Role of Microorganisms in Soil Genesis and Functions

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N. Kaviya, Viabhav K. Upadhayay, Jyoti Singh, Amir Khan,
Manisha Panwar, and Ajay Veer Singh

1 Introduction

Soil shows an important task in maintaining the ecosystem and provides supports to the plant roots and bestows them with essential minerals and nutrients, and protects plants from erosion and other destructive physical, biological and chemical activities. Soil have the profound habitat for different and varieties of living organisms such as insects and microorganisms. Microorganisms play foremost role in soil formation and soil ecology because they as 'natural soil engineers' regulate the flux of nutrients to plants and prop up nitrogen fixation, and ultimately promote detoxification of naturally occurring inorganic and organic pollutants in soil. Microorganisms associated with soil assist in liberation of essential nutrients from primary minerals, and thus released nutrients which are required essentially for both microorganisms and as well as for plants (Uroz et al. 2009). In soil, the phenomenon of weathering of rocks is a multifaceted interaction of three kinds of weathering processes (physical, chemical and biological). Plants, animals and microorganisms vigorously participate in the biogeochemical cycles which ultimately contribute to the process of pedogenesis through biological weathering (Gadd 2007). Microorganisms express imperative role in the weathering of rocks, and use released elements as nutrients (Calvaruso et al. 2006). They compel the important processes of mineral weathering, participate in process of soil structure formation and organic matter decomposition, and also play important role in nutrient cycling (Chorover et al. 2007; Feeney et al. 2006; Schimel 1995). Microorganisms, for instances bacteria, fungi, cyanobacteria and lichens have been considered as main entities for carrying out biological weathering of rocks (Gadd 2010). Hirsch et al. (1995) demonstrated that the byproduct of microbial metabolism in form of 'organic acids, produced by soil

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N. Kaviya · V. K. Upadhayay · J. Singh · A. Khan · M. Panwar · A. V. Singh (✉)
Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh
Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

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microorganisms can dissolve rocks. Microorganisms show impact on the dissolving rate of minerals, as the microbial metabolites facilitate in leaching out some substances from rocks or minerals (Lian et al. 2008a, b). The scientific study on types of microorganisms opens several ways for knowing the mechanisms of various steps of soil formation through biological process. Moreover, several current approaches particularly molecular techniques have been developed to explore and identify the both culturable and unculturable microbial diversity in soil assist in soil formation. Present chapter is focused on depicting the role of microorganisms in soil formation and the mechanisms for weathering process employed by such microflora with highlighting the current and advanced molecular approaches for determining microbial diversity in soil.

2 Microbial Diversity and Soil Microorganisms (Eubacteria, Archaea, Fungi and Algae)

The earth is considered to be around 4.5 billion years old and the microbial diversity on earth has a much prolonged evolutionary history than plants/animals. The microbiological process of life spans about 3.8 billion years of organic evolution, shortly after the surface of earth was still very hot (Delong and Pace 2001). At that time there was a "reducing atmosphere" comprised of methane, carbon dioxide, ammonia, and hydrogen and was devoid of free oxygen. The earth atmosphere was shifted from anoxic to oxic states by oxygenic photosynthetic progenitors. The two earth's most plentiful cellular life forms, *Prochlorococcus* and *Synechoccocus*, filling the ocean to varying degrees from pole to pole, generating oxygen as a byproduct of sunlight-driven photosynthesis (Gilbert and Neufeld 2014). Plenty of adverse conditions also influence the survival of microorganisms. Some microorganisms produce spores, when they encounter environmental stresses such as high temperature, and such form of tough structure survive for longer periods and engender new vegetative cell when exposed to favorable conditions. Bacterium such as *Deinococcus radiodurans* possesses the capacity to survive under higher/lethal doses of radiation, i.e., 3000 times greater than the mortal dose for humans. However, numerous microorganisms possibly developed in the subsurface of landmasses or beneath the sea surface where they were protected to some extent from 'UV radiation'. On earth, soil contains most diverse habitats and for diverse assemblages of different types of soil microorganisms comes under the category of two well known groups such as 'prokaryotes' and 'eukaryotes' (Fig. 2.1). Interpretation of microbial community's dynamics is likely the most challenging task because of the surprisingly huge microbial diversity in soil, and the variable and complex matrix where soil microorganisms are fixed. Torsvik et al. (1990) evaluated that 1 g of soil contains 4000 different bacterial "genomic units" which were determined on the basis of DNA–DNA reassociation. Approximately 5000 bacterial species have been confirmed from the soil (Pace 1999), while Giller et al. (1997) estimated about 1,500,000 fungal species exist on earth. Whereas as macrofauna, 3000 species of earthworms (Lee 1985), 1,00,000 species of 'protozoa' and 500,000 species of 'nematodes'

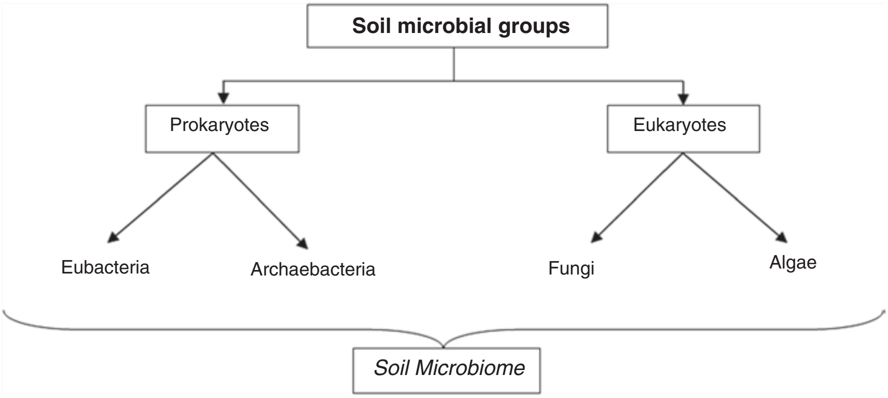

Fig. 2.1 Chief groups of microorganisms exist in soils

(Hawksworth and Mound 1991) have been reported in soil among soil flora and fauna, not to state the further invertebrate groups of the mesofauna (collembola, enchytraeids and mites) and macrofauna (ants, beetles, spiders and termites) (Giller et al. 1997). Microbial community composition may be one important control on soil processes. Soil microorganisms particularly both bacteria and fungi play very important roles in numerous biogeochemical cycles and as well help in cycling of different organic compounds. Soil microorganisms exert their effect on above-ground ecosystems as they contribute multifarious roles in plant health and nutrition, soil formation and soil fertility (O'Donnell et al. 2001). Prime factors considered to manage community composition are (Tiedje et al. 1999): (i) the key resources for growth i.e. fertility level e.g. various kinds of carbon or related compounds from plant litter, rhizosphere and invertebrates, key nutrients like N, P and K. (ii) Soil environment and its diverse characteristics (iii) Certain factors influencing organism dispersal, for instance soil structure, routes of dispersal, micro aggregate stability, and (iv) basis of population turnover such as nematode and protozoan grazing and controls on lytic enzymes. Plants also influence spatial distribution of soil bacteria and fungi. Anthropogenic activities such as intensive application of agricultural chemicals could negatively affect microbial diversity, and perhaps also present adverse effect on both above and below-ground functioning of ecosystem. Buckley and Schmidt (2001) reported higher amounts of 16S rRNA for all microbiological groups determined in uncultivated fields as compared to agricultural or cultivated fields, and this suggests a reduction in microbial activity in cultivated fields. Living organisms on earth are comprising three domains (i.e. Bacteria, Archaea, and Eucarya) and each domain containing two or more than two kingdoms. They come under the less complex cell constitutes called as prokaryotes (organisms without definite nucleus), consisting two groups of microorganisms such as eubacteria and archaebacteria. The eubacteria (also called as bacteria) comprises 'cyanobacteria', an important group formerly well-known as 'blue-green algae'. The cells are prokaryotic where the lipids present in membrane are primarily diacyl glycerol diesters, and the ribosomes having a eubacterial type of rRNA. Other important prokaryotic entities come under ‘archaebacteria’, where membrane lipids into cellular architecture are mainly isoprenoid glycerol diethers or diglycerol tetraethers. Archaea is subdivided into the two important known kingdoms, (1) Euryarchaeota, containing the methanogens and their phenotypically diverse relatives and (2) Crenarchaeota, which having the comparatively tight clustering of extreme thermophilic archaebacteria. On the other hand, the organisms with more complicated cellular structure possessing true nucleus are called eukaryotes (include plants, animal and fungi). The membrane of eukaryotic cells contains lipids mainly glycerol fatty acyl diesters and the eukaryotic kind of rRNA present in ribosome (Woese et al. 1990).

2.1 Eubacteria

Various kinds of soils have ‘Eubacterial group’ as the most dominant group of prokaryotic microorganisms. The number or population of bacteria depends on the depth of soil, and with the depth soil the microbial population decreases. Generally, ‘horizon A’ of a soil profile is rich in organic matters and holds high population of microorganisms than horizon B and C (SubbaRao 1997). Soil bacteria present various cellular forms such as cocci (spheres, 0.5 mm), bacilli (rods, 0.5–0.3 mm) or spirilli (spirals). However, bacilli are considered as most common in soil, while spirilli are found very rarely in natural environments (Baudoin et al. 2002). Soil bacteria further categorized in two important groups (a) ‘autochthonous organisms’ and (b) ‘zymogenous organisms’. Autochthonous, also referred as indigenous populations, are more uniform and stable in soil, since they derive their nutrition from native soil organic or mineral matter (*Arthrobacter* and *Nocardia*). But, zymogenous bacteria need an external substrate or nutrients unlike autochthonous organisms, and their activity in soils is uneven and they often form resting structure ‘propagules’ (*Pseudomonas* and *Bacillus*). The population of zymogenous bacteria increases when specific nutrient substrates are added to the soil and declines gradually as the added substrate is exhausted (cellulose decomposing bacteria, nitrogen-utilizing bacteria, *Nitrosomonas* and *Nitrobacter*). The most predominant species of soil bacteria comes under three orders namely, *Pseudomonas*, *Eubacteria* and *Actinomycetes* (Benizri et al. 2001). The most common bacterial genera are *Achromobacter*, *Arthrobacter, Azosprillium, Bacillus, Clostridium, Corynebacterium, Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Pseudomonas and Sarcina* (Lynch 1987). Although the bacterial population is also influenced by temperature and moisture, but certain bacteria have the capacity to survive under extreme climatic conditions. Bacteria can survive in area where the temperature is below freezing point such as arctic zones, and also can thrive in arid/desert soils, where temperatures go very high. They form a resting structure called ‘spores’ which assist in the survival of bacteria in most of adverse environmental conditions. Bacteria can be categorized into several groups on the basis of their temperature tolerance ability and these groups are assigned as mesophiles grow between

15–45 °C, psychrophiles grow below 10 °C and thermophiles grow between 45 and 65 °C. However, mesophilic bacteria encompass the immensity of soil bacteria (Barber and Lynch 1977). Other factors such as pH, farm practices, application of fertilizer and pesticide, and organic matter amendments also affect bacterial populations in soils. ‘Autotrophic’ and ‘heterotrophic’ bacteria are reside in a wide array of soils, where autotrophic bacteria such as purple and green bacteria build their own organic matter from CO_2 or inorganic carbon sources, while heterotrophic bacteria rely on pre-formed organic compounds or matter for their nutritional strength. ‘Photoautotrophs’ obtain their energy from sunlight that they grab and convert it into chemical energy with the help of bacteriochlorophyll pigment. Whereas, chemoautotrophs oxidize inorganic materials to obtain energy and parallely, they collect carbon from carbon dioxide (Tate et al. 1995). The cyanobacteria are gram-negative eubacteria identified by their capability to carry out oxygenic photosynthesis and having features common to bacteria and algae, and thus often named as “blue-green algae”. Other than chlorophyll, phycocyanin is additional pigment which gives a special blue-green color to cyanobacteria. Soil exerts excellent habitat for cyanobacteria where important or dominant cyanobacteria belong to the genera *Anabaena*, *Aphanocapsa*, *Chroococcus*, *Cylindrospermum*, *Fischerella, Lyngbya*, *Microcoleus*, *Nostoc*, *Scytonema* and *Oscillatoria* (SubbaRao 1997; Benizri et al. 2002). Heterocyst is special structure in some cyanobacteria having imperative role in nitrogen fixation. The rice fields are an excellent habitat for the growth of some cyanobacteria where they are able to fix atmospheric nitrogen (Prescott et al. 1996).

2.2 Actinomycetes

Among soil biota, actinomycetes are also important soil microorganisms having adequate characteristics to classify them into a different group of prokaryotes. Indeed actinomycetes are grouped with other bacteria under class ‘Schizomycetes’, but are restricted to order ‘Actinomycetales’. Actinomycetes exert certain resemblance to Fungi imperfecti or Deuteromycetes, which abundantly sporulates, and can be seen as distinct clumps or pellets in liquid cultures (Benson 1988). The abundant population of actinomycetes can be seen the presence of decaying organic matter. However, they are intolerant towards acidic conditions and the number of actinomycetes decreases below pH 5.0. Furthermore, waterlogged soil create unfavorable conditions for the growth of actinomycetes, but desert soils of both arid and semiarid regions maintain considerable populations, possibly due to spores resistance of towards desiccation. The proportion of actinomycetes in the entire microbial population enhances with the depth of soil. Therefore, actinomycetes can be isolated in ample numbers from C horizons of soil profiles. ‘*Streptomyces’* is the most common genus of actinomycetes while, *Nocardia* and *Micromonspora*, and in particular *Actinoplanes, Actinomyces* and *Streptosporangium*, are only encountered infrequently (Prescott et al. 1996; SubbaRao 1997). Temperature is another factor which influences the growth of actinomycetes, and temperatures ranged from 25 to 30 °C are much suitable. While in compost heap thermophilic actinomycetes (belong mostly to the genera *Thermoactinomyces* and *Streptomyces*) growing at 55 and 65 °C are most common and present in huge numbers.

2.3 Archaebacteria

'Archaebacteria' are primitive prokaryotes and are considered to be the first organisms to emerge on the earth. They have unique features for their survival in extreme hostile environments such as salt marshes and hot sulfur springs, where ordinary organisms cannot survive. They are phylogenetically very distant from 'eubacteria' and have typical characteristics. Moreover, these microorganisms are devoid of special cell-wall material 'peptidoglycan' a unique feature to describe and distinguish it from eubacteria, However, proteins and non-cellulosic polysaccharides takes part in formation of their cell wall structure. Branched chain lipids are act as main constituents of cell membranes that help archaebateria to tolerate extreme pH and temperatures. The rRNA component of archaea is also moderately dissimilar from those of other organisms (Huber et al. 2002). Archaebacterial group could be made-up of two subgroups referred as 'obligate' and 'facultative' anoxybionts, where obligate anoxybionts mainly includes methanogenic and halophilic species, those reside in habitat devoid of oxygen. However, facultative anoxybionts can be found in the presence and absence of oxygen (Kyrpides and Olsen 1999).

2.4 Fungi

Among the soil microorganisms fungi show the huge diversity and have distinctive features as they bear filamentous mycelium consisting of individual hyphae. Since fungi are heterotrophic in nature, but the type of organic materials exhibit a direct impact on fungal populations in soils. A unique trait of fungi emerges in acidic, neutral and alkaline soils, give them an advantage over population of actinomycetes and bacteria. Fungi are strict aerobic organisms and abundant fungal populations are present in arable soil. Moreover, fungi show a critical preference for different soil depths, and fungal species common at lower depths are rarely exist on the surface. Such distribution of fungal population is specified by the accessibility of organic substances/materials and by the ratio between oxygen and carbon dioxide in different depths of soil atmosphere. Fungi have been classified into 'phycomycetes', 'ascomycetes', 'basidiomycetes' and 'fungi imperfecti' or 'deuteromycetes'. Most common fungal isolates from soils are belong to the class 'Fungi Imperfecti' as they have very common trait to generate profuse asexual spores but not have sexual stages. The characterization of the members of these fungi is based on their septate mycelium and a special structure known as 'conidiophore', where this structure forms conidia or spores incessantly. However, other three classes of fungi show both sexual and asexual strategies for reproduction. Presence of non-septate mycelia is trait of phycomycetes and members of this group produce an imprecise number of specific spore cells known as 'sporangia'. In ascomycetes, species-specific number of meiotic spores is produced by the sporangium, while a higher degree of sporangium specialization known as ‘basidia’, is recognized in basidiomycetes. Fungi, specially ‘ascomycetes’ and ‘basidimycetes’, have the capacity to degrade complex organic compounds such as cellulose or lignin, but many members of these two fungi also live as root symbionts (mycorrhiza) and acquire photosynthetic product derived from plant partner in the form of simple sugar (Lynch and Hobbie 1988). ‘*Botrytis*’, ‘*Chaetomium*’, ‘*Aspergillus*’, ‘*Cephalosporium*’, ‘*Alternaria’*, ‘*Cladosporium’*, ‘*Cunninghamella’*, ‘*Fusarium’*, ‘*Gliocladium*’, ‘*Monilia*’, ‘*Rhizopus’*, ‘*Mortierella*’, ‘*Pillularia’*, ‘*Pythium’*, ‘*Rhizoctonia’*, ‘*Mucor’*, ‘*Scopulariopsis*’, ‘*Trichoderma*’, ‘*Zygorynchus*’, ‘*Verticillium*’, ‘*Penicillium’* and ‘*Trichothecium’* are the major genera of fungi present in soils (Hawksworth 1991a; SubbaRao 1997). Filamentous fungi show beneficial effect in soil as they participate in organic matter degradation and as well as assist in soil aggregation. ‘*Metarhizium*’, ‘*Alternaria*’, ‘*Dematium*’, ‘*Aspergillus*’, ‘*Gliocladium’* and ‘*Cladosporium’* are the some important genera of fungi having ability to produce certain substances related to humic substances in soil and hence assist in the maintenance of soil organic matter (Hawksworth 1991b).

2.5 Algae

In nature, the soil algae are ubiquitous when favorable conditions in form of moisture and sunlight are available. In soil system, the population of algae is not as abundant as bacteria and fungi, and structurally they may be unicellular (*Chlamydomonas*) or filamentous (*Ulothrix, Spirogyra*). As photoautotrophic organism algae utilize carbon dioxide from the environment and able to generate oxygen. Moreover, algae may also show the variety of their habitat, as they have also been found beneath the soil surface and at the location where sun light cannot be reached, besides the normal soil habitats. Though, such extreme locations express the lower population as compared to those of algae that dwell in normal habitat or soil surface (Metting 1988). ‘*Protosiphone*’, ‘*Chlorella*’, ‘*Chlrococcum*’, ‘*Oedogonium*’, ‘*Chlamydomonas*’, and ‘*Chlorochytrium’* are the important genera of green algae inhabit in most soils (Lynch 1990).

3 Biosphere and Microorganisms

In soil there are variety of spheres exist which influence the microbial interaction and relevant microbial mediated biogeochemical processes. Soils can be partitioned into different spheres of influence such as the rhizosphere, the detritusphere, or the drilosphere (Nunan 2017). The detritusphere is a vastly and relevant microhabitat in soils where litter fermentation and humification layers over the soil surface have substantial root, saprophytic and mycorrhizal activity, and associated grazing fauna (Haynes 2014). It is also depicted as ‘biogeochemical interface’, where soil makes contact with fresh plant litter and act as a very important biochemical hot spot for activity of microorganisms and soil material cycling in soil (Kuzyakov 2010). Accordingly, the detritusphere is compatible to explicate the regulation of important soil functions. Bacteria are typically considered to be more active entities in carrying out the degradation of labile organic compounds and also involve in early stages of plant litter decomposition. On contrary, fungi are considered to be more important organism in the degradation of composite substances, and actively involved in later stages of litter decomposition process (Paterson et al. 2008). Rhizosphere is an important sphere of soil frontier and first time described by 'Lorenz Hiltner' in 1904, who was a German agronomist and plant physiologist by profession. 'Rhizosphere' is the region where soil volume interacts in direct way with plant roots where the products of rhizodeposition stimulate the activity and population of microorganisms, thus shifting the balance between mineralization and immobilization of Nitrogen (N) (Clarholm 1985). Additionally, rhizosphere is an interface between biota and geologic atmosphere, where roots show extreme physical pressures on adjacent soils. Rhizosphere is also the chemical milieu where several biogenic chemical reactions intermingle with minerals, and this root surrounding region presents the unique territory for a broad group of microorganisms. Therefore, rhizospheres are primarily significant for soil formation, and as well as participate in the formation of the most tremendously weathered soils of earth (Richter et al. 2007). "Drilosphere", is an another soil sphere consist of the following (a) an inner microenvironment of the earthworm's gut, (b) the exterior part of earthworm contacted with the soil, (c) 'surface' and 'belowground' casts and (d) 'burrows' and 'chambers' constructed by the earthworm, and all of which are considered as 'microhabitats' for a variety of microorganisms such as bacteria and fungi (Condron et al. 2010).

The soils of drilosphere are rich in P, N and humified organic substances in contrast to the nearby soils and such kind of soils (Giri et al. 2005) are also reported to have a huge proportion of the entire soil 'denitrifying' and 'nitrogen-fixing' bacteria (Wolters 1991). Both the microbial community and enzymatic activity in the drilosphere can be influenced by the input of labile carbon and energy (Lipiec et al. 2015) and, moreover fresh earthworm cast aggregates with an elevated carbon input stimulate the activity and development of microorganisms. On contrary, the microorganisms in structural site built by earthworm are an inevitable part of earthworms usual diet (Pizl and Novakova 2003) and consequently through the direct trophic effect such structural sites may influence both the microbial loads and activity (Andriuzzi et al. 2016). Hence, the enhanced functional microbial diversity and the better enzymatic activity in the majority of earthworm-influenced compartments 'drilosphere' make the soils less prone to degradation. Moreover, the biological and physical functions of the both 'drilosphere' and 'casts' influence numerous ecological processes at the local (burrow) and landscape scales, and help in improving and as well as conserving the soil quality (Lipiec et al. 2016).

4 Role of Microorganisms in Chemical Transformation

A process of conversion in which organic compounds transforms from one form to another form, it affects existence and toxicity of the compound and known as chemical transformation (Smitha et al. 2017). Soil dwelling bacteria and fungi play a major task in chemical transformation by means of their direct and indirect activities. This process is known as biotransformation. Microbes developed such mechanisms to acclimatize environmental changes. Biotransformation occurs by means of enzyme as well as non enzymatic way. A huge diversity of microorganisms are important because diverse microorganisms contains different enzymes and resides in various physiological pH, which leads to the wide range of biotransformation, these biotransformation are the major source of nutrient recycle in environment.

4.1 Phosphorus Transformation

'Phosphorus' is found in both organic and inorganic form and known as second most essential nutrient it participates in various cellular metabolic activities in plants and microorganisms. In phosphorus cycle, microorganisms bring many changes in phosphorus transformation such as alter inorganic phosphorus solubility, convert organic phosphorus in inorganic form by means of mineralization. Many bacteria such as *Pseudomonas* and *Bacillus* take part in inorganic phosphorus solubilization by secreting a wide range of organic acids (acetic acid, glycolic acid, formic acid and succinic acid) (Chalot et al. 2002). Many fungi such as *Fusarium* and *Penicillium* also secrets various organic acids that solubilizes insoluble form of phosphate (Sollins et al. 1981). Mineralization of organic phosphorus occurs by the action of phosphatases (Phytase, Nucleotidase, Sugar phosphatases, Nuclease and Phospholipases), hydrolyzes phosphorus-ester bond and releases orthophosphate. These phosphatase categorized into three groups according to their optimal pH, if enzymes having optimal pH '5', '7', and '9.5' then they can be categorized in 'acid', 'neutral' and 'alkaline phosphatases', respectively (Tabatabai 1982). Some fungi produce huge amount of 'acid', 'neutral' and 'alkaline phosphatases and take part in organic phosphorus mineralization (Bae and Barton 1989).

4.2 Nitrogen Transformation

Nitrogen availability for plants is the major area of concern to maintain sustainable ecosystem. Majorly nitrogen is present in three forms such as ammonium, nitrate and organic nitrogen. Soil microorganisms utilize this organic nitrogen in their metabolic activities and converts into ammonium and nitrate form by a process called nitrogen fixation. Nitrogen fixation occurs in symbiotic association by *Rhizobium*, *Bradyrhizobium* in association with leguminous plants as well as asymbiotically by aerobic bacteria such as *Azotobacter*, *Beijerinckia*, anaerobic bacteria *Clostridium*, organotrophic bacteria and free-living cyanobacteria. In nitrogen cycle, a diverse and huge group of bacteria are involved in ammonification. But small group of microorganisms i.e. chemoautotrophic bacteria (ammonium oxidizers and nitrite oxidizers) are able to convert ammonia into nitrate in nitrification process (Kaplan 1983). Nitrate and nitrite reduce into ammonia through bacteria such as *Mycobacterium* and into nitrogen by denitrifying bacteria such as *Pseudomonas*, *Bacillus*, *Thiobacillus* (Payne 1981). Fungi are versatile in chemo-heterotrophic metabolism by means of their specific enzyme (Cromack and Caldwell 1992), and metabolize various substances for nutrient and energy (Wainwright 1992). Ectotrophic mycorrhizal fungi participate in ammonification of organic nitrogen hence takes part in nitrogen transformation (Lakhanpal 2000).

5 Soil Genesis and Function of Microorganisms

The term 'soil' is referred to as "critical zone" of earth as it plays an imperative function in controlling environment and life sustainability on earth. The term 'mineral soil' is used for those soils derived from weathered rocks and minerals. Soil is an excellent habitat for various kinds of organisms where they contribute in the organic matter decomposition and subsequently formation of humus. As the plant leaves are fall onto the soil surface where several pertinent soil microorganisms can "attack" and decompose plant tissues. The organic matter derived from plants or leaves is utilized as rich energy source for microbial growth, and increasing their population in the soil. Soil microorganisms use easily degradable materials (simple sugars and carbohydrates) present in the plant parts and leave more resistant substances (such as fats and waxes) behind, which are not easily degradable. The material left at last is not simply decomposed, and such tough residues involves in the humus formation. Humus in soil acts as a gluing agent, and effectively holding primary soil particles together to generate secondary aggregates and thus, soil microorganisms and the humus assist in the soil formation and development.

5.1 Microorganisms in Rocks and Minerals

Weathering of rock is one of the most significant geochemical processes which taking place on the earth and results into the pedogenesis, maintenance of soil productivity, regulating atmospheric composition and global climate change. The weathering is the process of breakdown and disintegration of rocks and minerals which are commenced by certain physical agents and chemical processes, leading to the formation of regolith (parent material). A complex interaction of physical, chemical and biological activities participate in rock weathering processes. In physical weathering the rocks are disintegrated and are broken down to relatively smaller pieces, without creating any new substances. However, in the process of chemical weathering disintegration of rocks and minerals occurs by various chemical processes and it takes place mostly at the surface of rocks and minerals with vanishing of certain minerals and formation of secondary products. On other hand, in biological weathering the biological or living agents are accountable for both disintegration and breakdown of rocks and minerals. Plants, animals and microorganisms actively involved in the biogeochemical cycles which add to the pedogenesis through biological weathering (Gadd 2007). Microbial and plant root assisted biological weathering of rock plays a key task in maintaining and supply of various inorganic compounds/elements which essentially required as nutrients by plants (Chang and Li 1998). The roots of plants loosen the rock material and the process for crack formation commence. 'Root-pry' is an important phenomenon which occurs by big crack created through root expansion. Soil associated macro fauna including earthworm, snail and burrowing animals (such as rodents) also contribute in the process of biological weathering (Lian et al. 2008a, b). The weathering of major types of rocks through microorganisms results into the releasing of various types of elements, which can be required as nutrients (Calvaruso et al. 2006). Bacteria, cyanobacteria, fungi and lichens are the important microbial groups, and they all have been considered as agents for biological weathering of rocks. Rock surfaces, cracks and the pore spaces of sand, stone and granite are the unique territory for microorganisms where they sometimes form 'biofilms' that ultimately contribute to the disintegration of the rocks (De ta Torre et al. 1993; Puente et al. 2006). Hirsch et al. (1995) demonstrated that organic acids secreted by microorganisms helps in dissolving of rocks, and enhance the rate of rock disintegration. Rocks are the main resource of metals in the form of minerals and ores. Metal bio-reduction is considered to be an imperative feature for microbes to endure in such environmental conditions. Cations, anions and inorganic nutrients are needed for rock inhabiting microorganisms and plants. And these nutrients are released during the process of rock biodegradation (Chang and Li 1998). One important activity carried out by bacteria through rock weathering is the accessibility of trace element in the soil; and such bacteria improve the trace element uptake by plants. This is attained by microbial alteration of the absorptive properties of the roots such as improving and increasing the root length, surface area and the amount of root hairs indirectly or directly contributes to the translocation of trace elements by different processes. Microbial mediated mineral weathering process occurs by range of bio-deteriorating mechanisms that comprises uptake of elemental, redox reactions, production of acids, metabolites, chelating compounds and polymers (Welch and McPhail 2003) (Fig. 2.2). Bacterial communities participate in dissolving the primary rock-forming minerals to get essential nutrients and also perform as nucleation sites for the precipitation of secondary minerals. Plant roots in association with the microorganisms disrupt sheet silicates and hence expose new surface area where the further process of biochemical weathering occurs (April and Keller 1990). In general, the weathering process of silicates carried out by microbial communities is a key biological mechanism for nutrient requirements. The sequential steps of mineral weathering can also be influenced by a nutritional potential of minerals with the microbial organisms having capacity for the formation of beneficial minerals. Numerous environmental factors such as lighting, humidity, nutrients and rainfall amount influence the process of biological weathering. Moreover, microbial mediated degradation of rocks is dependent on other certain factors including location, climate and season.

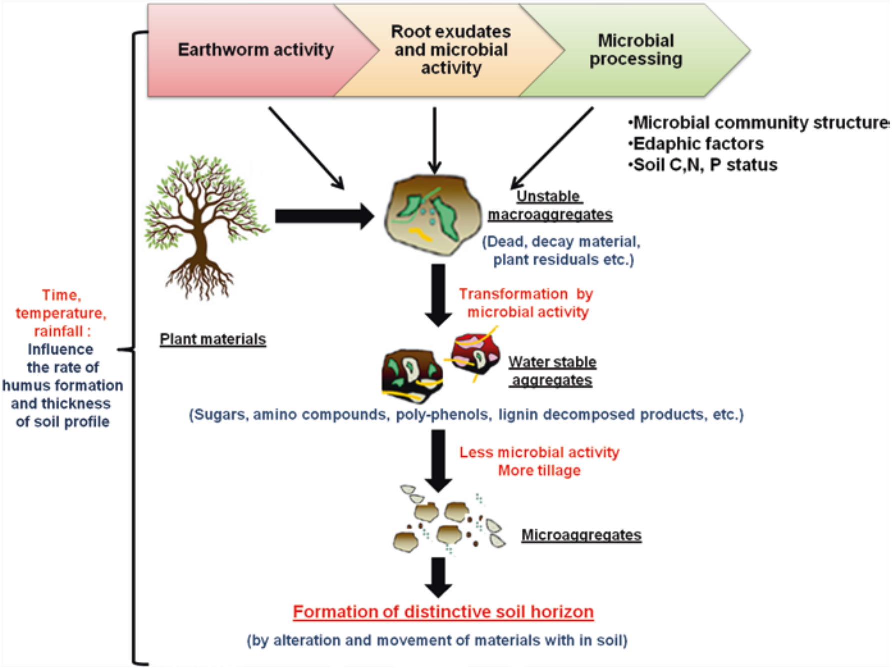

Fig. 2.2 Schematic representation of soil aggregates formation by biological and physical processes

Weathering process carried out by microorganisms can be either aerobic or anaerobic in nature, and may takes place in acidic, neutral and alkaline conditions (Berthelin 1983). Several factors such as wind, water and bird droppings can play a key role in the transfer of spores of both bacteria and fungi results into early colonization of rocks. Microorganisms may be entrained within the rocks through various processes including rainfall, snowmelt, and some aeolian transport (Cockell et al. 2009). Establishment and growth of microbial communities are also regulated by the elemental composition and physical properties of the rock (Gleeson et al. 2006). Lichens are the mutual association between fungus and a photosynthetic partner (either green algae or cyanobacteria), which are also helpful in weathering of rocks. Few lichen activities such as hypal penetration, contraction and extension of lichen thallus due to microclimatic wetting and drying helps in physical disintegration of rocks (Arino et al. 1997; Moses and Smith 1993). Chemical disruption of rocks is due to the production of respiratory CO_2, secretion of a variety of organic acids and salts which results in dissolving of minerals and formation of different biochemical compounds responsible for metal chelation. Weathering process of ‘sandstone basalt’, ‘granitic’ and ‘calcareous rocks’ through lichen, and the mode of action shown by lichen in rock weathering process are also well documented (Chen et al. 2000). Experimental works performed on hornblende granite in New Jersey (USA), depicted a three to fourfold enhancement in the rate of weathering of those rock surfaces which were covered by lichen as compared to non-covered/barren rock surfaces (Zambell et al. 2012). Chemical and mineral composition of rocks and 'micro-topography' influence the fungal rock colonization. 'Epilithic' and 'endolithic' fungal communities produce several compounds such as carbohydrate and osmolytes in response to 'desiccation' which extend the water retention period, and ultimately results into expanding the degree of chemical reactions responsible for silicate weathering. Moreover, mechanical strength of the hyphae is increased by melanin pigmentation, which is helpful to penetrate the crevices of an rock surface and also, provide shield from metal toxicity (Sterflinger and Krumbein 1997; Gadd 1993). Studies of Puente et al. (2004), revealed the fluorescent pseudomonads and *bacilli* were prominently responsible in the weathering process of limestone, igneous rocks and marble. Moreover, weathering of 'biotite' and 'anorthite' are potentially exhibited by bacteria associated with root and mycorrhiza (Balgoh et al. 2008). Bacteria also produced metal chelating organic compounds 'siderophores', assist in the weathering of iron and magnesium containing silicates (e.g. biotite) (Frey Klett et al. 2007). Bacteria and fungi presented a remarkable increase in the dissolution rates of apatite, feldspar, biotite, quartz and other related minerals (Barker et al. 1997).

5.2 Mineralization, Humification and Soil Aggregation

A process of progressive dismantling of organic material such as fertilizer into inorganic components is known as mineralization. Microorganisms are the major entities that take part in process of mineralization. The process of mineralization can also be influenced by numerous environmental factors such as oxygen availability, pH, temperature and water potential. Mineralization of organic fertilizers into nitrates governed by different microorganisms in three steps process such as aminizations, ammonification and nitrification. Aminization is referred to breakdown of huge and complex forms of proteins into short structural compounds such as amino acids, amines and amides. An organotrophic bacterium such as *Rhodospirillum rubrum* performs aminization by producing an enzyme 'proteases'. However, the process of formation of ammonia through organic compound is known as ammonification. The important bacterial groups that carry out this process include *Bacillus, Streptomyces* and *Proteus.* By mineralization process, microorganisms recycle nutrients in the soil and augments soil fertility and health (Buscot and Varma 2005) (Fig. 2.2). Secondly, 'humification' is a process governs by bacteria and fungi lead to the conversion of dead organic matter (leaves, twig) into humus. Humification influences diverse soil characteristics such as fertility, water availability, pore size and pH of soil. Decayed parts of plants, animal excreted substances containing organic compounds such as carbohydrates, protein, lignin and resin, which used by microbes as energy source and changed into humus. Fat, waxes and lignin are undegradable by many microorganisms but white rot fungi are able to metabolize it, which are the precursor for humus formation. Humus releases nitrogen containing compounds in soil and increases the soil fertility, also increases water holding capacity through increased porosity (De Macedo et al. 2002), which leads to the well soil structure and recycle nutrients in the environment. It enhances the cation exchange capacity of soil and thus increasing nutrients chelation activity (Szalay 1964). In the process of humification microorganisms produces mucilaginous substances, which increases soil adhering capability as well as allows better aeration (Huang et al. 2008). Therefore, microorganisms exhibit a key role in humification process and hence maintain structure, health and fertility of soil (Figs. 2.2 and 2.3).

Another important aspect is 'soil aggregation' where soil particles reside together in the form of stable clumps because of moist clay, gums, organic matter and fungal hyphae. Soil containing plenty of aggregates is called well-aggregated soil and is an important marker for soil health and environmental sustainability since soil aggregation stabilizes organic material, improve water holding capability, nutrients and air within soil micro-sites (Balesdent et al. 2000). A number of study exerted that microorganisms produce various sticky substances that can interact with soil particles (organic material, clay material and polyvalent metal) and leads to the development of soil aggregates (Davinic et al. 2012). Chenu (1993) reported

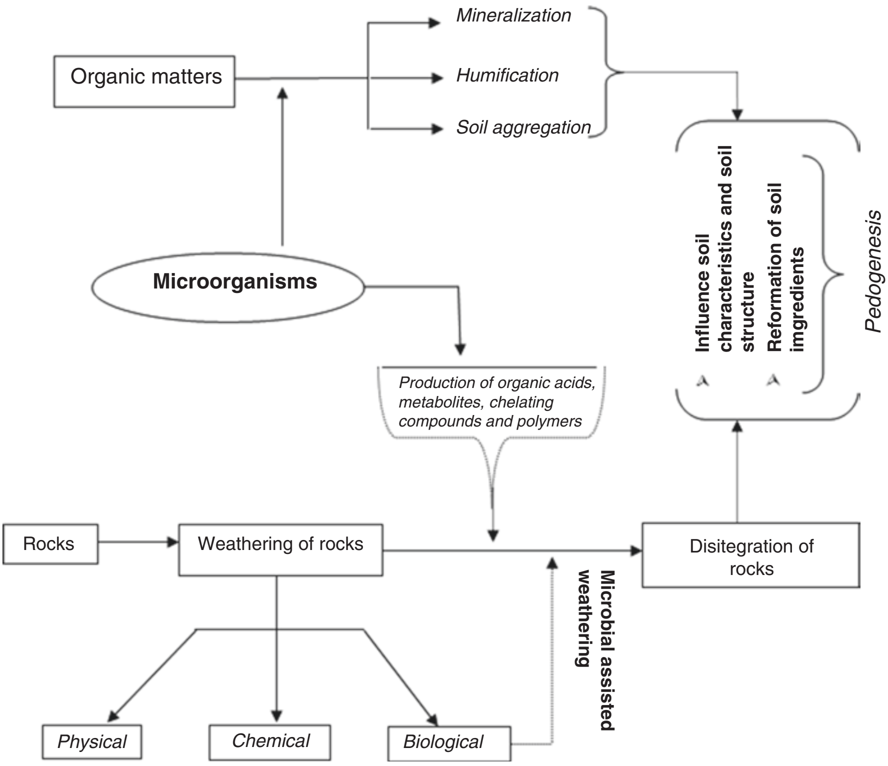

Fig. 2.3 Microbial mediated steps of organic matter decomposition and weathering of rocks for pedogenesis

increased water holding ability of soil aggregates due to addition of polysaccharides. Furthermore, bacteria have the ability to develop electrostatic charge, which holds small aggregates of soil together (Kallenbach et al. 2016). Fungi forms long hyphae which help in aggregation of soil by cross linking between soil particles and hence length of the hyphae is related to soil aggregation (Davinic et al. 2012). Moreover, arbuscular mycorrhizal fungi produce a glycoprotein ‘glomalin’ contain the trait of gluing the soil particle which leads to the phenomenon of soil aggregation (Chotte 2005).

5.3 Microbial Metabolism in Soil

Soil has been declared as porous medium comprising of organic materials, variety of minerals, water, gases and numerous communities or varieties of microorganisms. Diverse forms of microorganisms reside in soils present various kinds of cellular metabolism that ultimately influence the soil environment. Microbial metabolism is the process through which microorganisms uptake source of energy and nutrient, which are needed for their survival and reproduction process. Most of the soils broadly can be divided into two categories; (a) A mineral soil contains less than 20% organic carbon and, (b) an organic soil possesses at least this amount. The significance of organic matter within soils cannot be underrated. Soil organic matter (SOM) is an important source because it assists to retain the nutrients, holds the improved water capacity and maintains soil structure. Microbial mediated degradation of plant materials results into the evolution of CO_2 and the incorporation of the plant used carbon into additional microbial biomass. On contrary, a small fraction of the decayed plant material remnants in the soil medium as soil organic matter. Nitrogen (N) is another important element of the soil, but most of the soils are nitrogen deficient. Therefore, each year tons of nitrogen fertilizer is applied to agricultural field which consequently led to the soil highly polluted. Soil nitrogen is often considered in relation to the soil carbon content like the organic carbon to the nitrogen ratio (Nannipieri et al. 2017). The Nocardioforms, Coryneforms and the true filamentous bacteria such as Streptomycetes are important constituents of microbial community in soil (Madigan et al. 2011). These gram positive bacteria take part in the degradation of the hydrocarbons and additionally some members of these bacterial groups actively participate in degradation of pesticides and its residues (Quejigo et al. 2018). The filamentous actinomycetes, mainly of the genus *Streptomyces,* produce an odor causing compound called ‘geosmin’, which confers soils their characteristic odor. Supply of essential elements such as carbon (C), oxygen (O), hydrogen (H), nitrogen (N), phosphorus (P) and sulphur (S) from the soil are required for the growth of all living organisms. In soil the ‘recycling’ of these elements is a key process and also the basic measure to avoid exhaustion. Soil microorganisms take part in breaking down of dead organic matter and their conversion in to the forms which could further be utilized by other organisms. In this context, microbial enzymes act as key ‘engines’, and dominantly assist in driving the bio geochemical cycles (Falkowski et al. 2008). The carbon cycle globally is dominated because of the equilibrium between photosynthesis and respiration process. The shifting of carbon from the atmosphere to soil carried out by ‘carbon fixing’ autotrophic organisms, principally plants and as well ‘photo’ and ‘chemoautotrophic, microorganisms that produce atmospheric carbon dioxide (CO_2) into organic matters. The fixed carbon is then reverse to the atmosphere through employing various pathways that account for the process of respiration of both types of microorganisms i.e., autotrophic and heterotrophic microorganisms. Numerous environmental factors including mineral nutrients, available water content, carbon and energy sources, ionic composition, pH, temperature, oxidation-reduction potential, spatial relationships, relation/interaction between microbiota and genetics of the microorganisms show their influence on the natural environment, activity and as well as population dynamics of microorganisms in soil (Nannipieri et al. 2017).

5.4 Microorganisms in Polluted Soil

Presence of toxic chemicals and other pollutants in the soil is in elevated concentrations is accountable for polluted soil, to be of hazard for plants, wildlife, humans and the soil itself. Because of the polluted soil, most of the arable land of world is turning to desert land and becoming non-arable at continually increasing rates, mainly in part affected from global warming and due to rampant application of agricultural fertilizers and pesticides. Soil pollution can exert a number of harmful effects on ecosystems and human, plants and animal health also. In bulk soils, some of the heavy metals severely affect the growth, cellular architecture and the metabolism of microorganisms, where heavy metal cause several functional disorder, which causes denaturation of protein or the disintegration of cell membranes (Lei-ta et al. 1995). However, the polluted soil become enrich with specific microorganisms which particularly degrade the pollutant and convert into non-pollutant or less harmful matter. Polluted soil is the specific site for those microorganisms who have adapted towards several environmental pollutants in form of heavy metal, pesticides and other chemicals. Therefore, polluted soil is a fine milieu for exploring the effect of environmental pollution on community structure of microorganisms, microbial biomass, and enzymatic activities of microorganisms (Kandeler et al. 2000). The environmental pollutants present in the soil, alters the soil composition and pH of the soil which directly influence the growth of specific microbiome which can adapt or survive in the conditions of heavy metal stress, salts and other extreme conditions due to the presence of pollutants. Depending on their concentrations, these pollutants can have devastating effects on ecosystems, as well as cause severe damage to humans and other animals. Microbial wealth of soil is generally considered synonymous to higher bio-availability of nutrients, and therefore soils are biologically more reactive to any management input(s). Microorganisms are the eminent natural agents for pursuing biodegradation reaction of several toxic compounds (such as pesticides, polycyclic aromatic hydrocarbons and munition wastes etc.) and remediate soils effectively. Numbers of bacterial classes such as gamma proteobacteria (gr. *Pseudomonas*, *Aerobacter*, *Acinetobacter*, *Moraxella*, *Plesiomonas*), betaproteobacteria (*Burkholderia*, *Neisseria*), alphaproteobacteria (*Sphingomonas*), *Actinobacteria* (*Micrococcus*), and *Flavobacteria* (*Flavobacterium*) are considered to be dynamic microbial bio-degraders (Mamta and Khursheed 2015). Several petroleum compounds show the health hazards to humans and other animals. Study of Mirdamadian et al. (2010) revealed that microorganisms namely *Bacillus* spp., *Rhodococcus* spp., *Pseudomonas* spp. and *Micrococcus* spp. were found to have capacity to degrade petroleum compounds.

6 Molecular Approach in Soil Microbiology

Soil presents the renowned hub for the range of microbial diversity and contained with various biospheres where microorganisms perform several metabolic activities and maintain the soil characteristics. To study the soil microorganism's dynamics, modern molecular approaches have been opened vast ways to explore the numerous diversity of microorganisms present in the environment, in which most of the organisms are non-cultivable due to its unknown growth requirements. Some advanced techniques are mentioned in Fig. 2.4 for characterizing the microbial diversity and

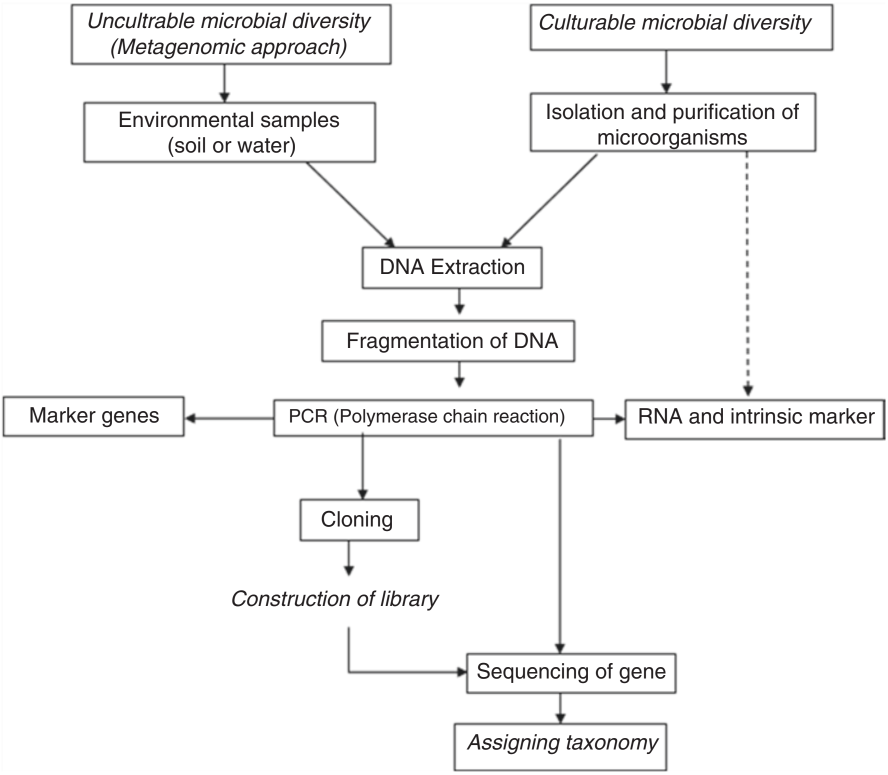

Fig. 2.4 Molecular techniques for determining culturable and unculturable microbial diversity

richness of culturable and unculturable microorganisms (Fig. 2.4). These microbial diversity take part in soil related functions such as decomposition of organic matters, nutrients recycling, production of organic acids and other related function lead to the soil genesis.

6.1 Marker Gene

In phylogenetics, group of orthologous genes can be used to define between taxonomic lineages as marker genes. Marker genes are genetic trait that can be detectable or a particular DNA segment that can be identify and track in the genome. Marker genes can serves as standard genes for another gene of interest called the target gene (Ren et al. 2016). These assets of the marker genes can either encoded by DNA and it can be detectable upon gene expression or be contained by a DNA itself. Generally two kinds of marker genes are presents i.e. intrinsic marker genes and recombinant marker genes. Intrinsic marker genes are naturally present within the genome of an organism such as *rrna, pmoA, narG, mcrA, chiA* etc. They are generally used for structural and functional diversity studies and examination of biofilm architectures (Jansson et al. 2000, Prosser 2002). The expression of intrinsic marker genes can be studied by detecting transcripts of these genes in nucleic acids, which are directly extracted from soil. Recombinant marker genes are those genes which are placed into an organism by utilization of techniques of genetic engineering. These are used for *in situ* gene transfer and their examination of growth survival and their activity in different environmental conditions. The fate of the microorganisms in the soil and the expression of selected activities in complex microbial communities can be studied using recombinant marker genes. Chromogenic marker genes, fluorescence marker genes, antibiotic resistance gene etc. are some examples of recombinant marker genes which directly or indirectly influence the different stages of soil genesis.

6.2 RNA and Intrinsic Marker

The genes encode for the ribose nucleic acids (RNA) especially its small subunit (SSU) is the important marker used in environmental microbiology. These SSU acts as intrinsic markers that are present in living microorganism, as they all possess the ribosome. Ribosome contains highly conserved sequences and participates in protein biosynthesis. However, presence of such highly conserved sequences in the RNA, certain part of the gene encoding this contains the highly variable segments that doesn't perform any functional role, rather can be act as intrinsic marker (Tourasse and Gouy 1997). This variability in the gene segment involved in tracing the biological evolution and phylogenetic relationship. The importance of rRNA encoding genes was recognized by Woese and Fox (1977) mainly for building the universal tree of phylogeny for all forms of life. In one of the pioneer study, fluorescent labeled rRNA targeted oligonucleotide was used for the detection of predominant genotype which is independent of cultivation by means on *in-situ* hybridization (Pace et al. 1986). The specificity of the different gene probe enables detection of different phylogenetic groups or species quantification without the desired cultivation. In addition, biofilms producing ability and symbiotic association tendency can easily be studied with this intrinsic marker. The SSU rRNA gene with an optimum size of 1540 nucleotide is more suitable for ecological study. In previously published literature symbiotic bacteria from marine ecology was successfully characterized by direct isolating and sequencing the 5S RNA from the larger subunit of prokaryotic RNA (Amann and Ludwig 2000). Thus all literature strongly supports the use of RNA as an intrinsic marker and can be best studied with polymerase chain reaction easily.

6.3 Cloning

Past the down to earth inquiries of the ways to improve vectors for library development and the ways to expand profitable existing libraries, a specialized inquiry that we find especially fascinating: what amount of the arrangement decent variety show in unique DNA separates is caught in built libraries, and what influences this? Despite the fact that less a worry for useful display, it is important to include the elements which impact library representativeness; explaining these elements may prompt improvement of effective procedures for getting maximum capacity of natural metagenomes. Already utilized shotgun sequencing to look at inclination in a fecal library of human (Lam and Charles 2015) likewise show the after effects of 16S rRNA quality sequencing to inspect predisposition in soil library of corn field (Cheng et al. 2014). Investigation at the phylum-level demonstrated that in spite of the fact that the fecal library contrasted significantly in the relative plenitude of phyla contrasted with its comparing extricate, the relative wealth of phyla in the soil library of corn field appeared to be like its concentrate. The abnormal state of host sullying could be because of special intensification of layout amid PCR in light of contrasts in DNA adaptation: however display in little amounts, straight DNA might be all the more effectively opened up finished supercoiled DNA or shut roundabout plasmid DNA (Chen et al. 2007). The way that specific taxa are under or over represented, won’t not represent a boundary to screen, but rather it helpful to realize which arrangements are not prone to caught under libraries. An investigation which have contrasted shotgun sequences of unique examples relating with marine water metagenomic libraries (Temperton et al. 2009) have demonstrated AT-rich groupings are represented in libraries. The particular components influencing the “clonability” of DNA, and the systems that prompt DNA prohibition, still should be tentatively decided. The steadiness of outside DNA in *Escherichia coli* is affected by duplicate number of vector and, therefore, single-duplicate fosmids might be perfect as library spin, in spite of the fact that the achievement of some utilitarian screens might be subject to a higher quality measurement. Plasmid vectors those are not cos-based give an elective where cloning is significantly not so much troublesome as extensive section DNA require not be segregated and bundling and transduction are not required; the weaknesses, in any case, are that a littler embed measure implies that bigger operons won't be unblemished, and if the plasmid retain a big duplicate number—valid for customary cloning vectors—it may prompt more noteworthy embed unsteadiness and prohibition.

6.4 Metagenomics

The evolutionary history, functional and ecological biodiversity can be understand by genomic analysis of complex environmental samples instead of laboratory cultivation and/or isolation of individual specimens. Examples of environmental samples include soil, water, sediments, passively collected aquatic, terrestrial and benthic specimens, gut contents and faeces. More than conventional Sanger DNA-sequencing technology, the advanced technique of Sanger DNA-sequencing technology led to large-scale, broad-scope biosystematics projects with wide applications like barcode of life initiative (Hajibabaei et al. 2007). The primary purpose of identification of unknown specimens are to generate vast DNA libraries which can be employed by DNA barcoding which further enrolls standardized species-specific genomic regions of DNA. For example, across Animilia, the cytochrome c oxidase subunit I (COI) gene region is capable of discerning between closely related species (Hebert et al. 2003). Likewise, 16S ribosomal RNA (16S rRNA) sequences is generally used for identification of bacteria (Welch and Huse 2011). However for fungal studies, the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA is employed (Nilsson et al. 2008). While, the regions of plastid DNA including maturase K (matK) and ribulose-bisphosphate carboxylase (rbcl) required for the Plant DNA barcoding (Burgess et al. 2011). For biodiversity analysis, a number of other marker genes have been employed at different phylogenetic depths or in taxonomic groups. Furthermore, for processing of complex environmental samples, the traditional DNA-sequencing method cannot be used because it can sequence specimens individually (Shokralla et al. 2012) and all samples often contains mixture of DNA of individuals present in soil. The conventional sequencing method is most efficient for the development of huge DNA barcode reference libraries but due to higher number of individuals from environmental sample is afar the scope of its capability. But by the use of next-generation sequencing (NGS) technologies, it is very much possible to recover DNA sequences from huge number of specimens from bulk samples of an environment which have the capability to read DNA, parallel from multiple templates; something that do effectively and with ever-lowering costs (Hajibabaei et al. 2007). These NGS technologies have huge potential and can generate parallel millions of sequencing reads (Esposito et al. 2016). This type of sequencing capacity generates numerous sequence reads from the fragmented library of a specific genome (i.e. genome sequencing) from a pool of cDNA library fragments generated through reverse transcription of RNA like RNA sequencing or transcriptome sequencing or from PCR amplified molecules like amplicon sequencing. These all generated sequences are without the need of a conventional method or vector-based cloning approach that is not easy to amplify and distinct DNA templates (Shokralla et al. 2012). NGS technologies based upon the diverse chemistry which includes base incorporation/detection tools and there are two main steps such as: 1st is amplicon library preparation and 2nd is diagnosis of the integrated nucleotides (Glenn 2011; Zhang et al. 2011). NGS technologies can be classified into two major groups in which one is PCR based and another one is non-PCR based. One of the groups are PCR-based technologies, includes four commercially available platforms: HiSeq 2000 (Illumina Inc., San Diego, CA, USA), AB SOLiD™ System (Life Technologies Corp., Carlsbad, CA, USA), Roche 454 Genome Sequencer (Roche Diagnostics Corp., Branford, CT, USA), and Ion Personal Genome Machine (Life Technologies, South San Francisco, CA, USA). The second group, called 'single molecule' sequencing (SMS) technologies, are non-PCR based and do not include an amplification step prior to sequencing. Two single-molecule sequencing systems have been recently announced: PacBio RS SMRT system (Pacific Biosciences, Menlo Park, CA, USA) and HeliScope (Helicos Bio-Sciences Corp., Cambridge, MA, USA) (Shokralla et al. 2012). In recent years, mass sequencing techniques of environmental samples has been utilized in full swing for ecology and biodiversity research. By the use of NGS technologies, analysis of environmental samples from various ecosystems like marine, terrestrial, freshwater, gut microbiota and soil can be performed (Buée et al. 2009). The numbers of studies solicit to reply the query that which type of flora and fauna are adjacent in environmental area of interest. By the use of sequence data processed by NGS techniques, researchers have been able to observe the modest changes in community structure and dynamics which may occur due to natural environmental fluctuations or anthropogenic sources (Fierer et al. 2007). The numerous studies have examined soil bacterial diversity by analyzing 16S rDNA amplicons (Singh et al. 2012; Rousk et al. 2010). Results of these studies suggest that agricultural management of soil may significantly influence the microbial diversity (Roesch et al. 2007). There are numbers of other studies which mainly focused on fungal diversity of soil in forest and agricultural system by examining ITS amplicons (Acosta-Martínez et al. 2008). The selected functional gene amplicons or total RNA serve as one of the alternate approach to target soil microbiota (Fierer et al. 2007; Leininger et al. 2006).

6.5 PCR

PCR (Polymerase chain reaction) based molecular approaches deliver a rapid and sensitive substitute to conventional culture techniques. PCR-based fingerprinting techniques comprised of three important steps: a) the nucleic acids extraction b) the rRNA/rDNA amplification c) using fingerprinting techniques analysis of PCR products (Agrawal et al. 2015). PCR-based 16S rDNA sequence profiles of an organism gives information regarding microbial diversity, identification and the phylogenetic relationship predictions (Pace 1997). So, various 16S rDNA-based PCR techniques such as DGGE (denaturing gradient gel electrophoresis), ARDRA (amplified ribosomal DNA restriction analysis), TGGE (temperature gradient gel electrophoresis), T-RFLPs (terminal restriction fragment length polymorphisms), SSCPs (single-strand conformation polymorphisms) and RISA (ribosomal intergenic spacer analysis) can give deep information about community dynamics and composition of an ecosystem in terms of richness, evenness and can be utilized to compare diverse species exist in an environmental sample (Agrawal et al. 2015). Another PCR technique for instance ‘qPCR’ is relies on the real-time recognition of PCR product by reporter molecules, which flourish upon amplification as PCR product amplified in every amplification cycle. qPCR technique is rather distinctive among other methods of community analysis dynamics, in that it allows to produce comparative and fast quantitative estimation of the availability of exact phylogenetic groups of microbial populations present in soil of interest (Fierer et al. 2005).

7 Conclusion

Microorganisms are ubiquitous and present everywhere and even in extreme environments, and carry out various significant functions. Wide diversity of microbial organisms and their particular habitat present a suitable sphere where microorganisms play important role in different process such as recycling of elements and other activities. Pedogenesis is the process of soil formation from rock weathering and other aspects such as organic matter decomposition on earth surface. Besides soil degradation, the reformation of soil is an important criterion which maintains the soil ecosystem and sustains the life of approximately all living organisms. Microorganisms take part in weathering of soil via synthesis and secretion of numerous organic acids and decomposition\partial organic matters decomposition through multiple enzymatic and other processes for releasing the inorganic minerals and provoking soil structures formation. Soil is hub for microorganisms as ‘natural engineers’ which carry out the several important processes for maintenance of nutrient cycling and essential mineral (such as phosphorus and nitrogen) transformation for maintaining the integrity of nutrients in soils. Recycling of important elements in soil through microbial assisted activities promote the sustainability of earth inorganic and organic matters and thus maintains the soil structure and its multifarious properties. The decaying plant or animal residues/parts ultimately helps in formation of upper portion of soil and this unique consequence is met through the activities of soil microorganisms. Soil microbiomes also participate in humification, aggregation and stabilization of soils and hence promote the fertility of soils which directly related to improved plant growth and provoke various magnitudes of microbial related activities. Soil also presents the diversity of both culturable and unculturable microorganisms which are important in formation of new soils from recyclable materials, and the study of such microorganisms opens the doors for several scientists worldwide to explore and investigate the molecular techniques for identifying total microbiome of particular site or sphere.

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