to impact nonproducing organisms (Horcajo et al. 2012). NCDAAs function as signalling molecules to mediate bacterial communication in extreme conditions like nutrient deprivation (Pietrosemoli 2013). NCDAAs can communicate with cells that synthesize NCDAAs as well as with neighbouring cells, and with cells from different bacterial species. This rapid diffusion of NCDAAs aids a quick and harmonized response from a bacterial population. The molecular processes that modulate the synthesis of NCDAAs are yet to be completely deduced (Aliashkevich et al. 2018).

Interconversion of L-amino acid to D-amino acid is mostly done by racemases that change the chiral α-carbon atom in amino acids (AAs) through a deprotonation/reprotonation mechanism. The first racemase explained in 1951 was a bacterial alanine racemase (Alr). As of now, many comparable enzymes in bacteria, archaebacteria, and eukaryotic organisms are discovered (Alvarez et al. 2014). Bsrs aids microbes in producing NCDAAs, which modulates structure of cell wall and biofilm disintegration (Espaillat et al. 2013). PLP-dependent racemases and PLP-independent racemases are distinguished by the usage of pyridoxal-5-phosphate (PLP). Pyridoxal-5-phosphate functions as a cofactor in racemases. PLP-dependent enzymes include amino acid racemase like Ala, Ser, and Arg racemases, and a few broad-spectrum racemases. Pro, Glu, and Asp racemases belong to the PLP-independent category. Bsr, unlike monospecific racemases, can produce D-AAs from a variety of non-proteinogenic and proteinogenic L-AAs (Hernández and Cava 2016).

BsrV interchangeably racemizes 10 of the 19 naturally occuring chiral AAs, including non-branching aliphatic AAs (Leu, Ser, Gln, Ala, Asn, Cys, and Met) along with positively charged AAs (Lys, Arg and His). It also catalyses the racemization reaction of a variety AAs that aren't normally found in proteins (Bernardo-García et al. 2016). BsrV was found to be ineffective on the chirality of negatively charged (Asp and Glu) or aromatic (Phe, Tyr and Trp) AAs, and to have a lower affinity for branched aliphatic (Ile, Val, and Thr) AAs. In vitro, biochemical research exposed that the enzyme can racemize 12 AAs (ser, ala, leu, met, gln, cys, his, val, asn, arg ile, and lys), with L-lys having the highest efficiency (Kcat/KM of 52.9 $mM^{-1}s^{-1}$) (Espaillat et al. 2013). NCDAA's adaptability encourages scientists to dig further into multi-specific racemases, which helps in understanding NCDAA-controlled activities and the finding of novel antibiotics to battle harmful bacteria (Conti et al. 2011).

2 Role of Bsr in Microbes

During the stationary phase of *Vibrio cholerae*, BsrV generates mostly D-Met and D-Leu. Interestingly, based on the bacterial species, these NCDAAs are integrated into the peptidoglycan by substituting the 4th and/or 5th residues of the disaccharide subunit N-acetylglucosamine-(β1-4)-N-acetylmuramic acid-L-Ala-D-Glu-(γ)-di-amino acid- D-Ala-D-Ala. In *Vibrio cholerae*, BsrV produces mainly D-Met and D-Leu during the stationary phase (Alvarez et al. 2018). Such NCDAAs editing slows peptidoglycan biosynthesis thus allowing coordination between biosynthesis of microbial cell wall and *V. cholerae* population expansion when resources are scarce. The integration of NCDAAs into cell walls reduces peptidoglycan synthesis, allowing synchronisation of cell wall metabolism and growth arrest in stationary phase, preserving cell wall integrity (Alvarez et al. 2018). Bsr in *V. cholerae* is expressed under the modulation of the stress σ factor RpoS (Cava et al. 2011a). However, the synthesis of NCDAAs is not widely conserved in the genus (Cava 2017).

Bsrs with enzymatic properties comparable to alanine racemase has been a topic of research in many organisms, like *Proteus mirabilis*, *Vibrio cholerae*, *Oenococcus oeni*, and *Pseudomonas taetrolens* (Radkov and Moe 2018). Thus far, only a small number of Bsrs have been biochemically characterized, but bioinformatic analysis shows that BsrV-like racemases exist in approximately 3% of all sequenced bacteria, being restricted to Gram-negative *Proteobacteria* (Hernández and Cava 2016). Today, the data on the biological functions of Bsrs is scarce. A strain KT2440 of *Pseudomonas putida* encodes different catabolic mechanisms of L- and D-Lys, and the Alr racemase connects the two pathways (Revelles et al. 2005). Modulation of peptidoglycan by D-AAs is not just confined to *V. cholerae* because bacteria like *Bacillus subtilis* synthesizes many D-AAs when compared to *V. cholerae* (mainly D-Tyr and D-Phe) in the stationary phase, moreover, these D-AAs influence structure and synthesis of peptidoglycan in ways similar to D-Leu and D-Met in *V. cholerae* (Lam et al. 2009). Hence, evolutionarily diverse bacteria have a similar strategy for modulating peptidoglycan synthesis in the stationary phase by the production and discharge of D-AAs. Furthermore, the growth of *B. subtilis* was delayed when they are grown in exogenous concentrations of D-AAs produced by *B. subtilis* in stationary phase. Henceforth, D-amino acids may have the means to curtail growth and peptidoglycan production as population density increases beyond a threshold. It was also established that exogenous presences of D-Met that are synthesized by *V. cholerae* were integrated into *Escherichia coli* peptidoglycan at the similar position in the peptide bridge. Hence, *E. coli* can incorporate NCDAAs into its murein sacculus though they do not produce them. Therefore, D-amino acids can act as paracrine-like effectors that modulate peptidoglycan dynamics in a many species that does not normally synthesize them (Cava et al. 2011b).

3 bsrV Gene

Besides the couple of genes that code the *V. cholerae* Ala and Glu racemases, the *V. cholerae* genome has one more gene (VC_1312, gene location: chromosome I:1, 370, 336-1, 395, 013) that encodes an alleged amino acid racemase that use PLP (Cofactor) (Cava et al. 2011b). Moreover, the VC_1312 has high sequence homology (E-value of 1×10^{-25}, 28% identity) to the chief Alr of *V. cholerae*, AlrV (VC_0372) (Lam et al. 2009). The new *V. cholerae* racemase (VC_1312) that is vital for the production of the D-AAs as exposed by the genomic data and comparative genomics showed that BsrV orthologs are present in other species of organisms as well (Fig. 1). These findings point to the fact that the production of Bsrs may be a common feature among various bacteria (Espaillat et al. 2013). Although highly conserved the dispersal of BsrVs among the Vibrionaceae family is not a hallmark of all Vibrios. Many racemases activity-based experiments established that BsrV orthologues are not present in some Vibrionaceae species (Alvarez et al. 2018).

Since bsrV gene exhibits homology with ala racemase genes, this Broad-Spectrum Racemase (BsrV), however, is annotated in UniProt database as Alanine Racemase 2 (ALR2_VIBCH), because it was believed to be only capable of racemizing alanine. *V. cholerae* produces another alanine specific racemase (ALR1_VIBCH). Thus, the current UniProt annotation of these two proteins (ALR1 and ALR2) does not distinguish between the D-AAs they produce: ALR1 and ALR2 are both allocated Enzyme Commission Number (EC = 5.1.1.1) indicating that they produce D-Ala. The ALR1 and ALR2 share a similarity in protein sequence and structure, and both use PLP to catalyze the racemization reaction, yet they are experimentally reported to exhibit different substrate specificity (Pietrosemoli 2013). Many bacteria may have mis-annotated racemases, which have broader substrate specificity than what was initially believed. This mis-annotation is likely because of the high sequence and structure similarity of racemases and the use of PLP (cofactor), which is a general feature in multiple bacterial proteins (Horcajo et al. 2012).

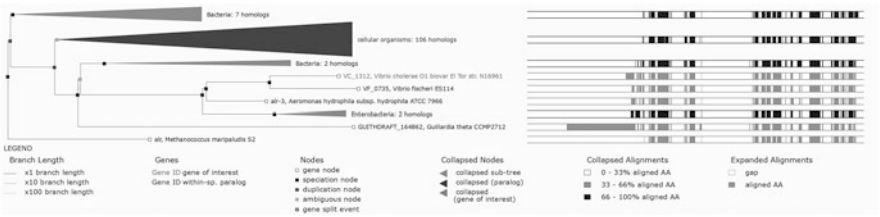

Fig. 1 Gene tree. Source: Ensembl Bacteria release 47—April 2020 (Yates et al. 2020)

4 Structural Determinants of Multispecificity

The unique molecular determinants (missing in monospecific racemases) that establish the structural foundation for multispecific activity of BsrV have been explored thanks to the 3D structure of this family and intensive bioinformatics, mutational and biochemical investigations. According to studies, BsrV has a somewhat broad entry channel that permits many substrates to enter. The substrate selectivity hinges on the content of amino acids present in the entry channel; negatively charged amino acids (AAs) are excluded to prevent the building up of negative charges at the catalytic entry site. In BsrV, the tunnel linking the active site and the entry are likewise longer. BsrV is unique in having an N-terminal extension that stabilises dimerization by generating a hinge-like structure between monomers (Espaillat et al. 2013). This conformational switch in the BsrV N-terminus leads to a different subunit disposition than that described for Alrs, resulting in a major divergence in the position and orientation of both enzymes' active sites (Figs. 2 and 3).

The size and other characters of the active sites in these two enzymes also differ. The substitution of a Pro residue for the Tyr residue in Alr that coordinates the PLP cofactor increases the dimensions of the active site in BsrV, permitting it to accommodate larger substrates. In contrast to the N-carboxylated Arg and Lys residues that do this in most Alrs, a tetracoordinated Cl^- ion stabilises substrate binding in BsrV. In this scenario, the catalytic Tyr and Lys residues are conserved in both Alr and Bsr,

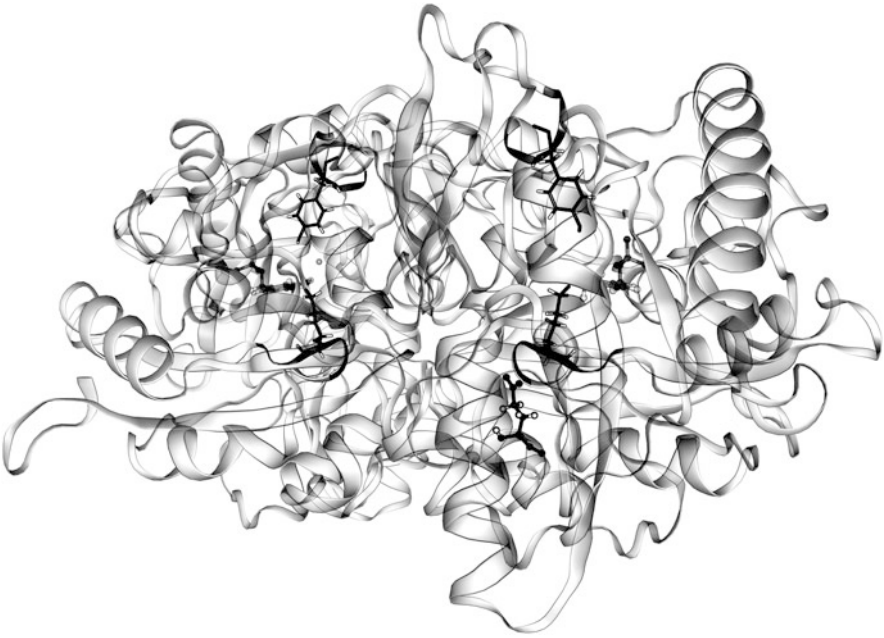

Fig. 2 Structure of BsrV. Source: https://swissmodel.expasy.org/ (Waterhouse et al. 2018)

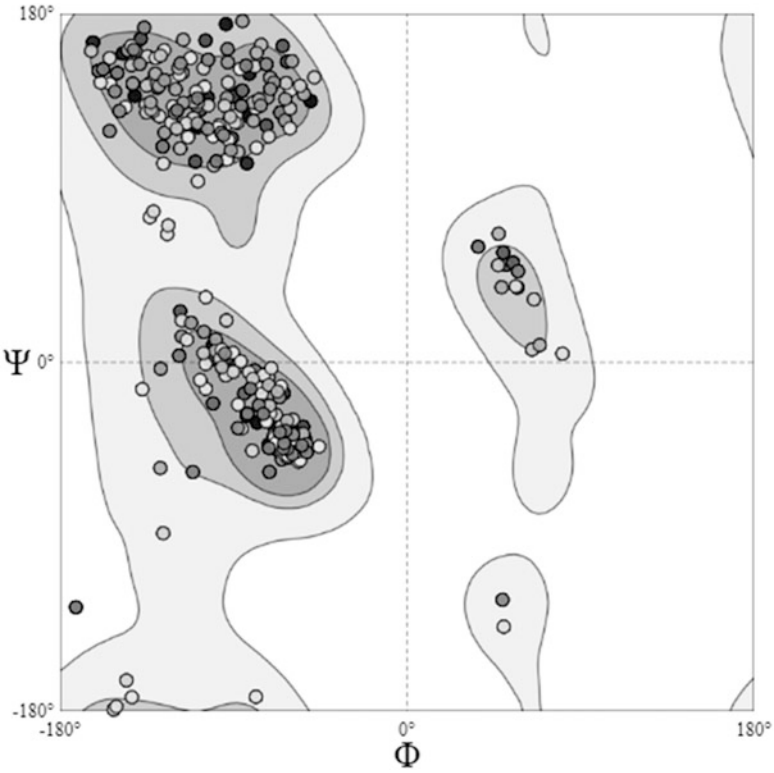

Fig. 3 Ramachandran plot. Source: https://swissmodel.expasy.org/ (Waterhouse et al. 2018)

indicating that the mechanism of the reaction is comparable in both racemase families (Table 1). Only Gram-negative bacteria have this type of Bsr, according to bioinformatic data on Bsr and Alr families. Furthermore, multi-sequence alignment algorithms aided in the identification of Bsr multispecificity's molecular footprints and determinants (Alvarez et al. 2014).

5 Catalytic Efficiency of BsrV

Kinetic studies revealed that BrsV's catalytic effectiveness (as measured by kcat/Km) varied based on the substrate (which is an amino acid), with lysine, methionine and arginine acting more readily than any other. The use of molecular docking methods exposed that BsrV has two overlapping methods for stabilising amino acids (AAs), one for aliphatic residues and the other for basic AAs. Aliphatic AAs such as Leu, Cys, Gln, Ser, Ala and Met are possibly stabilised at the BrsV active site by polar interactions of carboxylate moiety and Arg173, Tyr299, and Tyr318. In BsrV, the amino acid side chains are also stabilised by hydrophobic interactions with

Table 1 Important sites of BsrV

Feature key	Position (s)	Description/actions
Active site	74	Proton acceptor
Binding site	173	Substrate
Active site	299	Proton acceptor
Binding site	347	Substrate; via amide nitrogen
Site	391	Specificity determinant that enlarges the space within the active site of Bsr compared to Alr, allowing the accommodation of a wider range of substrates

Source: https://www.uniprot.org/uniprot/Q9KSE5 (UniProt Consortium 2019)

Met347. Similar interactions at their carboxylate end stabilise basic AAs like Lys, Arg, and Orn, aside from H-bonding to Tyr394 and electrostatic interactions with Asp268 and the phosphate group of PLP (Espaillat et al. 2013). Bioinformatics tools has revealed multiple functions and gene ontology annotations for BsrV (Table 2).

6 Protein-Protein Interactions

The STRING database, which provides a full description of protein function, can be used to better understand BsrV protein-protein interactions (Fig. 4; Table 3). All protein partners with which BsrV directly binds are required for a thorough description of BsrV function. In terms of function, 'association' can refer to direct physical binding, as well as indirect interactions such as participation in the same metabolic pathway or cellular process. STRING ('Search Tool for the Retrieval of Interacting Genes/Proteins') combines and grades these relationships by comparing them to a common reference set, and provides proof through a dependable and user-friendly web interface (Von Mering et al. 2005). The combined score is calculated by combining the probabilities from various evidence channels (Gene Fusions, Neighborhood in the Genome, Cooccurrence Across Genomes, Co-Expression, Experimental/Biochemical Data, Association in Curated Databases Co-Mentioned in PubMed Abstracts) and correcting for the chance of observing an interaction at random (Szklarczyk et al. 2019).

Table 2 Gene ontology of BsrV

Gene product	Functions	GO term	Reference	Assigned by
UniProtKB: Q9KSE5 (bsrV)	enables alanine racemase activity	GO:0008784	GO_REF:0000002	InterPro
UniProtKB: Q9KSE5 (bsrV)	enables catalytic activity	GO:0003824	GO_REF:0000002	InterPro
UniProtKB: Q9KSE5 (bsrV)	part of periplasmic space	GO:0042597	GO_REF:0000044	UniProt
UniProtKB: Q9KSE5 (bsrV)	enables amino-acid racemase activity	GO:0047661	GO_REF:0000003	UniProt
UniProtKB: Q9KSE5 (bsrV)	enables alanine racemase activity	GO:0008784	PMID:10952301	TIGR
UniProtKB: Q9KSE5 (bsrV)	involved in peptidoglycan biosynthetic process	GO:0009252	PMID:10952301	TIGR
UniProtKB: Q9KSE5 (bsrV)	enables isomerase activity	GO:0016853	GO_REF:0000043	UniProt
UniProtKB: Q9KSE5 (bsrV)	part of periplasmic space	GO:0042597	GO_REF:0000043	UniProt
UniProtKB: Q9KSE5 (bsrV)	enables amino-acid racemase activity	GO:0047661	GO_REF:0000104	UniProt
UniProtKB: Q9KSE5 (bsrV)	enables pyridoxal phosphate binding	GO:0030170	GO_REF:0000104	UniProt
UniProtKB: Q9KSE5 (bsrV)	part of periplasmic space	GO:0042597	GO_REF:0000104	UniProt
UniProtKB: Q9KSE5 (bsrV)	enables pyridoxal phosphate binding	GO:0030170	PMID:21873635	GO_Central
UniProtKB: Q9KSE5 (bsrV)	enables alanine racemase activity	GO:0008784	PMID:21873635	GO_Central
UniProtKB: Q9KSE5 (bsrV)	part of cytosol	GO:0005829	PMID:21873635	GO_Central
UniProtKB: Q9KSE5	involved in D-alanine biosynthetic process	GO:0030632	PMID:21873635	GO_Central

Source: https://www.ebi.ac.uk/QuickGO/ (Binns et al. 2009)

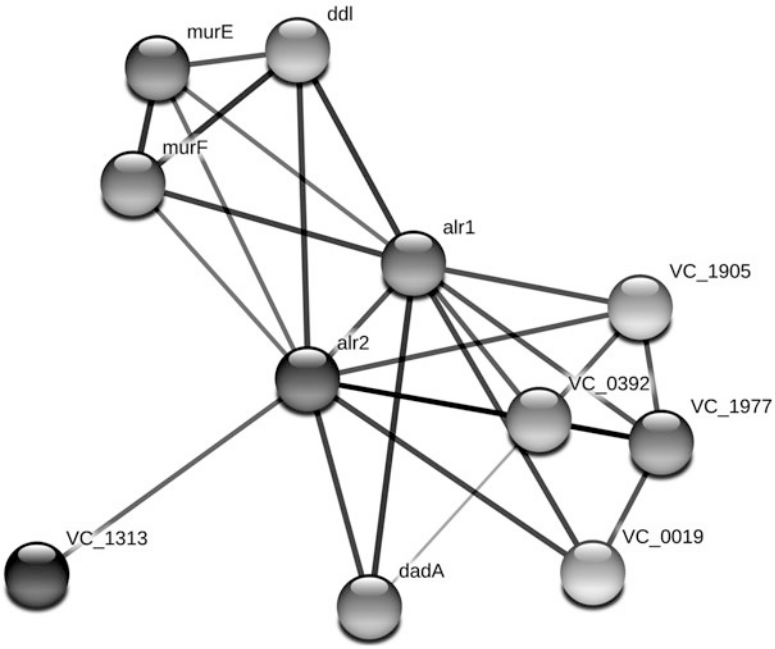

Fig. 4 Protein-protein interaction of BsrV. Source: https://string-db.org/ (Szklarczyk et al. 2019)

Table 3 Predicted functional partners of VC_1312 (alr2/bsrV)

Identifier	Protein	Molecular function	Score
VC_A0572, ddl	D-alanine—D-alanine ligase	Ligase	0.933
VC_0786, dadA	D-amino acid dehydrogenase	Oxidoreductase	0.923
VC_0019	Valine-pyruvate aminotransferase	Aminotransferase	0.907
VC_0372, alr1	Alanine racemase 1	Isomerase	0.843
VC_1905	Alanine dehydrogenase	Oxidoreductase	0.839
VC_1977	Aspartate aminotransferase	Aminotransferase	0.835
VC_0392	Aminotransferase, class V	Aminotransferase	0.807
VC_1313	Methyl-accepting chemotaxis protein	Transducer	0.772
VC_2406, murE	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate—2,6-diaminopimelate ligase	Ligase	0.724
VC_2405, murF	UDP-N-acetylmuramoyl-tripeptide—D-alanyl-D-alanine ligase	Ligase	0.693

Source: https://string-db.org/ (Szklarczyk et al. 2019)

7 Applications

Chiral resolutions of racemic mixtures have a theoretical production of 50%. This yield can be enhanced twofold by combining step-wise racemization of the enantiomer that is not of interest. Several steps in racemization necessitate tough treatments procedures and are frequently incompatible with their usage as a result of the highly functionalized states of compounds crucial in the life sciences. As a result, using catalysis for racemization can be tremendously beneficial if these enzymes are used preferably. Racemases make racemization possible in just one reaction step (Femmer et al. 2016). Compared to other identified amino-acid racemases, BsrV's substrate range and activity towards non-natural substrates are broader (e.g., norleucine, ornithine, N-acetyl lysine methyl ester, homoserine, aminobutyrate and diaminobutyrate), indicating its tremendous potential in biotechnology and industry. Currently, making D-amino acid is a high-priced process that relies on inefficient chemical catalysts (Toth and Richard 2007).

D- Arg bactericidal effect against a wide range of bacteria makes it a potent chemical weapon for *V. cholerae* against microbes that share the same habitat. Virtually all Vibrios are resistant to D-AAs, suggesting that D-Arg could be used for an inter-species altruistic cooperation mechanism to aid in the multiplication of Vibrios in harsh polymicrobial environments, allowing Vibrio species to co-exist in a variety of marine and freshwater niches and thus appreciate D-Arg synthesis. The ability of a few bacteria to efficiently make suppressor mutations to counteract negative effects of D-Arg may help to throw light on the development of different sets of NCDAAs to target various cellular processes and reduce the appearance of competing microbes to some extent (Cava 2017). This data related to Bsrs play a key role in the manufacture of new drugs for tackling pathogens.

Bacteria having Bsrs are usually Gram-negative bacteria present in environments like water, soil or animal hosts (Aliashkevich et al. 2018). BSRs are periplasmic when compared to the cytoplasmic monospecific racemases. This periplasmic location can prevent NCDAA-mediated interference with protein synthesis (Lam et al. 2009) or facilitate their discharge to the ecosystem where they carry out vital functions as signalling molecules. Today D-amino acid has turned out to be a hot topic in microbiology since the discovery of various taxonomically dissimilar bacteria that can discharge high amounts of D-amino acids (D-AAs) to the ecosystem. The environmental presence of D-AAs may have a tremendous influence on evolving microbes by shaping the organisms that inhabit a niche, their existence and social interactions (Hernández and Cava 2016). It was experimentally observed that in many co-cultivation systems, *V. cholerae* inhibited *Caulobacter crescentus* by D-Arg production. Thus, NCDAAs production by multispecific racemases can be tactic being used by vibrios and other species to succeed in competitive milieus (Alvarez et al. 2018).

8 Conclusion

The Bsrs finds application in various fields like industry besides functioning as a modulator of microbial populations. These enzymes are very flexible because of their broad specificity. The broad specificity is an exciting feature that researchers may look into as this can lower the cost of many industrial processes if Bsrs are used in the right way. Standardisation of industrial processes using these enzymes is an important aspect that needs to be looked into by bioprocess enthusiasts. The finding of this formerly ignored family of Bsr highlights the necessity to keep exploring the existence of novel multispecific enzymes in nature. Methods like computational predictions, crystallography, structure characterization, and biochemical assays or a combination of all these methods will allow the finding of such families of flexible enzymes having excessive potential for both the biotechnological and pharmaceutical fields.

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Variability of the Tannase Gene from Extreme Environments Uncultivable Microorganisms

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Abstract Tannase or tannin acyl hydrolase (EC. 3.1.120) has diverse potential applications in the food industry, because of this, it is necessary to count with different versions of the enzyme. Microbial origin enzymes have acceptance in the biotech industry. Moreover, metagenomics has opened new paths of investigation by allowing analysis without precedents of genome heterogeneity and uncultivable microorganisms. This study was done under the following objectives: (1) to amplify the Tannase gene from metagenomic organisms from soil of Coahuila locations with extreme environments; and (2) to determine the variability of the tannase gene in metagenomic samples. Soils of Cuatro Ciénegas, Ojo Caliente, La Azufrosa, Paila and Dunas de Viesca, Coahuila, México were sampled. Metagenomic DNA was isolated from these soils using methods based on CTAB (cetyl trimethylammonium bromide) and TENS. PCR was run using specific primers to amplified a segment of the tannase gene, and the variability if this gene section was determined using the DGGE technique. The results indicated that amplification of tannase gene from seven soil samples from Ojo Caliente, two from Paila, three from Dunas de Viesca, two from Cuatro Ciénegas and two from La Azufrosa was accomplished, noting the greatest variability in samples 2 and 3 of Ojo Caliente with the highest number of alleles. Whereas the most distant according to the allele variants that the tannase gene possesses, were samples 11 and 15 of Dunas de Viesca and La Azufrosa, respectively. This suggests that in the semi-desert region of Mexico presence of

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metagenomics organisms with the tannase gene exist and that the same gene presents a variability of alleles.

Keywords Tannin acyl hydrolase · DGGE · Mexico · Metagenomic analysis

1 Introduction

Tannins are important polyphenolic compounds present in the bark of the plant, wood, leaves, fruits, roots and seeds. However, the bitterness of wine, fruit juices, and tea is due to tannins that are considered anti-nutritional agents that precipitate digestive enzymes and other proteins (Aharwar and Parihar 2018). Tannase or tannin acyl hydrolase (EC 3.1.1.20) has potential applications in beer and fruit juice clarification, in the making of drinks with soft coffee flavor and as a grape wine flavor improver (Aguilar et al. 2007). This enzyme catalyzes the hydrolysis of ester bonds of tannic acid, releasing glucose and gallic acid (Banerjee et al. 2001). This inducible enzyme is produced by various microorganisms, although it can also be obtained from insects and plants. However, the microbial origin enzyme is preferred since it is more stable than its analogies obtained from other sources (Natarajan et al. 2011). In addition, the microorganisms can produce the enzyme in great quantities on an ongoing basis and these organisms can be submitted to fermentation techniques or to genetic manipulation, giving as a result an increase in the activity titration of the enzyme (Kazemi et al. 2011).

The molecular and physicochemical properties of tannases depend on the microbial source and production conditions. The molecular weight of characterized tannases is in the range of 38–320 kDa, depending on the source. Most fungal and yeast tannases have been reported to be multimeric proteins formed by two to eight subunits (Chávez-González et al. 2018). In the sequencing of the tannase gene of *Aspergillus oryzae*, it was found that it does not have introns and that it encodes for a 588 amino acid protein with a molecular weight of approximately 64 kDa. The analysis of the native protein indicates that the tannase gene product is translated as a sole polypeptide that is cleaved by posttranslational modification in two subunits joined by a disulfide bond (Zhong et al. 2004). Other researchers have gotten to the conclusion that the mature protein consists of four pairs of the two subunits, forming a heterooctamer with a molecular weight of approximately 300 kDa (Hatamoto et al. 1996). Since then, the tannase gene of various microorganisms have been identified by structural homology, but only in few studies of their sequences has been confirmed at protein level.

Moreover, only 2% of the microorganisms in the world have been proven to be sources for enzyme production, although exotic microorganisms from extremophile environments are considered as an important source of enzymes, and their specific properties are awaited to result in the application for new processes (Sarmiento et al. 2015). Within the microbial diversity, there is a possibility of finding microorganisms that produce new enzymes with better properties and that result adequate for commercial exploitation. The diversity of habitats has defied nature to develop in

organisms, molecular adaptations equally numerous. Thus, the microbial diversity is an important resource for products or biotechnological processes (Pessoa et al. 2019).

Microbiology traditionally was based on the isolation of microorganisms from their culture by traditional techniques, however this procedure is not very appropriate to understand the microbial environment, also it offers ambiguous information about the identification, characterization and physiology of microorganisms, with these limitations less than 1% of microorganisms have been able to be studied (Pace et al. 1986). It is estimated that the microorganisms that are uncultivable equal to a 99% of the total microorganisms (de Las Rivas et al. 2019). Genomics contributes to the biosynthesis of recombinant enzymes of great purity, which give greater quality to the final product and optimizes the processes of food production (Aguilar et al. 2007). Metagenomics studies the cultivable and uncultivable microorganisms and has opened new paths of investigation by allowing analysis without precedents of genome heterogeneity and understand the evolution in the environmental context (Handelsman 2004; Mardanov et al. 2018). As well as by having access to a lot more microbial diversity than seen in a culture medium plate. Metagenomics is leading to a new configuration of microbiology, examining its application in ecology and microbial biotechnology (Fierer et al. 2007).

Coahuila is one of the States of the Mexican Republic with a great environment diversity, of which stand out: extremely warm, medium dry and extremely semi-desertic areas. It is here, where microorganisms with a high potential of enzyme production have to be because of survival at the adverse conditions that nature offers them, this must oblige them to specialize themselves for their survival like any other organism. The soils constitute a very complex habitat for many species of microorganisms, since their system is very complex (Tecon and Or 2017). The soils are characterized by their spatial and temporal heterogeneity; it houses a great richness of vegetal, animal and microbial species (Pessoa et al. 2019). The soil is a very appropriate environment for the development of eukaryote (algae, fungi, protozoa) microorganisms, as well as prokaryotes (bacteria in Archaea), although viruses are also found.

The present work was done under the following objectives: (1) to amplify the tannase gene from metagenomic organisms from soil of Coahuila locations with extreme environments; and (2) Determine the variability of the gene in metagenomic samples.

2 Experimental Methods and Materials

2.1 Soil Sampling

The sampling was done in five locations of the Coahuila semi-desert, where the environment is extreme for life development, of which are described as follows:

Cuatro Ciénegas (26°58′30.02″N, −102°4′20.27″W) at this location semi-dry to semi-warm weather prevails, with a scarce rain regime. The soil has a superficial layer rich in organic material that rests upon limestone and a material rich in lime that is clayey, presents a high content of salts in some parts of the soil. This location is characterized by having salty soils and endemic biodiversity.

Ojo Caliente (25°36′53.91″N, −100°50′13.02″W) is located in a transition zone and resents a weather variation from dry, arid and semi-warm to semi-dry, semi-arid and mild; where rain regime is intermediate the majority of rains are winterly. Here the soils are chalky with little capacity to retain nutrients and by consequence are very poor in organic material, the color of this is yellowish brown, and its development is slow and does not reach more than 10 cm. In this zone besides the arid conditions, it is characterized by thermal streams that cross the area and emerges to the surface.

Azufrosa (25°53′11.87″N, −100°52′1.70″O): the weather of this place is very similar to the previous one; the soil presents wide and deep cracks during the drought period. The rain regime is intermediate o scarce. It presents a very hard, clayey, massive, black, gray and reddish soil; this location is characterized by having waters with a high content of sulfur. Like the Ojo Caliente zone, here some natural thermal streams cross.

Paila (25°33′22.78″N, −102°27′31.16″O) has a very dry, semi-warm weather. This weather is very associated to marine weathers; however, rains are very scarce, wide desert plains predominate, the average temperatures pass 30 °C with the minimum lower than 12 °C. The soil is typical of arid zones, a surface layer of a clear color and very poor in organic material, the subsoil can be rich in clay and carbonates. The soils, vegetation and weather are typical of the Coahuila semi-desert.

Dunas de Viesca (25°20′31.66″N, −102°48′20.19″O) has a very dry weather and semi-warm. In Mexico it is considered one of the most extreme places of the country because its environment characteristics. The most part of the year it presents average temperature of 35 °C maximum and 25 °C in the summer and 18 °C maximum and 5 °C minimum in the winter, the soil are fine sands, a product of erosion.

In each of the locations, the soil samples used were identified with a continuous number from 1 to 10 and with an initial letter from the place of provenance, all the samples were obtained from the surface to a depth of 10 cm and associated to a different vegetal species. The soil samples were homogenized and were passed to a sieve of 240 μm = 60 meshes to eliminate residues of greater size; and there were stored in closed recipients and conserved in refrigeration at a temperature of 4 ± 1 °C.

2.2 DNA Isolation from Soil Samples

DNA was isolated using the CTAB technique (Graham 1994) with some modifications for this type of samples. 0.5 cm^3 were taken from the soil and placed in a nucleic acid extraction matrix for fungi, adding 1800 μL of extraction buffer TENS (50 mM Tris [pH 8.0], 20 mM of disodium ethylenediaminetetraacetic acid, 100 mM of NaCl, 1% [w/v] of sodium dodecyl sulfate, and 20 μL of bovine seric albumine at 20%).

The samples were placed in a water bath at 65 ± 3 °C for 25 min and then they were homogenized using a cell homogenizer (BioSpec, Inc.) at a speed of 5 by 50 s. Afterwards the samples were centrifuged at 13,684 g for 10 min. After this, with a micropipette 1000 μL of the supernatant was taken and the tube was discarded. This product was transferred to a new 2.0 mL tube previously sterilized and identified with the sample number and 1000 μL of the extraction buffer CTAB at 2% (1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris-HCl pH 8) was added and was centrifuged in a Sorvall Heraeus ultracentrifuge at 13,684 g for 10 min. Afterwards with a micropipette the supernatant was extracted and the tube was discarded. The supernatant was transferred to a new 1.5 mL tube previously sterilized and identified with the sample number and 800 mL of the mixture chloroform: isoamyl alcohol (24:1) was added.

The supernatant was mixed with the mixture of chloroform: isoamyl alcohol (24:1) with gentle inversions for 1–2 min and was centrifuged in a Sorvall Heraeus ultracentrifuge at 13,684 g for 10 min. After this, the aqueous phase was transferred to a new 1.5 mL tube previously sterilized and identified with the sample number. The washing of the supernatant and the mixture of chloroform: isoamyl alcohol (24:1) was repeated, the gentle inversions were done for 1–2 min and the sample was centrifuged in a Sorvall Heraeus ultracentrifuge at 13,684 for 10 min. After this, the aqueous phase was transferred to a new 1.5 mL tube previously sterilized and identified with the sample number. Then, 50 μL of ammonium acetate at 7.5 M and 800 μL of cold ethanol at 96% were added. They were mixed with gentle inversions and placed in a freezer at −20 °C for 12 h. Afterwards, the sample was centrifuged in a Sorvall Heraeus ultracentrifuge at 13,684 g for 5 min and the supernatant was discarded. Then, sample was left to dry for approximately 15 min. The pellet (DNA adhered to the bottom of the tube) was washed adding ethanol at 70% (v/v), it was mixed with gentle inversions and centrifuged using a Sorvall Heraeus ultracentrifuge at 6865 g for 5 min. Afterwards the supernatant was decanted and the tube was placed uncovered and in inverted form; leaving it this way until the smell of the ethanol disappeared. The resulting DNA was suspended with deionized and sterile water, and then, the tubes were placed in a refrigerator for 12 h as minimum. From this stage, the samples were maintained in refrigeration. To determine the integrity of the extracted DNA, electrophoresis was done in agarose gel at 1%. The samples were fractioned at 60 V for 10 min, later the voltage was increased up to 95 V for 45 min. Afterwards the DNA separation was observed in a Spectroline^{R} Ultraviolet Transilluminator Longliffete$^{M\ filter}$ with the help of a UVP Mini Darkroom GDS-8000 System Labworks 4.5 digitalizer.

2.3 Amplification of Tannase Gene by Polymerase Chain Reaction

The conditions for amplification of DNA by PCR were the following: total reaction volume was 50 μL which contained 5 μL of 10× buffer, 500 mM KCl, 15 mM $MgCl_2$, 200 μM dNTP's, 0.5 μM of each primer TTH 1F 5′—CGA CTA CGA GAA CCG TTT CTA CGT TGC TGG—3′ and TTH2 R 5′—GCA GCA CAG TAG TAA GGC TCA CCG ATG ATA GAG—3′ and 2.5 U of the Taq polymerase (Difco Laboratories, USA). An initial cycle at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primers annealing at 58 °C for 30 s, DNA polymerization at 72 °C for 45 s and a final extension at 72 °C for 15 min. The DNA samples amplified by PCR were separated by electrophoresis in agarose gels (2% w/v), using the buffer TBE (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA [pH 8]) and a solution of ethidium bromide (10 μL/mL). The gel was placed in an electrophoresis chamber at 90 V for 45 min. Afterwards, the separation of amplified DNA was observed in a Spectroline^{R} Ultraviolet Transilluminator Longliffet^{M} filter with the help of a UVP Mini Darkroom GDS-8000 Labworks 4.5 digitalizer.

2.4 Separation of Fragments by Denaturing Gradient Gel Electrophoresis (DGGE)

The fragments amplified by PCR were separated by DGGE using a gradient of urea-formamide following the indications described by DCode™ Model 475 Gradient Delivery System Catalog number 170-9042 of BIO-RAD Manual. A solution of 7% of Bis-acrylamide was prepared. Denaturing solutions of 0%, 30% of Bis-acrylamide 23.33 mL, 50× TAE 2 mL, dH_2O 74.66 mL were obtained. Denaturing solution of 100%, 30% Bis-acrylamide 23.33 mL, 50× TAE Buffer 2 mL, Formamide (deionized) 40 mL, and Urea 42 g, then, it was filled to 100 mL. The solutions were sonicated for 15 min and were passed through paper filters of 0.45 μm so later be stored in amber bottles. These solutions were mixed in necessary proportions to generate the "low" and "high" solutions, which determine the range of lineal denaturation gradient. To polymerize the mixture and generate a gel of 1 mm of width, a 20 mL of "low" solution and 20 mL of "high" were added to 30 μL of ammonium persulfate (APS) at 10% recently prepared and 70 μL of N,N,N′,N′ tetramethylethylenediamine (TEMED), later, they were placed in syringes to be mounted on a gradientador of the equipment DCode^{TM} of BIORAD, once the sandwich was prepared, the gel was left to polymerize for at least 4 h. The electrophoresis ran parallel to the gradient for which the equipment was mounted according to the manual specifications. The runs were done in TAE buffer preheated at 60 °C at 120 V and approximately 40 mA during 12 h. The gel holes were filled with 20 μL of the sample (PCR products) with approximately 500 ng of product

(Brons and Elsas 2008). Later the run was done with the staining of the gel submerging it in a solution of BrEt (1 μg/mL), the bands were visualized in a Spectroline^{R} Ultraviolet Transilluminator Longliffet^{M} filter, with the help a UVP Mini Darkroom GDS-8000 System Labworks 4.5 digitalizor.

2.5 Analysis of Genetic Variability of the Tannase Gene

Once the banding was obtained, this was passed to binary code where for each resulting allele the absence (0) and presence (Aharwar and Parihar 2018) of the band was counted. With the binary code the genetic diversity, average number of alleles, numbers of effective alleles, polymorphic information content (PIC) and Nei's unbiased heterozygosity were determined. In addition, the clustering and principal coordinates analyses were done and the optimum sample number was estimated to determine the genetic variability of the tannase gene using the *Info-Gen* software.

3 Results and Discussion

3.1 DNA Isolation

The total DNA isolated from 50 soil samples of Cuatro Ciénegas, Ojo Caliente, Azufrosa, Dunas de Viesca presented a high level of contaminants, which may be humic acids and proteins, according to that reported by various authors (Volossiouk et al. 1995; Ikeda et al. 2004). By this reason, modification of the DNA isolation process was done, doing a double pass of lysis first with CTAB and then followed by TENS, in this way, a better quality of total DNA was obtained (Fig. 1).

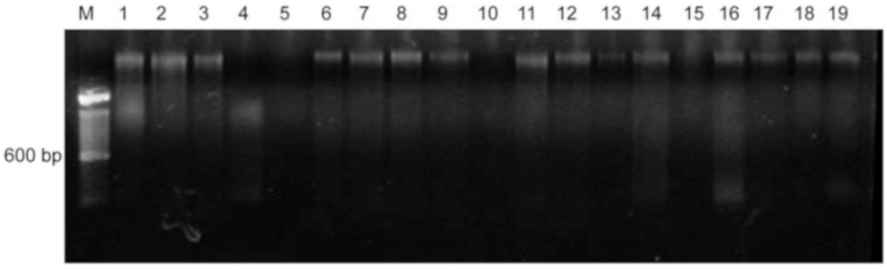

Fig. 1 DNA Integrity obtained from soil samples of five region of the semi-desert of México using a combination of CTAB and TENS methods. Lane M = ladder 100 bp molecular marker, lanes 1–19 total DNA isolated from soil samples

3.2 Amplification of the Tannase Gene Fragments

The tannase gene was amplified only from some soil samples (Fig. 2), this may be explained because the soil samples were collected close to plants and one may expect that when tissue dies from plants that have hydrolysable tannins, this is decomposed by microorganisms that present the tannase gene and close to plants with more tannins would be more microorganisms with different variations of this gene. The samples from Ojo Caliente were the ones that presents the highest number of tannase amplifications, in this place a microclimate exists that let the growth of many diverse plants, while the rest of the places stand out for having very high temperatures and sandy soils, this suggest that these factors impact in the presence of microorganisms with the tannase gene.

3.3 Denaturing Gradient Gel Electrophoresis (DGGE)

The PCR products obtained were loaded to a gel of DGGE to determine the presence of different alleles of the tannase gene in metagenomic microorganisms. As shown in Fig. 3. In addition, at enough quantity of PCR products, even in low levels, differences can be visualized.

The TTH F1 and TTH R2 primers for PCR amplification were selected based on the fragment size (600 bp) that they amplify, with this primer pair was possible the amplification of at least one fragment in 16 soil samples from different regions of the semi-desert. The band pattern that was formed during the evaluation of the 16 samples produced a total of 17 polymorphic loci. Analysis of the amplified and separated by DGGE bands showed a 95% of polymorphism. The power of the DGGE technique is based in the genetic variations that exist in the same gene, within metagenomic species, and this method is highly sensible for the study of DNA fingerprinting of any organism (Rosales-Serna et al. 2005).

The cluster analysis of the polymorphic band patterns in each sample was done with the Braun Blanquet distance, which was selected since it presents a high cophenetic correlation (Fig. 4). In the dendogram, it is observed that samples

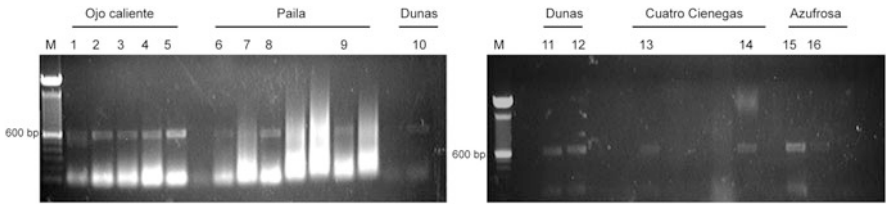

Fig. 2 Tannase gene fragments amplified by PCR, using the primers TTH 1F and TTH 2R, lane = ladder 100 bp molecular marker, only the samples that amplified a fragment of approximately 600 bp were taking as positive and are numbered

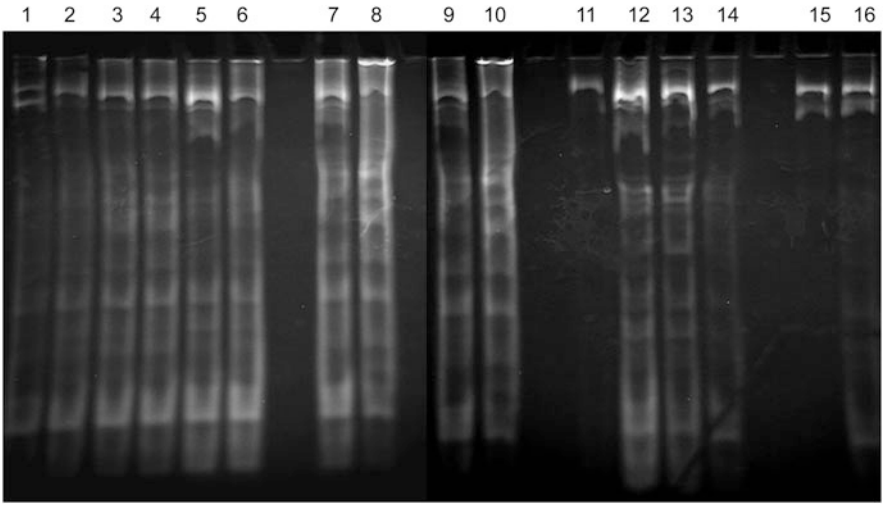

Fig. 3 Band patterns obtained by DGGE from the samples 1–6 from Ojo Caliente, 7–9 from Paila, 10–12 from Dunas de Viesca, 13 and 14 Cuatro Ciénegas; and 15 and 16 from Azufrosa

2 and 3 of Ojo Caliente have the same alleles. While the most distant samples according to the number and type of alleles, were 11 and 15, respectively, obtained from Dunas de Viesca and La Azufrosa. These locations have the particularity of being sandy soils and with very scarce vegetation; however, it is observed that at microbial level different variants of the tannase gene have been developed. The maintenance of the genetic diversity within specie is of primary importance in nature with the objective of preventing a potential extinction of the specie. In this case, it is necessary to determine if the different alleles of the gene are present in the same or in different species of microorganisms.

Analysis of the tannase gene sequences deposited in data bases, suggests that the alleles found in this study can be from microorganisms, maybe fungi and bacteria that have not been identified. The phylogenetic trees based on the sequences of amino acids of tannase suggest an evolutive relationship. This suggest that sequencing of the tannase gene variants must be done even when it is observed that there is a close relationship between the members of the super tannase family.

The total variation percentage explains by the analysis of principal coordinates was of 42.7% (Fig. 5). It could be observed that samples 11 and 15 have a great impact in the ordering of the samples at the first axis level and in the case of sample 11 also has impact in the ordering at the second axis level, as already mentioned it belongs to the soil from the Dunas de Viesca location. In Mexico this location is considered one of the most extreme places of the country for its high average temperature and for its edaphic characteristics with fine sands, product of erosion. The latter suggests that only some very specialized microorganisms can survive under these conditions, which without a doubt can affect the type of tannase alleles that these organisms present.

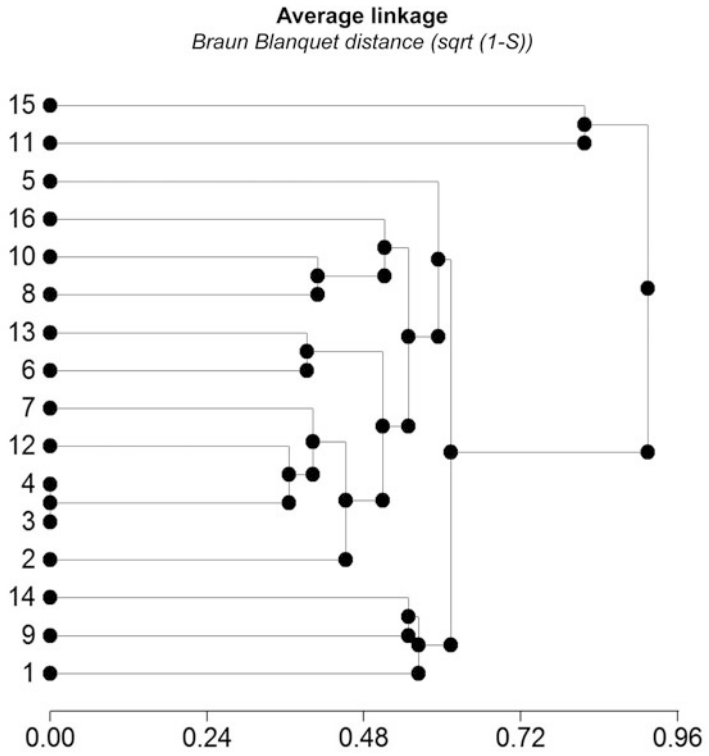

Fig. 4 Dendogram of the analysis of groups from DGGE using the Braun Blanquet index sqrt (1 − s) Group 1 = (15A* y 11D*), Group 2 = (5Oc*), Group 3 = (16A*), Group = (10D*, 8P), Group 4 = (13CC* y 6Oc*), Group 5 = (12D*, 7P, 4Oc* 3Oc*), Group 6 = (2Oc*) y Group 7 = (14CC*, 9P*, 1Oc*). **A* = *Azufrosa* **Oc* = *Ojo Caliente*, **CC* = *Cuatro Ciénegas* **P* = *Paila y* **D* = *Dunas* of the individual samples in the five regions of the semi-desert of México

3.4 Optimal Number of Samples for Determining the Genetic Variability of the Gene

The optimal number of samples for determining the genetic variability of the tannase gene in microorganisms of metagenomics was estimated to be 7–8 since if the number of samples increases, the same values of polymorphic loci (a), Nei's unbiased heterozygosity (b), number of alleles per loci (c), effective number of alleles (d), and generic diversity (e) are obtained (Fig. 6). Exceeding the optimal number of samples only a minimum gain in the genetic diversity values will be obtained and the costs would increase. The number of soil samples selected to estimate the genetic diversity of the alleles of the tannase gene has a great importance, since the genetic diversity estimation variance increases with reduced number of examined genotype. When the populations to be sampled have a different size, a strategy would be repeated random sampling and when similar individuals tend to

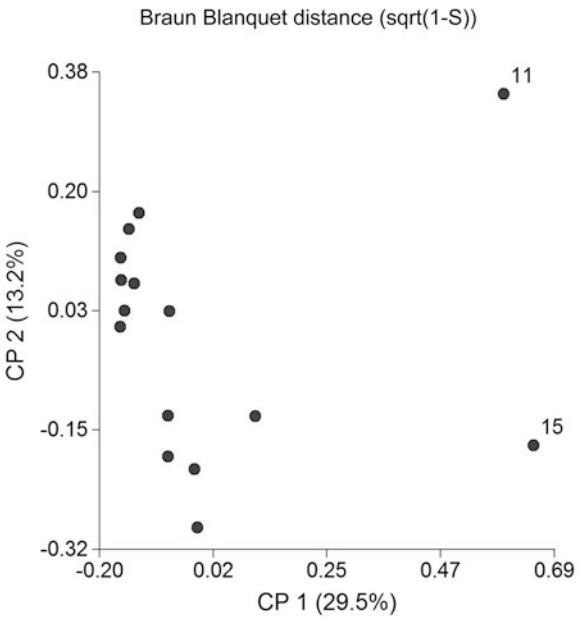

Fig. 5 Ordering in the grid of the first two main coordinates of 16 samples from soil, based on the type and number of alleles of the tannase gene amplified by DGGE

group according their genetic diversity, geographic criteria and/or their ecotypes, is proposed to use a stratified sampling for the collection of the samples (Wang et al. 2006). The presence of uncultivable microorganisms in soil and plants is considerable in the semi-desert of Mexico. Also some of these microorganisms have the tannase gene as a part of the biochemical machinery for the transformation of tannins present in their habitat (Lara-Victoriano et al. 2017). This enzyme in some way contributes to survival in the extreme conditions that the desert offers like: extreme temperatures, low food resources, sandy limestone soils, sulfurous, chalk present in the soils of the semi-desert of Mexico. The latter allows the design of strategies exploitation of the great genomic richness that the semi-desert of Mexico may offers.

4 Conclusions

By the DGGE technique and the metagenomic analysis, the diversity present in alleles of the tannase gene in populations of uncultivable microorganisms of the Mexican semi-desert, was evidenced. These uncultivable microorganisms offer a source of alleles of the gene that encode the tannase enzyme, some of the variants of the enzyme may have various applications in the food industry.

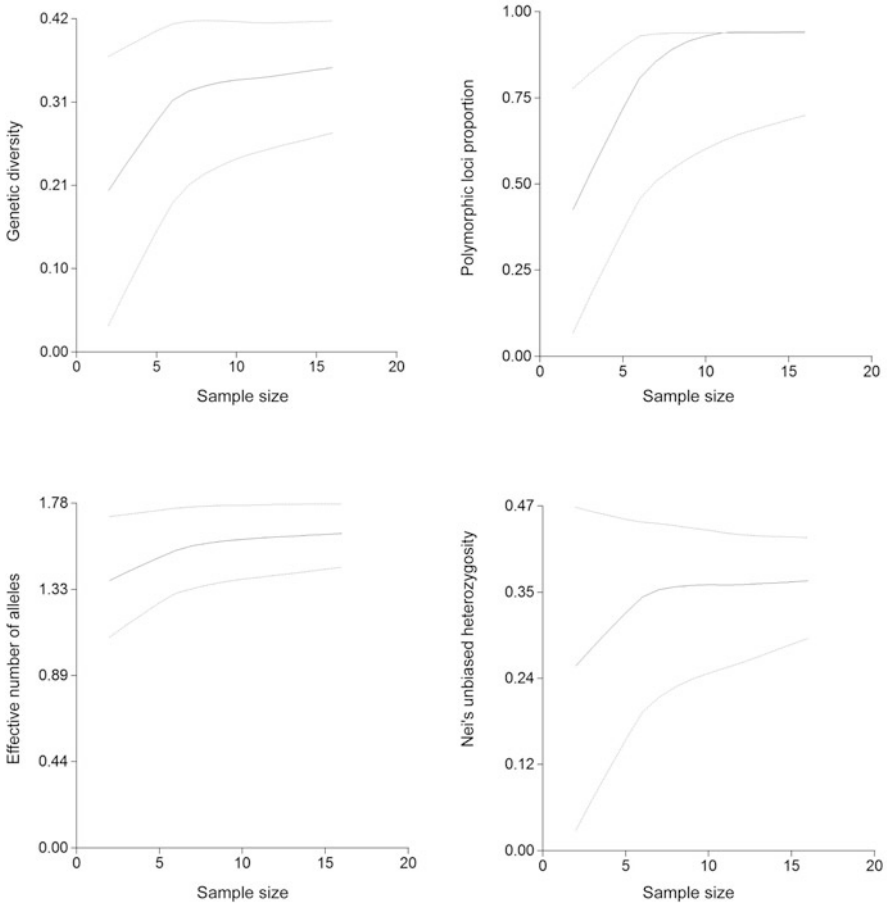

Fig. 6 Minimum soil sample size for calculating the genetic diversity measurements of the tannase gene

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Search for Haloenzymes

Luisa Peña-Cortes

Abstract The second of the Sustainable Development Goals proposed for 2030 called Zero Hunger, seeks to end all forms of hunger and malnutrition, to ensure access for all people, especially children, to sufficient and nutritious food throughout the year. This task involves promoting sustainable agricultural practices through support for small farmers and equal access to land, technology and markets. In addition, the promotion of international cooperation is required to ensure investment in the infrastructure and technology necessary to improve agricultural productivity.

Keywords Hunger · Soil · Osmotolerance

1 World Hunger

The second of the Sustainable Development Goals proposed for 2030 called Zero Hunger, seeks to end all forms of hunger and malnutrition, to ensure access for all people, especially children, to sufficient and nutritious food throughout the year. This task involves promoting sustainable agricultural practices through support for small farmers and equal access to land, technology and markets. In addition, the promotion of international cooperation is required to ensure investment in the infrastructure and technology necessary to improve agricultural productivity.

Food security is a fundamental need of all societies. The world population is projected to rise to ten billion people in the next 50 years. To meet the additional demand for food, an estimated 50% increase in the yields of the main crops will be required. While the world population is increasing, agricultural soils are declining about 1–2% per year. Low rainfall and high temperatures in tropical areas promote high salinity, becoming an important factor limiting plant growth. Salinity stress has

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resulted in a decrease of up to 70% in the yield of important crops such as wheat, corn, rice and barley. Problem is forecast to increase further in tropical regions due to global climate change, thus increasing production costs by US$12 billion per year globally (Etesami and Beattie 2018). This problem has driven the development of agriculture in saline soils (Zhou et al. 2017), based mainly on plant genetic improvement and genetic engineering, however, this approach has had limited success in the development of plants tolerant to salinity (Joshi et al. 2015).

2 Sodium Soils

Is estimated that about 932 million of hectares (Mha) in the world are affected by salts (Delgado-García et al. 2018), of these 434 Mha of land arid and semiarid from around the world are affected by conditions associated with sodicity (FAO 2000; Munns 2005). And natural or induced by the activity anthropogenic, in manner generally have one distribution environmental predominant in regions arid-semiarid, coastal and regions wet. Its formation is influenced by different factors environmental as the low rainfall, the variations of temperature, the activity microbial, the time geologic, the weathering of rocks, the quality inadequate for the irrigation, the intrusion of water sea in the earth, the rain mineral, the dissociation of minerals, generating one high conductivity electrical (>4 dS m^{-1}), a high percentage of sodium exchangeable and rate of absorption of sodium (SAR) characteristics own of ground salt and sodium (Delgado-García et al. 2018).

The main source of salt in the soil comes from the weathering and erosion of rocks and minerals primary formed in situ or transported by water or wind. Among the main causes that generate processes of salinization are irrigated with water saline, the level of water underground, the evapotranspiration, the percolation of the water to through the material salt and intrusion of the water sea (Metternicht and Zinck 2003).

The development of sodicity (or alkalinity) in the soil is a problem worldwide, the soil sodium is characterized by a high content of sodium interchangeable, the which forms a complex with the clays reducing drastically the permeability of the profile of the soil. Increasing the soil pH to levels above 8., causing low primary productivity in many soils around the world (Bhardwaj et al. 2019; Cubillos-Hinojosa et al. 2015).

The soil susceptible to salinization in Colombia include one extension of 63,900 km^2 that correspond to areas saline/sodic, of which 31,770 km^2 are areas with a high degree of intensity of salinization and usually are found in the ecosystems dry the valleys interandinos and the Caribbean region, these characteristics limit its agricultural use.

3 Osmotolerance

The osmotolerance is one of the critical factors for successful survival and colonization of microorganisms in saline environments. Research on these osmotolerance mechanisms remains insufficient. It is likely that the search for the saline soil microorganism to know its community structure will provide us with new genetic elements that provide information on the mechanisms involved in osmoadaptation (Ahmed et al. 2018).

Microorganisms are the natural inhabitants of numerous environments that have metabolic capacities to mitigate abiotic stress. Since microbial interactions with plants are a critical part of the resident ecosystem are believed to be the natural companions that modulate local and systemic mechanisms in vegetation to provide protection in unfavorable conditions outside conditions. Plant-microorganism interactions incorporate complicated mechanisms in the cell plant system. and interpret plant-microorganism relationships in terms of protection against abiotic stresses. Under the continuous pressure of increasing climatic alterations, it now becomes more imperative to define (Behera et al. 2015; Meena et al. 2017)

In recent decades, microbial communities have been isolated under conditions very adverse to life, such as hot springs, low atmospheric pressure, salt concentrations above 300 ppm, waters contaminated with heavy metals and toxic compounds, characteristics that can be in the bodies of water of Colombia; These conditions are extreme and the surviving microorganisms are called Extremophiles. Within this group are halophiles, which are adapted to grow optimally at high salt concentrations.

Traditionally, the problem of salinity in the soil has been managed by washing the soil and applying chemical amendments (Otero-García et al. 2002), but these methods generally lead to greater physical degradation of the soil and in some areas are technically and economically unviable, therefore it is necessary to evaluate other management options.

The ability of bacteria to transform nutrients and increase plant tolerance to saline stress is influenced by environmental conditions, including climate, soil characteristics, and interactions with the microbiome (Giongo et al. 2008). For example, the performance of phosphorous solubilizing microorganisms (PSM) is strongly affected by environmental factors, especially stress factors, losing the characteristics that promote plant growth (PGP) by increasing salinity. Therefore, the use of halotolerant microorganisms with PGP traits could significantly improve our ability to cultivate in environments with natural or induced salinity (Zhu et al. 2011). Bacteria isolated from saline habitats have been shown to be more efficient in improving plants' tolerance to salt than bacteria isolated from non-saline habitats (Etesami and Beattie 2018).

Halophilic plants play an important role in protecting ecosystems, developing various strategies to live in saline environments. These strategies include the production of compatible solutes to increase the osmotic pressure in the cytoplasm, the accumulation of Na+ in vacuoles, and the exclusion of Na+ from cells (Flowers and

Colmer 2008). They have also developed an ability to exploit the benefits provided by endophytes and microorganisms in the rhizosphere (Ruppel et al. 2013). Like plants, halophilic bacteria have developed various strategies to live in environments with high salinity, the most important strategy being the ability to accumulate compatible osmolytes to maintain intracellular osmotic balance. These compatible solutes are excreted into the cytoplasm either by the bacterial cell itself or taken from the environment. The osmolytes can be classified into two main groups; organic solutes (trehalose, ectoin, betaine, proline), inorganic solutes (K+, Mg 2+, Na+). Most bacteria lack intracellular systems for active water transport to cope with external osmotic stress. Therefore, they balance the internal environment by transporting or synthesizing a group of organic solutes (compatible solutes) without affecting the metabolic function of the cell. Compatible solutes can be classified according to their chemical nature into: (1) anionic solutes, (2) zwitterionic solutes, and (3) uncharged solutes. The "Salt in" strategy is used by two phylogenetically unrelated groups; anaerobic halophilic bacteria and aerobic halophilic archaea. These groups use the solute inorganic s to address the external osmotic stress. All intracellular machinery is adapted to the high concentration of salt in the external environment of the cell.

Strategies to mitigate osmotic stress appear to be genetically evolved through horizontal gene transfer. (Ahmed et al. 2018; Koonin and Wolf 2012; Miao et al. 2015). Most halophilic bacteria contain several plasmids that play an important role in bacterial flexibility and adaptation to different abiotic stresses (Dziewit et al. 2015). Bacterial plasmids carry genes that encode additional traits such as osmoregulation (Klümper et al. 2015; Oren 2012), resistance to antibiotics and heavy metals, root nodulation and nitrogen fixation, different metabolic transformations. Microbial plasmids also determine some specific phenotypic characteristics of bacteria, such as colony color (pigments) and mucoid colonial growth (Mukhtar et al. 2019).

4 Halotolerance Mechanisms

The understanding of halotolerance mechanisms are crucial for the comprehensive understanding of the microbiome of saline soils, and exploiting them for their applications in crops (Abbassi et al. 2019; Culligan et al. 2013; Xiao and Roberts 2010; Zhengbin et al. 2011). The genes that induce halotolerance have been detected in saline soils (Behera et al. 2015; Klähn et al. 2009; Naughton et al. 2009). However, the metagenomic approach provides a great opportunity to search for halotolerance genes (Chauhan et al. 2017; Kumar et al. 2016; Mirete et al. 2015).

The mechanisms by which bacteria increase tolerance to osmotic stress in plants can be very varied, either by inducing the accumulation of plant osmolytes (Dodd and Perez-Alfocea 2012), and thus maintaining turgor pressure and volume. cellular, especially in limited water conditions. The main types of osmolytes are sugar alcohols, complex sugars, tertiary amines, and sulfonia (Majumder et al. 2009). Root-associated halophilic bacteria have been shown to promote an improved

adaptive response in plants in soils with high salt concentrations, favoring the accumulation of osmolites, mainly proline (Jha et al. 2011; Upadhyay et al. 2012; Zarea et al. 2012).

Another mechanism is the production and secretion of exopolysaccharides (EPS), which confer a wide range of benefits to plants. For example, these high molecular weight compounds promote the stability of soil aggregates, which is one of the most important properties for controlling plant growth in semi-arid environments (Paul and Lade 2014). EPS, in addition to being made up of extracellular polysaccharides, also contain proteins and DNA, essential for the function and structural integrity of biofilms (Donlan 2002). Serving as a matrix that binds water, thus contributing greatly to the water holding capacity in the soil and on the root surface (Grover et al. 2011). In addition to water retention, a little-known function of EPS is their ability to bind cations (Na+), thus reducing the content of Na+ available for absorption by plants (Grover et al. 2011).

The alteration of ionic homeostasis in plants so that they can better tolerate salinity stress is another strategy used by microorganisms. Salinity stress results in the accumulation of Na+ in the leaves due to transport in the transpiration stream and even a K+ deficit. This physiological response is so consistent that the K+/Na+ ratio is used as an indicator of tolerance to plant salt. Using this strategy, microorganisms induce an accumulation of K+ ions and, in turn, a higher proportion of K+/Na+ (Etesami and Beattie 2017). From the physiological point of view, potassium is responsible for stomatal movements in response to changes in the water status of the leaves, preventing stomatal closure induced by salinity (Etesami and Beattie 2017).

5 Haloenzymes

Extremophilic microorganisms have established a variety of molecular strategies to survive in extreme conditions. The biocatalysts isolated by these organisms are called extremozymes and possess extraordinary properties of tolerance to salt, thermostability and adaptability to cold. The extremozimas pose new opportunities for biocatalysis and biotransformations as well as for the development of the economy and new research. During the last years thermophilic proteins, psychrophilic proteins, acidophilic and halophilic proteins have been studied.

Amylases, proteases, lipases, pullulanases, cellulases, chitinases, xylanases, pectinases, isomerases, esterases and dehydrogenases have a great potential of application for biotechnology, as in agricultural, chemical, biomedical and biotechnological processes. The study of extremezymes and their main applications have emerged in recent years (Dumorné et al. 2017)

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Waste Processes to Obtain Biogas and Bioethanol

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Abstract This chapter briefly explains the anaerobic digestion system to optimize biogas production and integrate digestion stabilization in rural semi-lot digesters, using agro-industrial waste mixtures to establish C/N ratios; with and without the inclusion of methanogenic inoculants. A second topic refers to obtaining second generation bioethanol from lignocellulosic wastes. General concepts of biogas and bioethanol production, related parameters and typical production schemes are described. On the other hand, it will focus on the application of fermentative processes as an activity of three different bacterial communities in biogas production and on induction systems using microorganisms to obtain bioethanol. The symbiosis between microorganisms will be presented, in the sense that in fermentation processes, metabolic actions of several microorganisms act together. In the final part, some practical applications related to the installation and start up of rural biodigesters are presented. In the case of lignocellulosic materials, pre-treatments, and processes for obtaining second generation bioethanol will be presented, as well as the current market trend. Similarly, it seeks to show the benefits for users, society and the environment, in the sectors of energy production, transformation of organic waste into high quality fertilizers, improvement of hygienic conditions, reduction or elimination of wood consumption and environmental advantages.

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1 Biogas Production

Biogas is understood as the combination of gases originating from the decomposition of organic matter (fermentation process) by microorganisms under anaerobic conditions (Kapoor et al. 2020). This process is called anaerobic digestion or biomethanization and the oxidizing bacteria: methanobacteria (Röske et al. 2014). The proportion of mixed gases depends on the digested substrate, being generally formed by 50–70% of CH_4, 30–40% of CO_2, and small proportions of H_2S, N_2, H_2, and others. These methane agents can be found in cow manure, in wastewater and in the sheep rumen (Budiyono et al. 2013; Kivaisi and Eliapenda 1994; Taherzadeh and Karimi 2008).

1.1 Operation Process of a Biogas Production Plant

A brief description of the operation process and general functioning of a biogas production plant is presented (Atelge et al. 2020; Lee et al. 2017; Taherzadeh and Karimi 2008; Wardani et al. 2020). Prior to the entry of the raw material into the plant for the biogas production process, the following steps must be followed:

Step 1: Elimination of contaminating components

The organic fuel to be fed to the plant must be analyzed for contaminants. The organic material (OM) used in anaerobic digestion (AD) should be biodegradable material such as food waste, vegetable waste, market waste, crop residues, sludge and sewage, animal excrement from pigs and cows, etc. (Daza Serna et al. 2016).

Step 2: Treatment of organic material

Once screened, the fuel must be treated to ensure a smooth consistency; this is because many AD units are fed by continuous flow reactor to achieve a better cost-benefit ratio. Once screened and treated as needed, the fuel is fed to a digestion unit for further decomposition by microorganisms (Aristizábal-Marulanda et al. 2020).

Step 3: At the Biogas production plant. The digestion units are expressed as a function of the temperature range in which the microorganisms grow (mesophilic or thermophilic) (Bouallagui et al. 2003, 2005).

Step 4: The different stages of decomposition. The decomposition of OM begins and involves four stages of chemical processes that are responsible for converting matter into usable biogas. DA is carried out in four steps, which occur partly simultaneously (Al-Zuahiri et al. 2015).

- Hydrolysis: The first step is hydrolysis, and it is the slowest step, where bacteria convert the most complex organic materials such as carbohydrates and proteins (long chain chemicals), into sugars and amino acids (individual molecules) (Dererie et al. 2011).

The waste is inside the sealed module and is sprayed with partially degassed percolate. The percolate is drained with fatty acids and pumped into the tank for gasification.

- Acidogenesis: The second step is acidogenesis, microorganisms break down individual molecules of sugars and amino acids into ethanol and fatty acids, in addition to producing CO_2 and H_2S as byproducts (Ylitervo et al. 2013).
- Acetogenesis: The third step is acetogenesis, in this third stage, ethanol and fatty acids are converted into H_2, CO_2 and CH_3COOH (Aristizábal-Marulanda et al. 2020).
- Methanogenesis: The last step is methanogenesis where methanogenic bacteria convert H_2 and CH_3COOH into CH_4 gas and CO_2 (de la Torre et al. 2019).

The gas mixture also contains H_2S, N_2, O_2 and H_2. As a percentage by volume, methane (Biogas) is approximately 60%, while carbon dioxide is 40%. Sulfhydric acid is usually less than 2% (Ituen et al. 2009).

1.2 Feeding Materials

Biogas plants can work with different renewable raw materials. With the help of a loader, the solid raw material can be poured into a cement or metal tank, which allows it to be filled approximately once a day. The raw materials are energy-rich and due to their high degree of reduction are suitable for use in biogas plants. The storage container can be equipped with a hydraulic unloader that continuously feeds the raw material through a conveyor belt. A scale under the conveyor registers the weight of the raw material.

Liquid manure is the most important basic substrate that some biogas plants can use. After storage, it is pumped through pipes directly to the mixing pump installed next to the conveyor belt. At the same time, the feedstock falls from the conveyor belt into the separators, which are equipped with two mixing rollers. In this way, the raw material is mixed just before the fermentation process (Fig. 1) (Leite et al. 2015). One or more fermentation tanks can be fed with fresh substrate according to the design requirements, even if they are not close by (Janke et al. 2015). Liquid waste from the food industry can be another substrate used, however, since the availability of this waste varies considerably, a storage tank must be installed for this purpose. Integrating the entire system serves to reduce odors and prevent epidemics.

The liquid waste is then heated to 240 °C in a tubular heat exchanger using a countercurrent process. After heating for 1 h, the hydrogenation of the substrates is completed, so that they can be poured into the fermenter, where the biogas is formed (Mirahmadi et al. 2010). The substrates are continuously agitated, to avoid the formation of layers of material on the top or bottom. A hot water heater brings the substrate to a temperature between 35 and 55 °C, to accelerate the formation of biogas (CH_4). The substrate remains in the fermenter for a period of 30 days before moving to fermenter for another 30 days to complete the biogas formation process

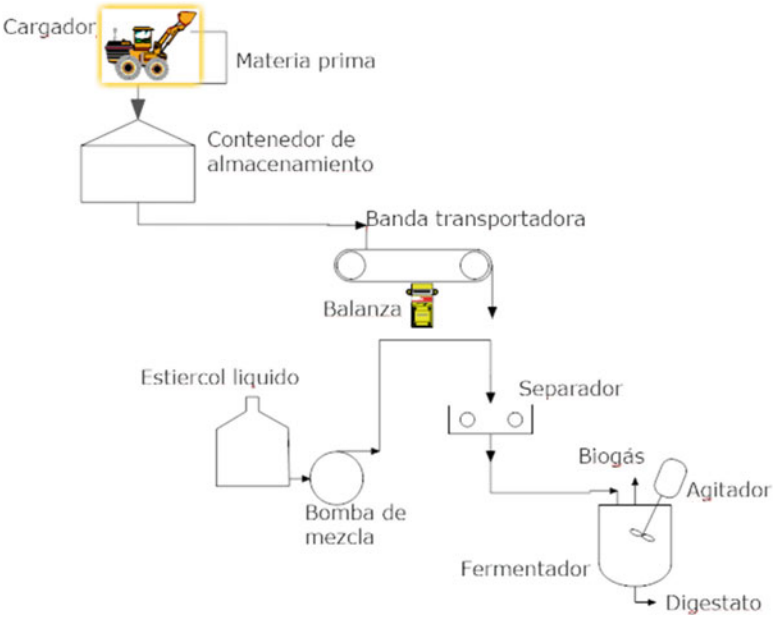

Fig. 1 Basic biogas production process

(Kotarska et al. 2019). When the fermentation is complete, the thin liquid substrate (digestate) is pumped into reinforced concrete tanks where it is stored until it can be taken to the field to be used as fertilizer.

1.3 Gas Produced

The controlled fermentation of biomass in biogas plants produces a gas that can be used to generate thermal-electric energy due to its high percentage of methane. The raw materials (substrates) used in biogas plants are products of agricultural liquid manure, agro-industrial waste, and liquid waste from the food industry (Kapoor et al. 2020).

If the fermenters are regularly filled with biomass, heated and shaken, the biogas is formed in a matter of days. The formation of gas is a complex and delicate process, the fats or organic carbohydrates contained in the substrates are what feed the fermenter to be digested by different types of bacteria (Wardani et al. 2020). This is the starting point to produce biogas, the contents are continuously mixed, the biogas rises slowly to the top of the digester. It consists of approximately 50–70% methane (green color), carbon dioxide, water vapor, H_2 and H_2S (Hagman et al. 2018). Because water vapor and H_2S are problematic, for the further utilization of the biogas, it is necessary to treat the biogas. First the gas is released from the water,

the condensed water is collected and pumped to another location. It is expected that the biogas will be accompanied by an aggressive hydrogen sulphide gas stream and will now be directed to a biological desulphurisation plant. By introducing air into the container, certain bacterial cultures to establish colonies on a metal chain placed inside the container (Moraes et al. 2015a, b).

There they break down the hydrogen sulfide into harmless sulfur, thus almost infertilizing the biogas. Finally, the biogas is fed to a compressor where it is brought to a pressure of 70 mbar required to burn it in order to completely condense any remaining water vapor and free the biogas from any suspended matter. The biogas is subjected to a washing and drying process, which is carried out with a steam at the freezing point, cooling the biogas to a temperature below 5 °C (Ben Yahmed et al. 2016).

In order to control the purification of the gas, it is constantly tested with an online measurement system which records the amounts of CH_4, H_2S, CO_2 and all this will guarantee a high degree of efficiency and safety. In case of an overproduction of biogas, it is necessary to operate a burner, to burn the methane preventing it from escaping into the atmosphere. Up to 30% of the waste heat from the water used for cooling the machines in the plant is used for heat exchange in the fermenter so that no additional heat is required (Dererie et al. 2011; Goshadrou et al. 2013; Ituen et al. 2009; Kotarska et al. 2019; Moshi et al. 2015). Once the production of CH_4 is stable and of high quality, it can be used in a gas engine to produce electricity, or the gas can be arranged for distribution. The electrical power generated by the generator in the case of using the biogas for electricity generation is converted to the main voltage level in the transformer, then the electricity can be fed into the grid.

2 Instrumentation and Control

There is a general agreement that the usual control parameters in biogas production plants in anaerobic digestion systems (e.g. pH control), are not sufficient or their measurement is not reliable. So far, none of the usual control loop systems provide a direct link to the biochemistry related to the microbiology of the anaerobic digestion system. Therefore, the design of a successful control strategy that is more closely related to the biochemistry of the digester with an analytical approach and evaluation of variables is necessary. Obtaining a model that relates all the biochemical and microbiological variables related to the anaerobic digestion process would provide the opportunity to tune controllers into control loops that can be implemented at the industrial level (Jiménez-Castro et al. 2020).

Like all living things, methanogens need an environment to live and thrive. Since in the process of acidogenesis acid (acidogenic bacteria) is produced, leading to a decrease in the pH in the digestion tank (Larsson et al. 2015), it is crucial to constantly measure the pH throughout the process to ensure the continuous well-being of the microorganisms and thus the production of methane (Mao et al. 2015).

A biogas production plant with an annual production capacity of 15,000 tons per year requires between 3 and 5 h of work per day. To reduce the amount of work, to a minimum, the use of effective measurement systems and process control technology is recommended. Thanks to a secure data exchange, it is possible for a person outside the plant to monitor and control the unit. For example, agitators can be started or stopped, container levels can be checked, or solids supply equipment can be monitored from remote locations (Mussoline et al. 2012; Shafiei et al. 2011).

As in other chemical processes, if the temperature is controlled during the digestion process, and is maintained at the top of the given specific range, the reaction rate will be at its highest value producing more gas in the same amount of time (Barta et al. 2010). While higher temperatures produce higher gas yields, they are also more difficult and expensive to maintain, making temperature a vital aspect to measure throughout the entire anaerobic digestion process (Holm-Nielsen et al. 2009).

It is vital to constantly measure biogas emissions (methane, carbon dioxide, small amounts of siloxanes, hydrogen sulfide, ammonia, hydrocarbons, and water), as production levels are good indications of production abnormalities, biomass quantities and microorganism welfare (Mladenović et al. 2018). The gases emitted should indicate the amount of biomass that remains to be decomposed. If a batch system is used, the measurement of gas emissions will provide a schedule for the complete digestion of the biomass and indicate when new biomass should be added to the digester. If a continuous system is used, the measurement of biogas production will indicate how efficiently the entire process is running (Sommersacher et al. 2013).

Monitoring for trace gases and volatile fatty acids can provide some measure of the metabolic state of an anaerobic system. In contrast to liquid phase sampling, gas analysis is susceptible to real time data acquisition. Monitoring both hydrogen and CO together, and volatile fatty acids, can provide significant insight into the metabolic state of the digestion process and has the potential to indicate process disturbances in real time. The entire process can be controlled locally through a PLC (programmable logic controller) system (Jouzani 2018; Shafiei 2018; Smajevic et al. 2012) or with a SCADA (supervisory control and data acquisition) system with support available through the internet (Werther et al. 2000).

3 Conversion of Organic Waste into Biogas and Fertilizer

The following materials are generally considered organic and can be processed in a digester:

Animal Manure

- Food waste
- Fats and oils

– Industrial organic waste
– Biosolids

Below is a summary of the process that must be performed on organic waste before it is sent to a biogas production plant:

– Collect organic waste
– Processing waste with standard machinery
– Previous classification
– Separate plastics and large impurities from the organic fraction
– Make a good mix, first of the green structural material
– Then several pre-selected waste loads
– Finally, thick structural material
– Load the mixture into the process module
– The module is closed and sealed

If the plant accepts more than one agricultural raw material, it is called a co-digestion plant. The organic compounds that feed the anaerobic digestion process are composed of carbon (C), nitrogen (N), oxygen (O). The microorganisms use these organic compounds as a substrate for growth and combine them with water (H_2O) to form carbon dioxide (CO_2) and methane (CH_4). The actual decomposition of the organic compounds into methane is not done by one microorganism alone but occurs in three stages through the teamwork of several microorganisms. The first microorganisms convert the organic compounds into an intermediate substance that other microorganisms can convert into organic acids. Anaerobic methanogenic (methane producing) bacteria convert the organic acids into methane (Akbulut 2012; Ren et al. 2006).

4 Anaerobic Digestion (AD)

Anaerobic digestion is sometimes referred to as methanogenesis. Composting is a treatment option for organic solid waste and is a process of controlled aerobic (in the presence of oxygen) degradation. The anaerobic digestion process is used in the treatment of domestic and industrial wastewater. Within this process primary (solid) and secondary (liquid) organic waste can be anaerobically digested. In simple terms AD is the decomposition of organic materials into gases (CH_4 and CO_2), with some water as a by-product (Cakir and Stenstrom 2005; Holm-Nielsen et al. 2009; Khalid et al. 2011; Marin et al. 2010).

Anaerobic digestion is the group of processes where microorganisms degrade biodegradable organic matter in the absence of oxygen. This can occur in swamps or even in the stomach of ruminants. Its importance in solid waste management lies in the fact that microorganisms can produce biogas, which is a mixture of methane and carbon dioxide. In practice, what is interesting in this process is the production of methane, because it is flammable and can be used for energy generation (heat and

electricity) and on the other hand, a nutritious digestate (wet mixture that is generally separated into a solid and a liquid and is rich in nutrients) is obtained that can be reused to apply to crops as fertilizer. The interesting thing is to use this process applying engineering and design of control systems, in a reactor(digester) without air influence, to produce biogas (by means of anaerobic bacteria) and digestate (Izumi et al. 2010; Kabouris et al. 2009; Kivaisi and Eliapenda 1994; Linke 2006).

4.1 Benefits of Anaerobic Digestion (AD)

Firstly, this is a renewable energy source, which reduces greenhouse gas emissions. On the other hand, subway reactors can be designed and built, requiring little space, eliminating the use of wood, reducing the volume of solid waste, avoiding disposal costs and finally, recovering value from solid waste, biogas and nutrients (Karagiannidis and Perkoulidis 2009; Kim et al. 2006).

4.2 Disadvantages of Anaerobic Digestion (AD)

The DA process is more sensitive, less stable and slower, and no energy is generated inside the reactor, because the energy is contained in the produced methane, which means that there is no heat generation. DA is a more technically complex process requiring higher levels of skill and investment. Finally, obtaining models that represent the dynamics of the process and can be used for control purposes is not an easy task (Ariunbaatar et al. 2015; Banks et al. 2011; Forster-Carneiro et al. 2008a, b).

4.3 Practical Parameters and Operational Conditions of Anaerobic Digestión

It is possible to start with the raw materials (input materials), but a distinction must be made between the solid content and the water content. The dry matter is also called total solids (TS), some of them are biodegradable and others not. The relevant fraction is the biodegradable organic fraction and is called volatile solids (SV: biodegradable fraction of total solids). The levels of total solids and volatile solids in solid waste differ depending on the type of waste. For example, for vegetable waste, the total solids content is between 5 and 20% of the organic solid waste, while the amount of volatile solids is between 76 and 90% of the total solids (Bouallagui et al. 2005; Chen et al. 2008; Gómez et al. 2006; Sawayama et al. 1997).

Depending on the type of solid waste, different amounts of methane produced can be expected, for example, lignin-rich organic waste has a methane yield of 200 L/kg of SV, while fruit and vegetable waste has a yield of 420 L/kg of SV. Lignin does not degrade under anaerobic conditions, so anaerobic digestion is not suitable for treating garden waste or wood waste (Bouallagui et al. 2009).

An important parameter in anaerobic digestion is the organic load rate (OCR), which quantifies the amount of substrate per reactor volume and time. Organic loading rate = mass of volatile solids, SV/reactor volume and time (kg SV/m3dia). A good daily loading rate for unshaken reactors is TCO $\leq$ 2, while with a shaken reactor this can be higher, and can be increased to a loading rate of 8, i.e. <2 kg SV/m3dia without shaking and 4–8 kg SV/m3dia with shaking. The pH range for anaerobic digestion is between 6.5 and 7.5. There is a risk of acidification of the reactor by acidogenic bacteria when the OCT is very high. However, in the acidic phases, the pH is low, while in the methanogenic phase it is a little higher. Methanogenic bacteria are rather sensitive to these parameters and will therefore be inhibited. In order to react to this situation the organic load rate must be reduced or lime or sodium hydroxide can be added to increase the pH level (Moraes et al. 2015b; Zuo et al. 2012).

Another factor that influences the process of anaerobic digestion is temperature. Temperatures below 15 °C are not ideal (not much activity occurs) and the organisms decrease their activity. Subterranean buildings or installations can buffer this temperature variation, but the anaerobic process is more comfortable in two temperature zones (ideal range): the mesophilic temperature between 30 and 40 °C and the thermophilic temperature between 45 and 60 °C. Operations in the mesophilic range are more stable and can tolerate large changes in parameters and consume less energy, however, they are slower in degradation and need more time. Thermophilic organisms are faster but the system is more sensitive to changes. Mesophilic units operate between 20 and 45 °C, with an optimal temperature of 37 °C. These are more common, because they are cheaper to build and maintain due to their lower operating temperature (Bouallagui et al. 2009; Forster-Carneiro et al. 2008b; Linke 2006; Wardani et al. 2020).

The hydraulic retention time (HRT) is another parameter that influences the anaerobic digestion process, and corresponds to the amount of time the input material remains in the reactor. An ideal time is between 10 and 40 days, the lower values correspond to the higher temperatures in the thermophilic range, because the process is faster (HRT = reactor volume/input volume per day) (Khalid et al. 2011).

Here is a confrontation with an optimization process. If for already defined input materials the volume of the reactor is small, then the HRT is low, which means that little biogas will be obtained, since there is little time for the process. If the reactor volume is large, then the HRT increases and you will have a higher biogas production, but at the cost of having a large reactor (more space and higher investment costs). Another parameter is the C/N (Carbon/Nitrogen) ratio, ideally a value between 16 and 25. A higher value implies limited nitrogen supply, which means food for the bacteria and therefore less biogas production. A lower value of the C/N

ratio can cause an accumulation of ammonia which can inhibit the anaerobic process (Bouallagui et al. 2003; Mel et al. 2015; Westerholm et al. 2012).

The last parameter corresponds to the size of the particles in the input material. The smaller the size the better, sizes smaller than 5 cm are ideal. What this does is increase the surface area of the material and allow microorganisms to degrade the material faster. For operations this means that generally the input material is fragmented using a crusher to get smaller particles. All anaerobic digestion systems adhere to the same basic principles, whether the raw material is food waste, animal manure or sludge from a wastewater treatment plant. Systems may have some differences in design, but the process is basically the same (Jeihanipour et al. 2013; Salehian and Karimi 2012).

4.4 Use of the Products of Anaerobic Digestión

The main products of anaerobic digestion are biogas and digestate. The substrate chain corresponds to different organic wastes, the collection and transport and the pre-treatment stage. Using an appropriate digester and with good operational conditions, the reactor will produce a good amount of biogas, which is saturated with water vapour and in the first instance the water vapour that moves through the pipes will condense and the humidity will be collected as water in the pipes and must be removed (process called dewatering).

Water can be removed at the lowest point in the pipes, if it is not removed it can block the pipes making the flow of biogas difficult. Because of this a siphon (water trap) should be installed at the lowest point in the gas pipes (Cesaro and Belgiorno 2015a).

There are different types of siphons that can be installed, Fig. 2 shows a simple elbow used to drain condensate into a biogas line. There are different types of traps that can be installed, they can be automatic or manual. Both have a T-shaped connection where the condensate can flow. The automatic trap empties when it is full, using a U-shaped drain, while the manual trap empties with the opening of a manual valve. The biogas produced in the reactor varies during the day according to the feeding models and according to the changes in ambient temperature (Jeihanipour et al. 2010).

Biogas production also continues during the night when less gas is used and therefore there are periods when the gas must be stored. This can be done in the same digester at the top of the fixed digesters, or in bags or balloons. Biogas can also be stored at high or medium pressure in gas tanks, through the use of compressors, requiring the use of energy, in three steps: conditioning, cleaning of the biogas and compression. In its uncompressed form, the energy content of one cubic meter of biogas corresponds to about half a liter of diesel. As a general rule, about 10 kg of organic solid waste (in wet weight) is needed to produce one cubic meter of biogas (Casaretto et al. 2019).

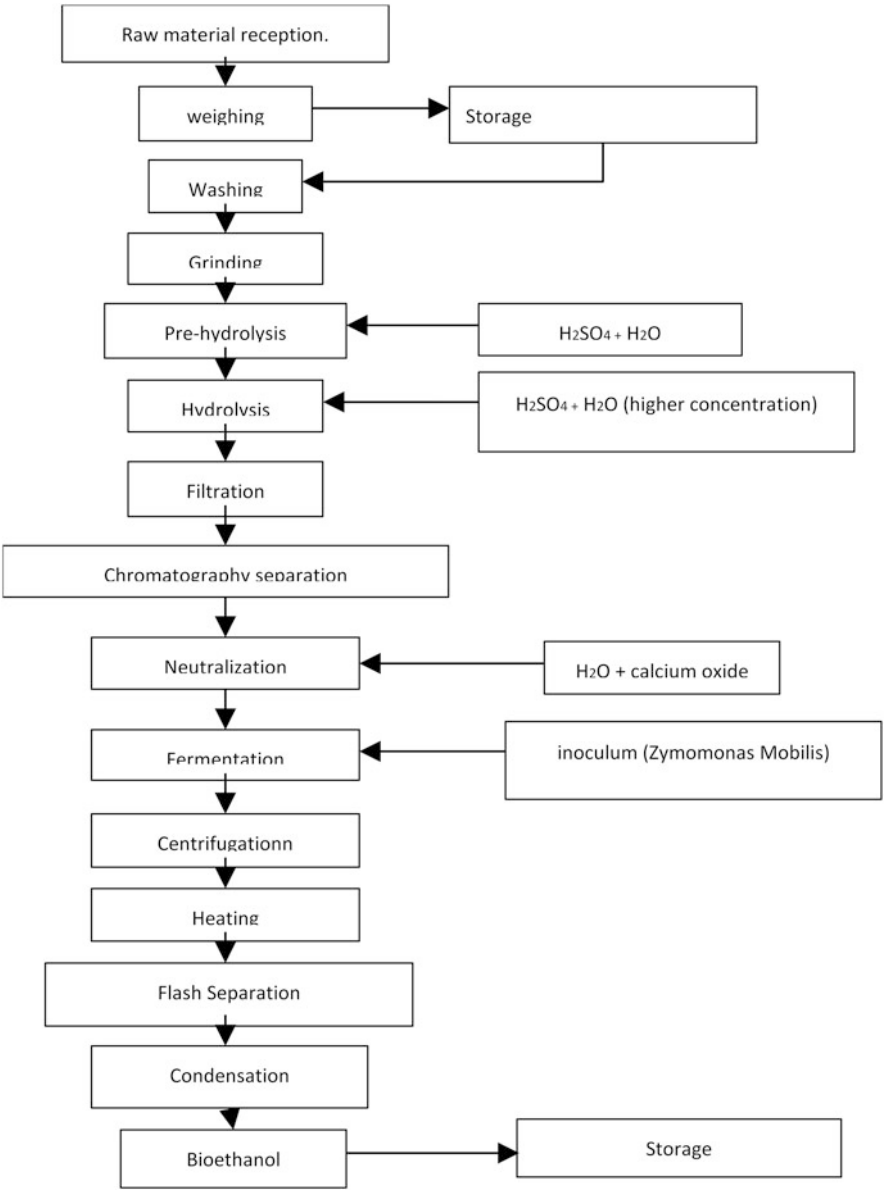

Fig. 2 Process flow diagram of bioethanol production from rice husks

As an example, the calorific value of diesel is 12 kWh/kg, it is needed to burn half a liter (0.5 kg) of diesel to generate the same amount of energy as one cubic meter of biogas (6 kWh/m^3). This means that burning half a liter of diesel generates the same energy as burning 1 m^3 of biogas (Silva et al. 2019).

For all its uses in machinery, engines or generators, the biogas needs to be conditioned or cleaned. The objective is to remove water vapor, and hydrogen sulfide which is very corrosive, and carbon dioxide which has no energy value. The removal of hydrogen sulfide is called desulfurization and can be done with ferrous oxide, which then transforms the hydrogen sulfide into iron sulfide. This can also be done with water purifiers, which at the same time can remove carbon dioxide.

If you think about the usefulness and use of biogas, the simplest way is to use the biogas near the anaerobic digestion facility for direct combustion, without the need for conditioning. The household ovens for cooking only need 200–450 L/h of biogas. Often when the direct use of biogas is not obvious, methane is introduced into the gas generators to produce electricity. However, with transformation losses only about 30% of the energy can be retained as electricity, the rest is lost as heat.

In addition to the gas, another production from anaerobic digestion is the digestate. In wet digestion systems, these are a kind of nutritive sludge with nitrogen, phosphorus, potassium and traces of other elements. Fifty percent of this nitrogen is available as ammonia that can be directly assimilated by plants and in general terms is a good fertilizer that can be used in agriculture. But if the operation is in the mesophilic range, you will not have high temperatures and if you have pathogenic substances these will not be sanitized. If it is not possible to use these digests in agriculture, then they must be treated before disposal, because they still have a high organic load and based on the regulations they are not allowed to be disposed of in surface waters (Atelge et al. 2020).

In a biogas system it is necessary to regularly check for leaks, using for example a pressure test, to see if the gas pipes are in good condition. The pipes also need to be checked and released if necessary. The siphons must be emptied and the sludge level in the outer compartment must be also regularly observed. The digester should not be overloaded, as this can lead to a process of acidification. From time to time the sludge must be removed from the digester (every 5–10 years), most of it will be found gravel and sand that accumulate at the bottom of the digester.

To undertake anaerobic digestion projects, it is necessary to have access to a good source of organic waste that is well sorted and accessible. Similarly, local skills and experience in the construction and operation of digesters need to be considered. Then, another requirement is the need and demand for gas or a small network that can be powered (institutional-state environment that allows power to be fed into the electricity grid). Likewise, the opportunity to compete with other energy sources is needed. Finally, there is a demand for digestate for use in agriculture.

4.5 Anaerobic Digestion Technologies and Practical Operation

There is a classification of the different types of anaerobic digestion technologies. A distinction can be made between dry and wet systems, between a batch and a

continuous system, between thermophilic and mesophilic operating systems, and between single-stage and multi-stage technologies. Wet reactors (operating with a low total ST solid content of 16% or less), continuous (the system is fed at a regular interval and at the same time an equivalent volume enters, an equivalent volume leaves the reactor), mesophilic and single-stage will be discussed (Bouallagui et al. 2003; Wardani et al. 2020).

Figure 3 shows a fixed dome reactor, which is a watertight gas structure built of bricks and covered with plaster and is often installed underground. It has an inlet pipe through which solid waste enters the digester, a digester with a volume of sludge and a space at the top to store the biogas produced. There is a compensation chamber (outlet) and a drainage system. The gas pipe is located in the highest part, with a valve to open or close the biogas passage. There are several designs of fixed dome digesters and sizes may vary. This is the most common type of reactor in developing countries (Bouallagui et al. 2009).

When the reactor is first started up, it is important to inoculate it with metonogenic bacteria. The easiest way to do this is to add cattle manure and water in a ratio of 1:1, or somewhat easier to use the digestate from another reactor that is already in operation. As a rule, about 10% of the reactor volume is needed with livestock manure to start the process, although more than 10% is good or even better (Bouallagui et al. 2009).

Operation The solid waste is mixed with water and introduced into the reactor and mixed with the material that was already inside the reactor, being subsequently degraded. The biogas is generated in the sludge by means of anaerobic digestion, the gas bubbles are moved to the top of the reactor, where they accumulate and begin to generate pressure. If the gas outlet valve is closed, the gas pressure will continue to

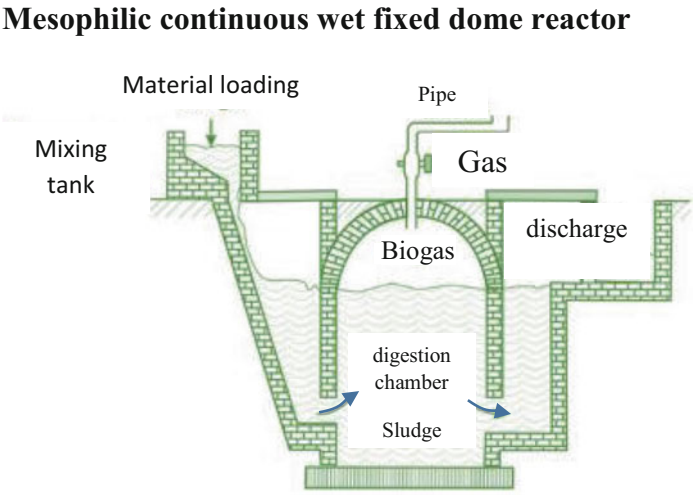

Fig. 3 Fixed dome reactor

increase and push the sludge down into the reactor and up into the dewatering chamber, where it is recovered as Biol (Yin et al. 2008).

When the gas is used, the pressure of the gas inside the digester decreases and the level of the sludge will be equalized generating a new balance.

Advantages Long life, no moving components, space-saving subway construction, low construction cost, easy to operate.

Disadvantages Need for specific technical skills (specifically at the construction stage), special sealing is required to ensure hermiticity of the structure, fluctuation of gas pressure depending on the volume of gas stored and its use.

It has similar characteristics to the previous reactor (inlet, digester to accumulate the sludge, outlet and drainage), the gas container is not a fixed unit, but a mobile unit, floating on a water jacket or in some cases floating directly on the sludge. When the gas pressure increases, the gas collection vessel (dome) moves upwards, and when the gas is used the weight of the hood pushes it back down. The hood is made of metal (painted to protect it from corrosion), fiberglass, plastic reinforced or galvanized sheet metal. In this design there is a guide rod, to ensure its stabilization while moving up and down. There is also a dividing wall that helps prevent circulation of the sludge from the inlet to the outlet directly. The digester is often positioned underground, while the gas container is located above ground (Duan et al. 2014; Khan et al. 2009).

Advantages Simple and easy to operate, the gas volume is directly visible (it is possible to see if the system is working or not), it has a constant gas pressure, or it is possible to increase it by adding some kind of weight to the gas tank or container (dome), relatively easy to build.

Disadvantages High material costs for the steel bell, and the steel parts are susceptible to corrosion, There are regular maintenance costs that increase when performing preventive maintenance against corrosion.

They are common in Latin American regions. They are long horizontal plastic and rubber tubes or balls (Fig. 4). The inlet and outlet lines are attached directly to the ball's shell. These balls need some kind of protection from the top (to avoid damage to its structure), as well as from the bottom, which is usually a compacted filling. A biogas accumulation system can be installed in the upper part of the biodigester.

Advantages Low construction cost, very simple to install, easy to build, easy to transport, higher digester temperatures in hot climates, easy emptying and maintenance.

Disadvantages Relatively short service life (5–10 years, depending on the material used for construction), susceptible to mechanical damage.

Fig. 4 Digestor tubular

5 Practical Applications in Biogas Production

Figure 5 shows a tubular type biodigester, 10 m long by one meter in diameter, located in the municipality of "Anzoategui" (Department of Tolima-Colombia)—Vereda Berdun high part and fed with pig excrement (28 pigs). The biogas is used for cooking food. The equipment used for measuring the parameters is a Multitec 540, reporting volume concentrations of 46.2%v for methane (CH_4) and 0.2 for hydrogen sulfide, %v (H_2S) (Mladenović et al. 2009; Xiang et al. 2018).

Biogas treated to meet pipeline quality standards can be distributed through the natural gas pipeline and used in homes and businesses, or it can be cleaned to produce compressed natural gas (CNG) or liquefied natural gas (LNG), which can be used to fuel automobiles. The digestate can be applied directly to the soil and incorporated into soils to improve soil characteristics and facilitate plant growth. It can also be processed into products to recover nitrogen and phosphorus and create concentrated nutrient products, such as ammonium magnesium phosphate and ammonium phosphate fertilizers (Ferella 2020).

If there are many animals on a farm, the resulting manure and wastewater can have significant environmental impacts if they are allowed to simply run on the land and into storm drains and surface waters. The waste depletes oxygen from the water as it degrades, which is harmful to aquatic life. Containment of animal waste is often required to protect water quality. Anaerobic digestion reduces the volume of this waste, produces methane and provides a byproduct that can be used as fertilizer (Ozgen et al. 2021).

Fig. 5 Biodigestor bag—Vereda Berdun upper part (Anzoategui-Tolima-Colombia)

6 Bioethanol Production

Bioethanol is a clear liquid that looks like water, it is mixed with gasoline (fuel additive) to produce a cleaner fuel that improves oxygen. Bioethanol is a chemical compound that can be used in different applications, such as alcoholic beverages, chemicals, pharmaceuticals and biofuels (Piccolo and Bezzo 2009).

6.1 Process Details for Bioethanol Production

Below is a diagram of the ethanol fermentation process.

The raw material is sent to the production plant and the handling of the material must be done, then comes the pre-treatment process, followed by the hydrolysis or fermentation process (Fig. 6) and then goes to the distillation process and finally through the evaporation process the ethanol is separated (Aruwajoye et al. 2020; Schläfle et al. 2017). The wastewater that comes out of the pre-treatment process can be used to produce biogas, which can be used to operate a boiler and the steam can be used for distillation purposes or to produce electrical energy. The following is the main equation in the alcohol fermentation process in a plant:

$$C_{12}H_{22}O_{11} + H_2O \overset{\text{yeast}}{\rightarrow} 4C_2H_5OH + 4CO_2 \quad (1)$$

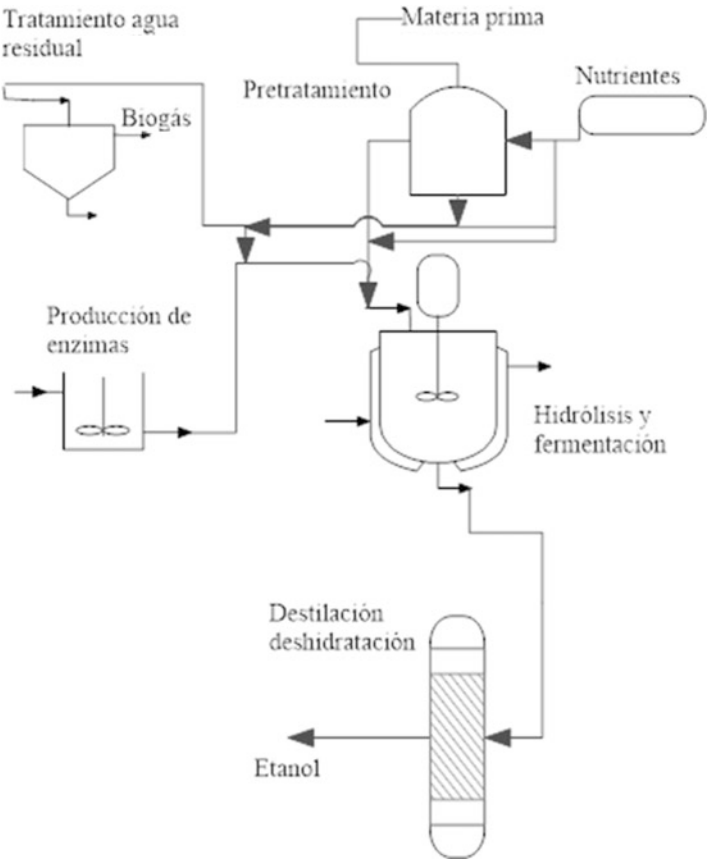

Fig. 6 Alcohol fermentation process diagram

$$\text{sucrose} + \text{water} \overset{\text{yeast}}{\rightarrow} \text{ethanol} + \text{carbon dioxide}$$

Sucrose reacts with water to produce ethanol and carbon dioxide, with the help of a yeast that acts as a catalyst in this process. The process can be given in feed-batch mode, where the diammonium phosphate (essential nutrient for the yeast), is fed intermittently into the reactor during the process. The stages of the process correspond to mixing, fermentation, and purification. The input consists of sugar syrup, yeast and nutrient which is fed intermittently into the reactor. The output consists of carbon dioxide, yeast, and ethanol (Balat 2011; Boluda-Aguilar et al. 2010; Rodríguez et al. 2010).

There are many crucial steps in the design of a bioreactor, first the type of reactor (e.g. stirred tank in batch feed), second the size and arrangement (a typical size for an industrial fermenter is between 50 and 100 m^3). The bioreactors use an agitation system that includes shock absorbers (buffers) and impellers. Using the mass flow

rate and density the working volume can be found. Smaller fermenters are more economical, the minimum number of parallel fermenters is 4, to ensure optimum process efficiency (Plácido et al. 2013; Zuo et al. 2012).

Proceso de operación de una planta de producción de bioetanol a partir de materia prima lignocelulósica:

Any sugar fermentable by a yeast can act as a raw material for alcohol fermentation. The ethanol manufacturing process depends mainly on the raw material. The most used steps in the production of bioethanol from raw materials containing lignocellulose are:

- Pretreatment of cellulose and hemicellulose to make it more accessible in the subsequent steps.
- Acid or enzymatic hydrolysis of polysaccharides in simple sugars (hexose and pentoses) to ethanol.
- Separation and concentration of ethanol.

6.2 Fermentation Process of Lignocellulosic Hydrolysates

Different microorganisms (e.g. *Saccharomyces serevisiae*) are used for glucose to ethanol fermentation. This microorganism is able to metabolize mono- and disaccharides (glucose, fructose, maltose and sucrose), but not pentoxes (xylose and arabinose), moreover it is not able to directly assimilate cellulose and hemicellulose (Lee et al. 2017; Oyeleke and Jibrin 2009).

There are pentose-fermenting microorganisms (Pichia Stipitis, Pachysolen Tannophilus and Candida Shehatae), however, they have a production rate of ethanol at least five times lower than the production of ethanol from glucose by *S. cerevisiae*, and on the other hand the tolerance to oxygen and ethanol is 2–4 times lower.

Zymobacter palmae Gram-negative strain is an anaerobic with potential to metabolize hexose, di- and trisaccharides bound to α and sugar alcohols (fructose, galactose, glucose, mannose, maltose, melibiose, sucrose, raffinose, mannitol and sorbitol). This strain produces approximately two mole of ethanol per mole of glucose without accumulation of byproducts and shows a productivity like *Z. mobilis* which is used to produce glucose alcohol in conjunction with the enzyme pyruvate decarboxylase and alcohol dehydrogenase (Dias et al. 2013; Nair et al. 2017).

The filamentous fungus *Fusarium oxysporum* is known for its ability to produce ethanol, but the conversion rate is low and produces significant amounts of acetic acid as a byproduct. In a bioethanol production plant, hydrolysis and fermentation can operate separately (HFS) or saccharification and fermentation can operate simultaneously (SFS) (Zuo et al. 2012).

HFS hydrolyzes the lignocellulosic raw material pre-treated to glucose and then ferments it to alcohol using different bioreactors for each process. After

pre-treatment of the lignocellulosic raw materials, the solid phase is separated from the liquid phase, which contains mainly sugars (pentoses and some hexose). After pre-treatment with diluted acid, the residual solid phase contains mainly lignin and cellulose. The cellulose is hydrolyzed by the addition of cellulolytic enzymes. Here both hydrolysis and fermentation take place at their optimum temperatures (50 °C for hydrolysis and 28–32 °C for yeast fermentation) (Carrillo-Nieves et al. 2019; Jafari et al. 2011; Moraes et al. 2015a).

Special microorganisms perform the fermentation of hexose and pentoses (present in the liquid phase) separately, because microorganisms that use pentoses metabolize pentoses and hexose more slowly than microorganisms that only assimilate hexose and are more sensitive to ethanol and inhibitors. The accumulation of released sugars (glucose, cellobiose) during enzymatic hydrolysis inhibits cellulase activity, with the effect of cellobiose being greater than that of glucose (Branco et al. 2019; Mirahmadi et al. 2010; Rastogi and Shrivastava 2017).

During SFS, hydrolysis and fermentation take place in a single bioreactor, with the sugars released in the enzymatic hydrolysis being used immediately by the microorganism. Relatively low concentrations of sugar will occur in the culture medium, and consequently the inhibition of the cellulase enzyme by the released sugars is reduced. The optimal temperature is 38 °C, with a compromise between optimal hydrolysis (45–50 °C) and fermentation temperatures (30 °C). Improvements can be achieved by selecting improved enzymes and yeast strains. In this technology *T. reesei* and *S. cerevisiae* are most frequently used (Bernier-Oviedo et al. 2018).

FSS Advantages increased hydrolysis rate by reducing cellulase inhibition by released sugars, lower enzyme demand, higher bioethanol yield, lower sterile condition requirement, shorter bioprocess time, reduced process, and separation equipment cost.

Disadvantages of SFS Incompatible hydrolysis and fermentation temperatures, microbial tolerance to ethanol, and enzyme inhibition by etanol.

Improvements can be made in order to optimize the process of obtaining bioethanol. For example, the inclusion of pentose fermentation in the FSS is an integration alternative; this process is called simultaneous saccharification and co-fermentation (SCFS). The two producing microorganisms must be compatible in terms of optimal pH and temperature. The development of microbial strains capable of growing at elevated temperatures can significantly improve techno-economic indicators. By co-fermentation of pentoses and hexose in a bioreactor, capital costs are reduced, as well as the possibility of contamination (Lynd 1996).

A second integration approach for the conversion of lignocellulose-containing feedstocks into bioethanol is consolidated bioprocessing (CBP). The production of cellulase and fermentation requires only one microorganism. Therefore, cellulase production, cellulose hydrolysis and fermentation are performed in one step. BPC involves four biological reactions in a single step: enzyme production (cellulases and hemicellulases), hydrolysis of carbohydrates into sugars, fermentation of hexose (glucose, mannose, and galactose) and fermentation of pentoses (xylose and

arabinose). CBP has the following advantages compared to other techniques for bioethanol production: enzymatic and fermentation systems are fully compatible and therefore bioethanol production costs are reduced, and no capital and operating investments are required (Aden and Foust 2009; von Sivers and Zacchi 1996).

6.3 *Feeding Materials*

The different types of biomass have potential as a raw material for bioethanol production. Due to their chemical composition (source of carbohydrates), they mostly form three groups: raw materials containing sugar (sugar beet, sugar cane, molasses, whey, sweet sorghum). Raw materials containing starch (corn, wheat, yucca). Lignocellulosic biomass (straw, agricultural residues, crop residues and wood) (Chovau et al. 2013; Hamelinck et al. 2005).

Raw materials containing sugar and cotton (first generation), compete with their use as food or fodder, which influences their supply. Therefore, lignocellulosic biomass (second generation), represents an alternative raw material for bioethanol production due to its low cost, availability, high distribution and is not competitive with food crops. Microalgae can accumulate starch as a reserve polysaccharide that can be used for bioethanol production (third generation), after the pre-treatment process, the residual biomass can be used as fertilizer. In the alcoholic fermentation process, there are three types of raw material (Azaizeh et al. 2020; Kumari and Singh 2020).

6.4 *Raw Materials Containing Sugar (Sucrose)*

Saccharin materials (mostly comprise sugar materials: they need little or no pre-treatment other than dilution (sugar cane, beet sugar, molasses, and fruit juices). Sugar cane and beet are the most important sugar producing plants in the world, they can be easily hydrolyzed by the invertase enzyme that is synthesized by most Saccharomyces species. Pretreatment for bioethanol production is not necessary, which makes this bioprocess more feasible than using starchy raw materials (Lu et al. 2011).

Sugar cultures only need a milling process for the extraction of sugars from the fermentation medium, and here bioethanol can be produced directly from juice or molasses. Total residual sugars in molasses can amount to 50–60%(m/V), of which approximately 60% corresponds to sucrose, making this substrate suitable for bioethanol production on an industrial scale. Sugarcane is less expensive than other raw materials used for bioethanol production (rapid processing and greater productivity) (Aden and Foust 2009; Jeihanipour et al. 2010).

The beet is used for the production of sugar, obtaining a fine and thick raw juice, as an intermediate product formed during the processing of sugar beet, as well as

crystalline sugar of high purity, which could be converted into bioethanol and/or bio-based products. Another sugar-containing material that can be used for bioethanol production is whey, a by-product of cheese making, which contains about 4.9% (m/V) lactose. Due to the relatively low sugar content a moderate size bioethanol plant requires a considerable volume of whey.

6.5 Raw Materials Containing Starch

Materials with starch, need treatment to decompose starch into glucose (corn, malt, barley, rice, oats, rye). Grain crops (corn, barley, wheat, or sorghum), and root/tubular crops (cassava, potato), contain large amounts of starch. Pretreatment of cassava tubers for bioethanol production includes the following operations: cleaning, peeling, chopping, and drying. Dry cassava chips are used for bioethanol production. The crucial enzyme for the hydrolysis of starch is α-amylase active at α −1.4, but not in links α −1.6 in amylopectin (Budiyono et al. 2013; Cesaro and Belgiorno 2015b; Ferreira et al. 2018; Safarian and Unnthorsson 2018; Sarkar et al. 2012).

To produce bioethanol from starch-containing raw materials, it is necessary to perform starch hydrolysis (mainly through α-amylase and glucoamylase), into glucose syrup, which can be converted into ethanol by *Saccharomyces cerevisiae* yeast. This step is an additional cost compared to the production of bioethanol from raw materials containing sugars. *Bacillus licheniformis* bacteria and genetically modified strains of the bacteria *Escherichia coli* and *Bacilus subtilis* produce α-amylase. *Aspergilus niger* and *Rhizopus* sp. produce glucoamylases. Under anaerobic conditions the *Saccharomyces cerevisiae* yeast metabolizes glucose into ethanol (Branco et al. 2019; Carrillo-Nieves et al. 2019; Rastogi and Shrivastava 2017).

The maximum conversion efficiency of glucose to ethanol is 51% by mass. Since yeast also uses glucose for cell growth and synthesis of other metabolic products, the maximum conversion efficiency is reduced. In practice, 40–48% by mass of glucose is converted to ethanol.

6.6 Raw Materials Containing Lignocellulose

Cellulosic material, requires pre-treatment, to decompose cellulose into glucose (wood, residual sulfite liquor, cane bagasse). First the lignin is removed, then the cellulose is hydrolyzed, and glucose is produced, which is finally fermented and converted into bioethanol. Lignocellulosic biomass is renewable and not competitive with food crops, and a considerable reduction in greenhouse gas emissions is achieved. It can be obtained from different residues or harvested directly from the forest and its price can be lower than that of previous raw materials. They form six main groups: crop residues (sweet sorghum cane and bagasse, corn residues,

different types of straw, rice husks), hard wood, soft wood (pine), cellulose waste (paper waste and recycled paper sludge), herbaceous biomass (grasses), municipal solid waste (Jafari et al. 2011; Moraes et al. 2015a; Zuo et al. 2012).

Lignocellulosic biomass contains 43% cellulose, 27% lignin, 20% hemicellulose and 10% other components. The heterogeneous structure of this biomass requires more complex chemical processes than uniform and consistent raw materials. Pretreatment and Hydrolysis: Hydrolysis of lignocellulosic biomass into monomeric sugars is necessary before microorganisms can metabolize them. This process is performed by acids, alkaline substances, or enzymes. The alkaline pre-treatment stage is necessary to obtain the conditions for efficient enzymatic hydrolysis (Chovau et al. 2013).

Pretreatment methods are divided into four groups: physical, chemical, physico-chemical and biological. In pretreatment, reduction of the degree of polymerization and of the crystallinity index, breaking of lignin-carbohydrate bonds, removal of lignin and hemicelluloses and increase in the porosity of the material must occur to ensure effective enzymatic hydrolysis of the lignocellulosic biomass. The choice of pretreatment depends on the nature of the raw material and the formation of by-products during the selected pretreatment. The harsh conditions used during pretreatments lead to the synthesis of toxic compounds, such as furans (2-furaldehyde: furfural) and 5-hydroxymethylfurfural, carboxylic acids (acetic, formic and levulinic acids) and phenolic compounds (aldehydes, ketones) (Dias et al. 2013; Lee et al. 2017; Nair et al. 2017; Oyeleke and Jibrin 2009).

Because these compounds are potential inhibitors of yeast, the following methods can be used to reduce their impact on process performance: removal of inibitors by solvent extraction, ion exchange, use of zeolites, use of highly tolerant inhibitor yeast strains, selection of an effective pre-treatment method that causes minimal degradation of sugars and subsequent inhibitor formation. Most detoxification methods only partially remove inhibitors, but they also contribute to sugar loss, which also increases the costs of the final process (Balan et al. 2013).

6.7 Conversion of Organic Waste into Bioetanol

The following is a brief description of the different processes that take place during the conversion of organic waste to bioethanol.

6.7.1 Pre-treatment Methods for Starches

– Acid Hydrolysis: Starches can be chemically treated by hydrolyzing in the presence of acid to obtain a saccharified starch.

$$\text{Starch material} \xrightarrow{H_2SO_4} \text{Saccharified starch}$$

In the acid hydrolysis of starch, high pressures and high temperatures are used, resulting in the formation of brown compounds due to the polymerization of sugars.

– Enzymatic hydrolysis: amylolytic enzymes are used for the conversion of starches to sugars.

$$\text{Starch material} \xrightarrow[\text{Amylolytic enzymes}]{\text{Sprouted barley grains}} \text{Fermentable Sugar}$$

Cellulose can be degraded to sugars using concentrated HCL.

$$\text{(a)} \quad \text{cellulose} \xrightarrow[\text{concentrated HCL}]{30-35\ ^\circ\text{C}} \text{Sugars (Glucose)} \& \text{Brownian Reactions}$$

$$\text{(b)} \quad \text{cellulose} \xrightarrow[\text{HCL diluted}]{150-160\ ^\circ\text{C}} \text{Sugars (Glucose)} \& \text{Brownian Reactions}$$

7 Fermentation Process

It is a biological process that converts sugars such as glucose, fructose and sucrose into cellular energy, producing ethanol and carbon dioxide as products, also known as alcoholic fermentation process. Molasses is a raw material that contains 50% of sugars and is suitable for the process of bioethanol fermentation and in the presence of enzymes is converted to glucose and fructose. Glucose passes through a pathway that produces pyruvic acid, and this when subjected to a decarboxylation reaction, produces acetaldehyde (Branco et al. 2019; Kotarska et al. 2019).

Yeast carries out this conversion in the absence of oxygen, so this is considered an anaerobic process, here the sugar is converted to bioethanol (Taherzadeh and Karimi 2008). The alcohol is named to indicate the source of the raw material from which it is made or to indicate the general purpose for which it is to be used. Grain alcohol (made from grains such as corn, wheat, and rice), molasses alcohol (produced from sugar cane molasses), industrial alcohol (used for industrial purposes), and fuel alcohol (used in combination with gasoline or other motor fuel) (Karimi et al. 2013).

7.1 Microorganisms Used in Alcohol Fermentation

The microorganisms used in the alcoholic fermentation process are the yeast, widely used in the industry to ferment the sugars present in the raw materials to form carbon dioxide and ethanol and the bacteria (*Zymomonas mobilis*) (Batista Meneses et al. 2020). Examples of yeasts are *Saccharomyces cerevisiae* (has a hard cell wall and is easy to multiply) and *Saccharomyces carlsbergensis*. Microorganisms are used to produce the product of interest, and in this way only the organisms of interest need to be present in the space that produces the product (the bioreactor), however, the organisms are present everywhere for example in the space where the bioreactor is installed, for this reason the surroundings must remain clean and only the microorganisms of interest are added to the bioreactor and this is done to multiply and produce the product of interest (Zhang et al. 2010).

In the surroundings there may be organisms with concentrations of 104 cells/ml in the air, there may be many types of organisms and it is not desired that they be present in the bioreactor which is designed to produce a particular product (bioethanol). What happens is that, if other microorganisms arrive, they may grow much faster than the organisms of interest, and they will compete for the food that is available in the bioreactor, and this results in a failure called bioreactor contamination (Koike et al. 2009).

All microorganisms should be removed from a bioreactor, for example, using very high temperatures (120 °C), for about 15 min. Liquid chemicals or vapors can also be used to sterilize the spaces. The characteristics of alcoholic fermentation yeast are

- The organism can tolerate high concentrations of sugar and alcohol.
- High sugar fermentation power: higher fermentation rate, the characteristics of the organism can be determined with respect to Ks and μmax: (kinetic parameters of the Monod equation: maximum growth rate, see application example 1). Alcohol is the product associated with growth, which means that the rate of alcohol formation is proportional to the rate of cell mass formation.
- High biochemical stability: this means that a uniform alcohol concentration is achieved, which is highly desirable on an industrial level.
- Tolerate a wide pH range with optimal acid, which makes its fermentation less susceptible to infection than bacteria
- Can grow under non-sterile conditions (this is the main advantage of the alcohol fermentation process).

In the mechanisms of the alcoholic fermentation process, two theories have been established

- Neuberg Theory (mainly theoretical bases)

$$C_6H_{12}O_6 \rightarrow \underset{\text{Glioxal metil hidratado}}{(2C_3H_6O_3)} \xrightarrow{+H_2O} \underset{\text{Glioxal metil}}{2CH_3\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{C}}-O} \xrightarrow{+O_2} \underset{\text{pyruvic acid}}{2CH_3COCOOH} \xrightarrow{-CO_2} \underset{\text{Acetaldehyde}}{2CH_3CHO} \xrightarrow{2H} \underset{\text{Alcohol}}{2C_2H_5OH}$$

$$C_6H_{12}O_6 \rightarrow \underset{\substack{\text{Glioxal metil hidratated}\\ \downarrow +2H\\ 2C_3H_8O_3\\ \text{Glycerol}}}{(2C_3H_6O_3)} \xrightarrow{+H_2O} \underset{\text{Glioxal metil}}{2CH_3\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{C}}-O} \xrightarrow{+O_2} \underset{\text{pyruvic acid}}{2CH_3COCOOH} \xrightarrow{-CO_2} \underset{\substack{\text{Acetaldehyde}\\ \downarrow \frac{1}{2}O_2\\ 2CH_3COOH\\ \text{Acetic acid}}}{2CH_3CHO} \xrightarrow{2H} \underset{\text{Alcohol}}{2C_2H_5OH}$$

There are glucose molecules (1 mol of glucose), then hydrate the methyl glyoxal, this is a pathway to pyruvic acid (2 moles), after a dexcarboxylation reaction is produced acetaldehyde and finally by hydrogenation takes place the reduction and alcohol is produced. This is one way in which alcohol is produced. Another way is where glycerol can be produced, and another way is where acetic acid can be produced, and ethanol is produced (Li et al. 2009).

Stoichiometry of conversion:

$C_6H_{12}O_6$	$\rightarrow$	$2CH_3CH_2OH$	+	$2CO_2$		
Sugar	$\rightarrow$	Alcohol	+	Carbon dioxide (g)	+	cell mass
(Glucose)		(Etil alcohol)				
100 kg	$\rightarrow$	51.1 kg	+	48.9 kg		

From the stoichiometric equation, 1 mole of glucose can produce 2 moles of ethanol and two moles of pure carbon dioxide (Balat 2011; Hoekman et al. 2011). Then 100 g of glucose, produce 51.1 g of ethyl alcohol. At industrial level, this fementation is carried out in closed vessels so that carbon dioxide can be collected, and at high pressure it can be converted into dry ice, which can be sent to cold producing industries. The theoretical yield of ethanol is about 51.1 % g/g. A typical analysis of the fermentation product is presented in the following Table 1.

7.2 Instrumentation and Control

In the industrial field, the liquid gas chromatograph corresponds to an equipment of daily use by the operators, as well as equipment for titration, pH monitoring and microscopes to monitor the yeast. During distillation, the alcohol is sampled and tested for purity. In the control room the operator can control the whole plant from the computer, he can start different processes: the pre-treatment process, the hydrolysis, the fermentation process, and the distillation process. It is possible to control at what temperature the process needs to run, how many gallons per minute it needs to run, or change the flow rates, the pH, and in general any variable that needs to be changed from the computer. An operator must be in the control room 24 h a day, 7 days a week (Zhang et al. 2010).

In the case of a Feed Batch Fermenter, the objective is to control the flow, the level, the temperature (at 30 °C), the pressure (from 1 to 1.1 bar), and finally the pH (from 4 to 5). The control strategies can be ratio control for nutrient flow and feed back control for the other variables of interest. There are four main objectives that need to be controlled: the control of the feed liquid entering the top using Feedback control, control of the flow of carbon dioxide and ethanol entering the bottom using Feedback control, control of the level of the liquid at the bottom to prevent it from overflowing and finally control of the pressure at 1 bar using Feedback control (Oberoi et al. 2011). For process control with centrifuge, the objective is to control

Table 1 Typical material analysis in the alcohol fermentation process

Product	g per 100 g of glucose
Ethanol	48.4
CO2	46.6
Glycerol	3.3
Succinic Acid	0.6
Cellular mass	1.2

Via Embden-Meyerof parnas: This is based on experimental facts and corresponds to a pathway for obtaining energy from glucose

the flow and level in the equipment. The flow can be controlled by adjusting the inlet current, while the level can be controlled by adjusting the outlet current. The objective of the control and instrumentation of a distillation column is to maintain the level in the column, the temperature (80 °C) in the column, to maintain the pressure at 6.1 bar in the column, and to maintain the external reflux ratio and the level in the reflux balloon.

7.3 Fermentation Process of Alcohol from Broken Rice

The raw material used is broken rice, which is sent to the milling machine, mixed, liquefied (liquefaction), heated to gelatinize the starch, then the enzyme is added in the saccharomization tank. The saccharified material, rich in sugar, is placed in the fermenter where some yeast and nutrients are added and where it undergoes the fermentation process and finally is sent to the distillation column for distillation (Mussoline et al. 2012).

The output of the distillation column is sent to the rectification column where the alcohol is obtained, and the water is removed from the bottom. The bottom distillate (stillage) coming from the distillation column is sent to a stillage separator, then to a mixer and finally to a drying unit where a dry green solid distillate is obtained which can be used by adding other nutrients such as good fodder (Daza Serna et al. 2016).

7.4 Fermentation Process of Alcohol from Rice Husks

The use of biofuels generates a new energy alternative and a solution to the dependence on fossil fuels, opening new possibilities for the controlled use of agricultural residues in favour of the environment. In recent years, the way to degrade and use biomasses, in this case rice husks, has been studied. The rice husk is suitable for the production of bioethanol, since it is composed of lignin (20–25%), cellulose (35–40%) and hemicellulose (15–20%), so it is considered a suitable substrate for the production of this, given its availability and its relatively low cost (Pellera et al. 2012).

Worldwide, the largest producers of bioethanol are the United States and Brazil; the Latin American countries that are producing bioethanol are, of course, Brazil, Argentina and Colombia. This relatively new fuel market offers great advantages, since the demand for the product is high, since the country is using a 10% bioethanol blend and the rest in gasoline, while in countries like Brazil, the blend is up to 27.5%. Figure 2 shows a block diagram representing the process flow corresponding to the production of bioethanol from rice husks, and Fig. 7 represents a process flow diagram to produce bioethanol from rice husks (Jouzani 2018).

7.4.1 Process Description

The process begins with the reception of the raw material that arrives in trucks, which are weighed in scales, then the rice husk is lifted by an ejector to the storage hoppers, to be processed the next day (Fig. 7). From the storage hopper it passes through pipes to the inside of the plant, where it arrives to a conveyor belt, which as it moves the husk is sprayed with water to wash the raw material. It is then taken to a hammer crusher, whose objective is to reduce the size of the particle to increase the exposure area and achieve better results in prehydrolysis and hydrolysis. Once in the Pre Hydrolyzer, the raw material has gained a percentage of water by washing, there is added H_2SO_4 diluted in a percentage of 7.65% and water. The pre-treatment process is handled at 30 °C and 1 atm. At the time of leaving the stream has already decomposed some of the cellulose, hemicellulose and lignin (Jouzani 2018).

The filtrate obtained enters a chromatographic separation column, which is packed with a calcium-based ion exchange resin. In this equipment, the separation is performed because the resin that is positively charged retains the SO_4^- ions from the sulfuric acid, letting the sugars pass through, therefore, these are part of the first current that leaves the column, and the acid is retained in the column for a while, when all the sugars have already passed through it and the column is saturated with SO_4 ions; distilled water is pumped and the acid begins to leave it, thus recovering more than 90% of the acid used (Taherzadeh and Karimi 2008).

The stream of sugars is sent to the centrifuge where water and lime are added to neutralize the remaining acid in this stream, this produces a quantity of gypsum. The acid that is recovered when the distilled water is pumped into the column is transported to an evaporator where the concentration is raised again and then recirculated back to the pre-hydrolysis and hydrolysis stages. The stream of sugars coming from the centrifuge enters the fermenter, where the fermentation is carried out with the help of the enzyme, *Zymomonas mobilis*; which are the bacteria in charge of the transformation of sugars into alcohol, in an efficient way (Zuo et al. 2012). After the fermentation is finished, the system is depressurized in such a way that the CO_2 is eliminated. The outlet stream from the fermenter is passed through another centrifuge, where the ethanol inoculum is removed, and then transported to a flash separator in order to obtain the ethanol vapors that are then condensed and discharged into the distillate stream.

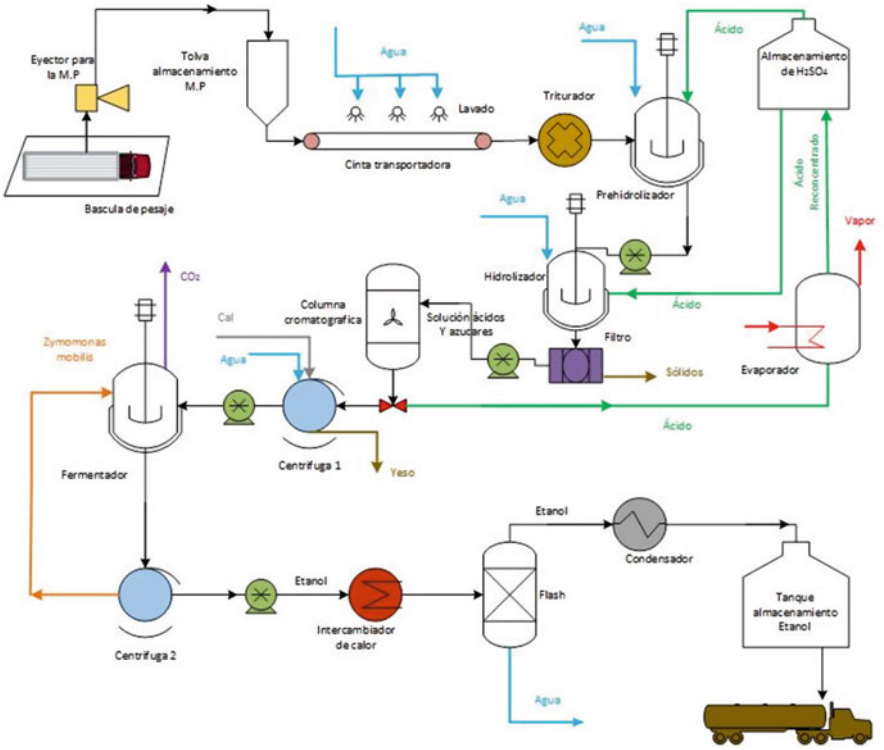

Fig. 7 Flow chart of the process of bioethanol production from rice husks

Before the flash separator there is a heat exchanger that takes the alcohol-water solution to the necessary temperature at the entrance to the separator so that the desired concentration is obtained at the exit. Finally, from the condensate equipment, the ethanol is passed to a storage tank, and from there it is loaded into the tank cars that will distribute the final product (Carrillo-Nieves et al. 2019).

7.5 Benefits of Fermentation and the Use of Ethanol

- A complete combustion is carried out (it burns more efficiently). Most of the time the ethanol is burned completely, to produce carbon dioxide and water. Ethanol has a lower ignition temperature than petroleum. A 10% ethanol-gasoline blend burns more quickly which produces less engine stress and less fuel consumption, while reducing carbon monoxide emissions by up to 30%. In the case of a hydrocarbon reacting with oxygen, in an incomplete combustion, solid carbon like graphite will be produced, and this deteriorates the spark plugs of the cars.

- Ethanol has a higher flammability range (it ignites and burns more easily).
- It produces less toxic emissions
- The by-products of oil combustion are greenhouse gases, which deplete the ozone layer. In the case of bioethanol use, ozone depletion is reduced.
- It is a renewable source, it is derived from plant material, these raw materials can grow rapidly to support the demand.
- It has positive implications for sustainable development.
- It is a neutral greenhouse.
- The carbon dioxide emitted is recycled back into the crop through absorption and photosynthesis

7.6 Disadvantages of Fermentation and the Use of Etanol

- If more than 15% ethanol is used in the mixture, some modifications need to be made to the machine, because bioethanol is corrosive and also absorbs water, and gets dirty easily, and will circulate through the parts of the machine such as the carburetor and the fuel injection system.
- Contaminants must be filtered out (a molecular sieve can be used) or they will cause corrosion or damage to the machine.
- Bioethanol has a lower heat of combustion than petroleum, which means that the energy released per gram of ethanol is lower than that of octane. This means that if you have two cars, one with an ethanol-gasoline blend and the other with only high-octane gasoline, and both are supplied with the same volume of fuel, the high-octane will go further.
- Large areas of land (farmland) are needed to cultivate and produce bioethanol. This land will be available for fuel production rather than food production and in most places, there is a shortage of dry land, water and fertilizer.
- Large energy inputs are needed to produce bioethanol. A considerable amount of energy is used in the production of fertilizers, that is, for the crops that produce the bioethanol, as well as for the distillation or refining of the ethanol.
- This process will only be effective if more energy is saved than is spent on the manufacture and use of bioethanol. This is the point where the industry is stuck, because if you put more energy into the input and you don't get more energy out, it would be worthless and very expensive to do the whole process.
- It is difficult to eliminate residual smelly fermentation liquors after the removal of the alcohol.

7.7 Practical Parameters and Operational Conditions of Fermentation

The factors that affect the alcohol fermentation process are:

- The efficiency of the pre-treatment.
- The optimal concentration of sugars: the cost of recovery or purification depends on the concentration of the product in the fermentation broth, if this is high the cost of recovery will be lower, if the concentration of ethanol in the fermentation broth is increased, more sugar will have to be used and a higher concentration of sugar is used, some kind of osmotic pressure is caused.
- Optimal temperature and pH: Yeast fermentation usually takes place at an acidic pH: 4.5–5 and at a temperature close to room temperature (30 °C).
- The addition of nutrients: it is necessary to add some magnesium, nitrogen, and phosphorus.
- The use of a vigorous yeast strain: a highly productive strain
- The maintenance of anaerobic conditions during fermentation: it allows the yeast to convert the sugars into alcohol. One thing to note is that the process of alcohol fermentation usually takes place in an open bed, in an acidic environment where fewer organisms can grow. Yeast can grow under aerobic conditions and produce cell mass and under anaerobic conditions produce alcohol. Initially, whatever dissolved oxygen will be used for the growth and metabolism of the yeast cell as soon as it is consumed the anaerobic condition prevails and the sugar is converted into alcohol.
- Finally, prompt distillation of the fermented product.

8 Fermentation Technologies and Practical Operation

The reactor is the heart of the bioethanol production process. Let us look at some types of reactors used in bioethanol production. The reactors can operate in three different ways, the first is a batch operation, the second is a semi-batch operation and the last is a continuous form (Cardona Alzate and Sánchez Toro 2006).

8.1 Agitated Tank Reactor (ATR)

It is a vessel, a reactor widely used at industrial level and consists of a cylindrical tank and can be small (1 or 2 L/reactor), medium (15 L) or very large occupying large spaces in the industry (Fig. 8). All bioreactors have an agitator to shake the reactor contents and keep the cells in suspension (Faraco and Hadar 2011; Jafari et al. 2011).

This reactor has several measuring instruments (temperature, pH, dissolved oxygen meters) and is highly controlled. The factories that produce the products (bioethanol) are the cells (culture) in the bioreactor, in this case that manufacture bioethanol, everything necessary is done by these microscopic cells, whose size ranges between 2 and 5 microns, although there may be larger (Mtui and Nakamura 2005).

Batch Operation or Process When the agitated tank is operated in batch mode, what is done is that everything is dumped into the bioreactor (the medium, the cells, the inoculum), and then the reaction takes place, and we wait until the bioproducts are formed and at the end of time everything is dumped out of the bioreactor and further processing takes place (downstream process steps to separate and purify the product). Many processes in the industry are carried out in batch mode, as it is very simple to operate bioreactors in batch compared to other types of bioreactors (Mahmoodi et al. 2018).

Continuous Operation or Process Here there is a continuous input of the substrate (nutrient broth), it is not necessary that it contains cells. There is a continuous output of the bioreactor, with the product produced (Bioetanol). The times are adjusted so that the product is produced properly, and this operation is called continuous operation. During the process there is cell growth, product formation, and nutrient flow, all happening simultaneously within the reactor (van Dyk et al. 2013).

Semi-Batch Operation or Process (Fed-Batch) Between the Batch operation and the continuous operation, there is something called semi-batch operation. There could be an intermittent input, and an intermittent output. You probably start with a batch, and for some well-known reasons, you add substrate may be in intervals (not continuously), this is a Fed-batch feed. You can remove some part of the reaction broth intermittently, or you can add or remove it inermittently, this kind of operation is called a Fed-batch operation (Safarian and Unnthorsson 2018). There are several reasons to operate in Fed-batch mode, for example, some products that are made by these cells may be toxic to the cells themselves, as is the case with bioethanol. Ethanol above 18% is toxic to cells, so it cannot be allowed to continue to grow. There are other products that can be toxic at much lower concentrations, and to manage the toxicity, a portion of the culture is removed, and fresh culture is added so that the concentration of the toxic product is maintained (Casaretto et al. 2019).

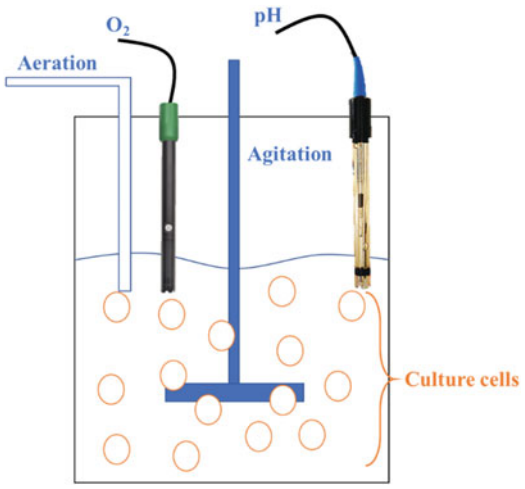

Fig. 8 ATR reactor

8.2 Air-Lift Bioreactor

The main difference with the previous one is that in this bioreactor there is no agitator, although it is necessary to keep the culture medium of the bioreactor in agitation, only this way the bioreactor will work properly. The agitation and mixing nature is caused by air pumped by a compressor (Liguori and Faraco 2016).

The air is lifted through the internal cylinder located inside the reactor and carries the cells along (the air keeps the cells in suspension) and is then recirculated.

8.3 Packed Bed Biorreactor

This bioreactor has a stationary bed (site where biorreactions take place and where microorganisms convert the reactant into the desired product) that is packaged and typically operated continuously. This type of reactor is more used in water treatment, where the bed can be sand through which the water to be treated flows and at the exit clean water is obtained. The fluidized bed is a variant of the packed bed, but the bed is not stationary, but is moving, is jumping (Kumari and Singh 2018).

9 Practical Applications in Bioethanol Production

Application example: A distillery industry produces 100 m^3 of rectified alcohol (containing 92% ethanol %v/v) in a chemostat from cane molasses (containing 50% sugar %w/w) using S. Cerevisiae. Find the bioreactor volume and the amount of cane molasses required per day. The characteristics of the yeast are the following (under anaerobic condition) (Fig. 9).

$$\begin{aligned} \mu_{\max} &= 0.05\ \mathrm{h}^{-1} \\ K_s &= 2\mathrm{g}\ \mathrm{L}^{-1} \\ Y_{X/S} &= 0.05 \\ Y_{p/S} &= 0.5, \\ y\ S_0 &= 350\ \mathrm{g/L} \end{aligned}$$

Solution:

- For a sterile environment: $X_0 = 0$
- Concentration of biomass in stationary state:

$$X = X_0 + Y_{X_{\acute{S}}}(S_0 - S) \tag{2}$$

That is, it must be found under steady state conditions, with which dilution rate D, the maximum cell mass production will be achieved.

That is, it must be found under steady state conditions, with which dilution rate D, the maximum cell mass production will be achieved.

$$\frac{dP}{dt} \alpha \frac{dX}{dt}$$

Then the maximum cell growth will give the maximum amount of product formation (Bioethanol). It must be found under what circumstances the maximum amount of cell mass is produced.

– Then, the Dmax value is calculated,

$$D_{max} = \mu_{max}\left(1 - \sqrt{\frac{K_S}{K_S + S_0}}\right)$$
$$= 0.05\left(1 - \sqrt{\frac{2}{2 + 350}}\right) = 0.0462\ \mathrm{h}^{-1}$$

– Substrate concentration in steady state

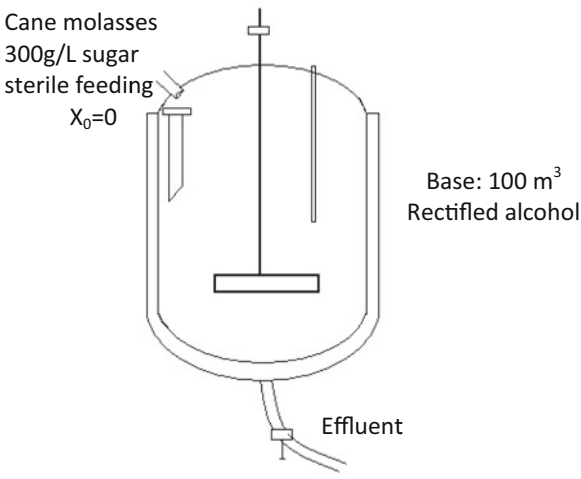

Fig. 9 CSTR for bioethanol production from sugarcane molasses

$$S = \frac{K_S * D_{max}}{\mu_{max} - D_{max}}$$
$$= \frac{2 * 0.0462}{0.05 - 0.0462}$$
$$= 24.31 \text{ g/L}$$

– Biomass concentration in steady state

$$X = Y_{X/S}(S_0 - S)$$
$$= 0.05(350 - 24.31)$$
$$= 16.28 \text{ g/L}$$

Calculation basis : $100 \text{ m}^3 = 92 \text{ m}^3$of ethanol production per day (92%v/v)
$$= 92,000 \text{ L of ethanol/day}$$

– Ethanol Density, $\rho = 780$ g/L
– Calculate the amount of ethanol that is produced

$$m_{et} = \rho_{et*V=780 \text{ g/L}*92,000 \text{ L}=71.76\times10^6 \text{g}}$$
$$= 71.76 \times 10^3 \text{kg de etanol por dia}$$

– Substrate required (sugar required per day)

$$Y_{p/S} = \frac{p}{S}$$

$$S = \frac{p}{Y_{p/S}} = \frac{71.76 \times 10^3 \text{kg etanol/day}}{0.5} = 143.52 \times 10^3 \text{ kg sustrato/day}$$

– Volumetric feed flow rate, F

$$F = \frac{S}{S_0} = \frac{143.52 \times 10^3 \text{ kg sustrato/day}}{350 \text{ g/L}}$$

$$= \frac{143.52 \times 10^3 \text{ kg sustrato/day}}{350 \text{ kg/m}^3} = 410.057 \frac{\text{m}^3}{\text{day}} = 17.08 \frac{\text{m}^3}{\text{h}}$$

– Time required by the continuous stirred tank reactor, CSTR

$$\tau_{CSTR} = \frac{S_0 - S}{-r_s}$$

$$-r_s = -\frac{ds}{dt} = -\frac{ds}{dX}\frac{dX}{dt}$$

$$-r_s = \frac{1}{Y_{X/S}}\mu X = \frac{1}{Y_{X/S}} D_{max} X = \frac{1}{0.05} \times 0.0462 \times 16.28 \text{ g/Lh} = 15.04 \text{ g/Lh}$$

– Calculate τ_{CSTR}

$$\tau_{CSTR} = \frac{S_0 - S}{-r_s} = \frac{V}{F} = \frac{(350 - 24.31)}{15.04} \text{ h} = 21.65 \text{ h}$$

– Calculate the volume of the reactor, V

$$V = F \times \tau_{CSTR} = 17.08 \frac{\text{m}^3}{\text{h}} \times 21.65 \text{ h} = 369.78 \text{ m}^3$$

– Cane molasses required

Substrate required/day = 143.52×10^3 kg Substrate/day, Sugar content of cane molasses 50% w/w, Cane molasses required = 287.04×10^3 kg/day. This is the amount of molasses required to operate the plant and obtain 100 m^3 of rectified ethanol. Most industries use CSTR (Continuous Stirred Tank Reactor) because they have the highest productivity.

10 Economic Overview of Bioethanol Production

The sustainability of the ethanol and biogas economy is based on four fundamental pillars: government participation and policies, corporate social responsibility, venture capital investment and energy conversion costs (Quintero et al. 2012).

Government Participation Governments' concern about energy dependence on hydrocarbons has forced them to generate policies to encourage and support projects that seek to minimize the dependency ratio. Throughout the world, various strategies have been developed by governments to promote the development of the bioethanol industry, policies that have had variable effects but that, in one way or another, contribute to energizing this economic sector. Brazil, the second largest producer of

bioethanol in the world after the United States, has doubled its agricultural frontier for the production of sugarcane for bioethanol and has almost tripled its domestic consumption of fuel alcohol in the last decade (Arora et al. 2019) (Fig. 10).

In the case of Brazil, an outstanding national program was developed that involved the local sugar production industry, research institutions, the automotive industry and the government with policies to encourage investment in infrastructure and regulations for the use and guarantee prices of bioethanol, guaranteeing demand by establishing minimum quantities of fuel mixture, a situation that links the demand for fuel alcohol to the consumption and expenditure of motor fuels (Piccolo and Bezzo 2009). This situation is thought to be favorable, in which the demand for biofuels is linked to the consumption of fuels by the automotive industry, and generates a great deal of uncertainty for this agroindustry when the world is undergoing global dynamics of reduction in the use of traditional fossil fuels or static demand effects resulting from dramatic changes in consumption, such as those experienced as of the second quarter of 2020 in reference to the global pandemic that affected many human activities (Balan et al. 2013; Sari and Ernawati 2019).

In the case of the United States, the world's leading bioethanol producer, its promotion policies include the fact that 95% of the gasoline consumed in its territory contains between 10 and 15% bioethanol, a production based on its large corn agro-industry, subsidizing bioethanol by between 10.6 and 15.9 US$/L, a condition that alters competitiveness in favor of national production, although it is estimated that the subsidies reach the surplus production and reach the export of bioethanol, affecting the stability of the domestic industry in other countries such as Colombia (Chovau et al. 2013).

Notes: A more detailed breakdown of fuel ethanol production by country for 2000–2012 can be viewed at the U.S. Energy Information Administration's (EIA) International Energy Statistics: Biofuels Production (Sunarno et al. 2020).

Geopolitical The energy sources available in each country will determine the autonomy and development of the industries that depend on energy sources to

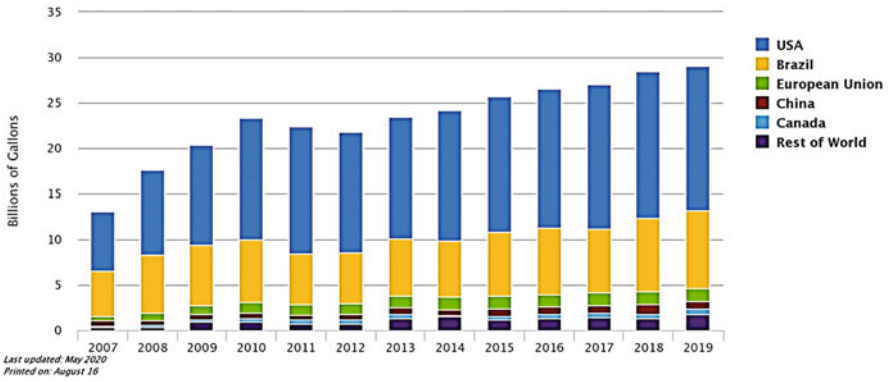

Fig. 10 Global ethanol production. Source: Renewable Fuels Association-2020

move their gears; the privileged standard of living of a good part of the societies of industrialized countries is based on the wealth of the subsoil and its hydrocarbons originated and treasured during thousands of years in it. For any state to talk about geopolitics will have to see as a prominent issue the production or supply of oil, that is why being a non-renewable resource or in some cases not available in their territories for exploitation (Quintero et al. 2008; Yu and Tao 2008). The search for independence from fossil fuels or to diminish the importance of suppliers is a state policy that has generated the development of the ethanol and biogas agro-industry, especially in Western countries such as the United States, Brazil and, incipiently, in the European Union, which have sought to reduce dependence on oil from the East and natural gas from Russia (Georgieva and Ahring 2007; Novo et al. 2012).

The importance of the issue is very visible when, for example, the U.S. Congress passed laws based on the average yield of gasoline, which should be 35 miles per gallon, an increase in the use of ethanol to 36 billion gallons per year by 2022, prohibiting the use of fuels that pollute more than synthetic fossil fuel (Quintero et al. 2012).

Corporate Social Responsibility (CSR) CSR is a modern way of doing business and organizations have identified this strategy to create and preserve market value and investment. Society is rewarding the efforts of corporations that demonstrate a focus of responsibility in social and environmental issues, in this direction the negotiation of energy and environmentally friendly fuels have a general acceptance from consumers and the state, that is, the profitability of these initiatives are marked by a consumer demand and subsidies, preferential tax rates that states create to encourage and energize these economic sectors (Barrera et al. 2016).

Risk Capitals The concept of risk capital is used about investments made in companies with cutting-edge or innovative concepts or proposals, which are going to enter markets with the uncertainty of the success of their business models. According to The Economist Newspaper Limited magazine, green energies, where ethanol and biogas production is located, have become an outstanding reference for the interest of large risk capitals and research institutions; ethanol being one of the green energies of greatest interest for risk capitalists (Brown 2015).

10.1 Used Raw Materials

According to the FAO, it is expected that the ethanol industry will make use of between 15 and 20% of the world's corn and sugarcane production by 2026. The growth and dedication of sugarcane is also projected to contribute 35% to the world's ethanol production by 2026, while corn will remain the main source of extraction, with 58% of its use in obtaining bioethanol (Eggeman and Elander 2005; Kabir et al. 2015).

Other alternative biomasses for obtaining bioethanol such as vegetable residues, fibers and cellulose materials are estimated to grow by 8% as contributors of

bioethanol sources in world trade, a figure that is linked to the reduction of extraction costs from these sources which, although they do not impact food security, constitute a different technological challenge (Dabwan et al. 2020; Padi and Chimphango 2020).

In the European Union it is estimated that the production of Bioethanol will stabilize at 7.3 billion liters, mainly obtained from wheat and sugar beet. In the United States, it is estimated that by 2020 there will be a demand for 61.6 billion liters of bioethanol, obtained mainly from corn. As for Asia, China is expected to reach a production of 1.8 billion liters of bioethanol produced mainly from corn, cassava, and soybeans (Lohrasbi et al. 2010; Padi and Chimphango 2020).

10.2 How Bioethanol Will Be Used

The growth of this agroindustry has been linked to the demand for gasoline and automotive fuels, so that the use and growth of its market will depend on the dynamics of fossil fuels. Until this condition is broken, and massive propulsion technologies are developed using only bioethanol, the bioethanol agroindustry will be dependent on the good for which it was developed (Chiaramonti et al. 2012; Chiranjeevi et al. 2018; Prasara-A and Gheewala 2017).

Figure 11 shows the ethanol production and consumption trend from 2000 to 2019. To the extent that less transport fuel is used, the need for bioethanol will also decrease; this situation is expected to be very strong in the European Union, Canada and Japan, which are increasingly generating policies to discourage the use of fossil fuels and create transport systems that use emission-free energy (He et al. 2014).

The demand for bioethanol will grow mainly due to consumption in developing countries such as Brazil, China, India and Thailand. Brazil is called upon to be the main driver of demand with an increase of 6 billion liters, which represents 35% of the global increase in bioethanol use. Due to the fuel ethanol blending policies of some provinces in China, demand is expected to increase by 1 billion liters, as well as Thailand where ethanol's share in fuel will generate an additional demand of 0.7 billion liters (Cara et al. 2007; Yu et al. 2018).

The bioethanol production process is being developed as a state policy strategy, as mentioned above, so that demand is met internally in most cases, which means that international trade in this biofuel has very little growth, with projections of stagnation after 2020 and setbacks after 2026 (Kumar and Sharma 2017; Martínez-Patiño et al. 2017; Solarte-Toro et al. 2018, 2019; Sritrakul et al. 2017).

10.3 Costs Associated with Bioethanol Production

The production of ethanol has become a survival strategy for the sugar industry that has been affected by the drop in sugar prices as a result of regulations and restrictions

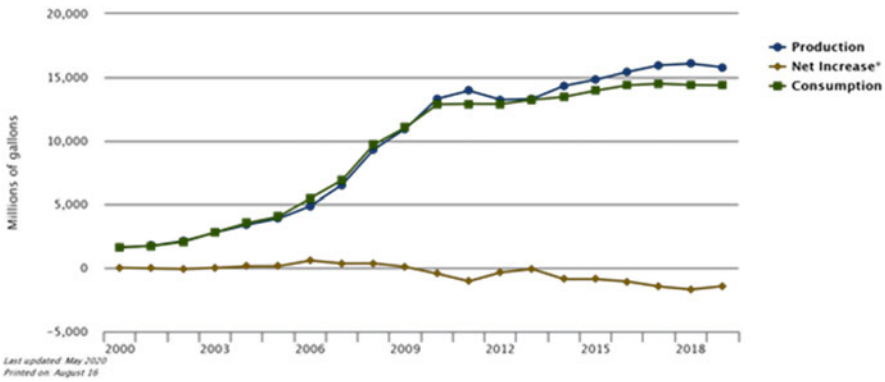

Fig. 11 U.S. production, consumption and trade of ethanol. Source: U.S. Energy Information Administration (EIA) Monthly Energy Review, Table 10.3

on food as well as cultural changes in consumption that have caused the demand for sugar as a sweetener to stop growing. The ethanol agro-industry is an integral part of the business strategy of the old sugar mills that took refuge in the renewable energy business and through technological reconversion are saving this agro-industrial line for the time being (Alvira et al. 2010; Chiranjeevi et al. 2018; Náthia-Neves et al. 2018).

Parity prices to define sugar or bioethanol production in this market are hardly stable, going through relevant variations over time due to climatic aspects, various production subsidy policies, tariff barriers to bioethanol or sugar imports make, for example, domestic prices higher than international reference prices and therefore parity in the definition of production is indifferent in many sugar producing countries (Manzanares et al. 2017).

Installation scales for ethanol production generate direct production costs that include such aspects as necessary investments in the distillery and its fermentation, distillation and auxiliary systems, including biomass treatment and conditioning for processing, and will vary depending on the scale of the assembly and to a very small extent on the source of the ethanol (Yu et al. 2018).

Ethanol from sugar cane has a production cost in the range of 0.27 to 0.51 USD/L. The production costs of ethanol from molasses indicate a production cost in the range of 0.43–0.64 USD/L. These costs may differ depending on the valorization of molasses as a raw material, since it is an important factor in the decision of sugar mills on the use and disposal of this co-product. By August 2020, the market reference prices for Bioethanol are Brazil*: 0.50, United States*: 0.52, Thailand*: 0.58, France*: 0.81, Sweden*: 1.43, Spain*: 1.71. Therefore, there is a margin for the agroindustrial business that will vary according to the local conditions of taxes, incentive policies and controlled demand (Sarto et al. 2019; Solarte-Toro et al. 2019; Sritrakul et al. 2017; Taherdanak et al. 2016; Venturin et al. 2018).

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