Chapter 11
Microbial Consortia and Their Application for the Development of a Sustainable Environment

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Abstract Abundant diversity of microbial communities existing in nature follows the rule of coexistence demonstrating interspecies and interkingdom interactions for their proliferation. These communities exhibit highly complex and interwoven web of metabolic communications. They also perform division of labor managing their metabolic load for the survival of different co-inhabitants of that area. These microbial consortia are involved in various interactions such as mutualism and syntropy, commensalism, and antagonistic behavior as a part of their struggle for existence. Due to their unique properties of division of labor, diversified metabolic capacities, and ability of co-survival, microbial consortia can be exploited for approaches applied for maintaining sustainability of the environment. They can be utilized in agricultural practices that can reduce the use of chemical fertilizers and pesticides. Microbial mixed cultures also have potential for waste management and bioremediation. Additionally, they can prove to be an attractive alternative as a source of biofuel and bioenergy generation.

Keywords Arbuscular mycorrhizal fungi (AMF) · Microbial consortia · Microbial interactions · Plant growth-promoting rhizobacteria (PGPR) · Soil ecosystem

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11.1 Introduction

Due to enormous human activities which have caused massive impact on environment, its sustainability has become a matter of great concern. Increased use of chemical fertilizers and pesticides has created questions regarding sustainability of soil ecosystems. Increasing efforts are being made to develop such processes which can lead to sustainable agriculture practices. Along with that, there is an increasing awareness about solving pollution issues through sustainable approach. Additionally, bioproduction sector also has their own problems regarding the sustainability of processes, which can cause economic load on the industries. Microbial consortia can prove to be an attractive alternative to solve these issues.

Microorganisms are the most diverse forms of living beings that thrive in any ecosystem ranging from extreme environments to water and soil. In all these ecosystems, different microbial communities flourish simultaneously creating complex web of biochemical interactions that function as a key to ecological architecture. Enormous diversified species in large number forms complex interactive webs of metabolic activities in natural ecosystems such as soil, gastrointestinal system, etc. (Shahab et al. 2020). Hence, it leads to a fact that “Mother Nature” has designed the microbial existence as a large consortium.

Microbial consortia refers to the coexistence or co-cultivation of two or more species in terms of growth, metabolism, and division of labor, creating conditions for their own composition (Jia et al. 2016). Even though, in recent times, microbial consortia have attracted the attention of bioprocess engineers by offering certain advantages, it was also used in the ancient eras, from wineries in Africa to biogas generation from undefined microbial mixtures used for heating bath in Assyria (Peng et al. 2016). These multiple microbes together possess the capacity to perform difficult metabolic tasks and provide a miraculous outcome. It is advantageous over single species-operated bioprocesses in certain aspects. Firstly, combining suitable species can fulfil complex tasks without much difficulty. Secondly, co-adaptability of multiple strains leads to co-stability and can resists environmental fluctuations. This is because of their internal beneficial interactions (Nuti and Geovannetti 2015). Additionally, metabolic requirements of different species can be fulfilled by the fellow microbial members in the consortia. This occurs when a metabolite produced by one member of consortia is utilized as limiting nutrient for other species, thereby manifesting complete mineralization of the components along with favoring their coexistence. Also, through gene regulation via quorum sensing, these microbial consortia demonstrate multiple unexplored biochemical mechanisms that contribute in their functionality (Odoh et al. 2020).

There is an enormous array of mechanisms that occurs continuously among the members of the consortia which ranges from highly beneficial or synergistic to extreme damaging or antagonistic. These interactions occur as a part of processes microbes develop while thriving for their existence in the natural environment, while dealing with space limitations, nutrient depletion, and other natural or co-inhabitant generated stressful environment. Hence, these interactive mechanisms can be studied and exploited as a tool for the search of newer metabolites, novel enzymes, and other components. Furthermore, study of these interactions can provide an insight into gene regulation which assists in altering the production yields of certain components at the industrial level.

Microbial consortia can be categorized broadly in two types: (1) natural communities and (2) man-made co-cultures (Canon et al. 2020). The former types are present in the natural ecosystem and are known to establish a spontaneous association where the dynamics are governed by the laws of natural selection. They are substantially complex, and the species flourishes according to the release of metabolites by one member which can be subsequently utilized as substrate by other members of the community. Natural communities are employed in number of processes such as methane production in activated sludge process, fermentation such as cheese or kimchi production, as well as waste management (Canon et al. 2020). The second type of microbial consortia includes artificially mixed cultures as well as synthetic co-cultures that are developed by human conciliation. They are customized mixtures framed according to the process requirements. Hence, it can be deciphered that these mixtures of microorganisms hold enormous applications in variety of fields which include agriculture (Sekar et al. 2016), bioproduction (Canon et al. 2020), bioremediation, and even in waste management (Al-Dhabi et al. 2019). In addition, the potentiality of these microbial consortia had recently opened up the gates for a newer aspect of synthetic biology that deals with designing and analyzing synthetic microbial consortia, having applicability in the field of bioprocessing. They are also added as a part of ingredients which are supplied as plant bio-stimulants (Woo and Pepe 2018).

Traditional bioprocesses are focused on exploiting the potentiality of monoculture for fermentation; however, when it comes to complex biotransformations, the mentioned approach faces some of the drawbacks, namely, extensive metabolic burden, cellular space limitation, and toxic intermediates (Shahab et al. 2020). This has diverted microbial engineers toward exploring the possibilities of utilizing microbial consortia to get the job done.

11.2 Microbial Interactions

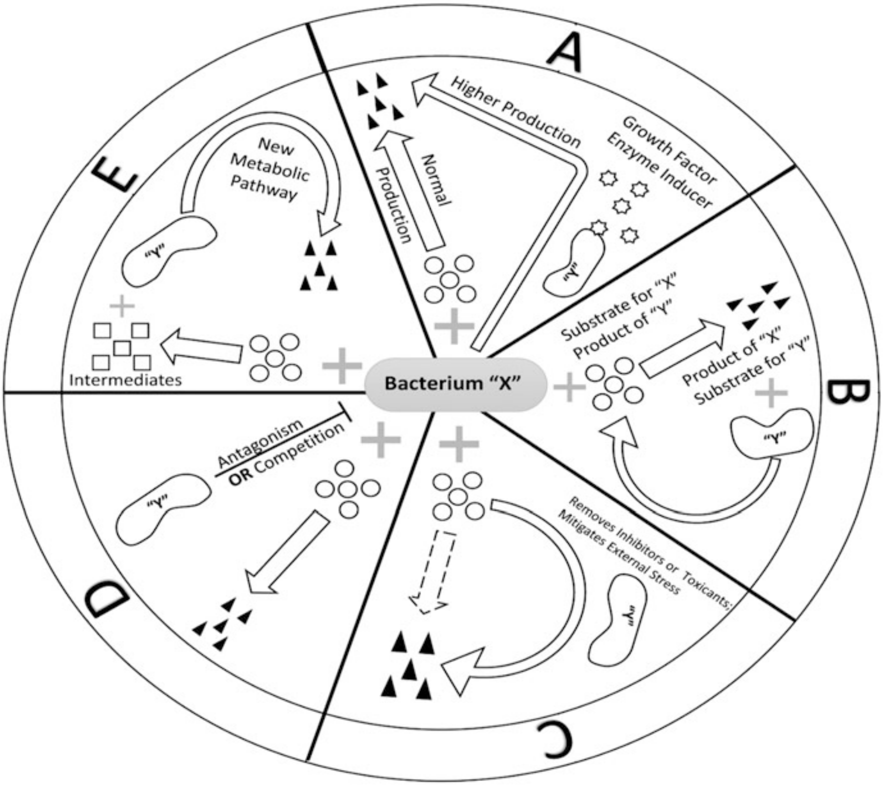
Cellular interactions among microbial species are ubiquitous in nature and are prime sculptors of ecosystem dynamics (Kong et al. 2018). Interaction between members of microbial consortia, either natural or artificially designed, is a pivotal factor that plays a decisive role in the success of any bioprocess. Furthermore, emergence and sustainability of metabolic interactions depend on factors such as nutrient availability, diffusion constraints, and microbial community spatial structure and structure. In their habitat, even though microbes tend to demonstrate individualism, the consortia respond to environmental stress as a unique organism (Odoh et al. 2020). Positive interactions are mutualistic and improve the fitness among co-cultures and favor product formation (Canon et al. 2020). When microbial species flourish in their natural habitat, they may have to overcome through negative interactions such as competition, parasitism, etc. However, when polycultures are applied purposefully, the designing is done to selectively enhance beneficial interactions, excluding the members involved in damaging mechanisms (Fig. 11.1).

11.2.1 Mutualism

Established as one of the most common interactions found in biosphere, mutualism refers to the interaction in which different species are dependent on each other for fulfilling their nutritional requirements or need for space (Kouzuma et al. 2015). Also termed as symbiotic relationship, such interactions are widespread in nature. The word "symbiose" was coined by De Bary in 1879, for dissimilar organisms that are living together, and his definition covered both mutualists and parasites (Hirsch 2004).

In agriculture, microbial consortia use symbiotic interactions which can contribute for enhancing soil fertility. In soil ecosystem, where bacteria accounts for 95% of the microbial community (Odoh et al. 2020), multiple microbial species colonizes around the rhizosphere either as free living, epiphytes, or endophytes. They establish close interactions with root system which may prove beneficial to plants and microbes both. The signature example of such interactions can be understood in terms of rhizobium-root legume relationship, where rhizobium is involved in nitrogen fixation benefiting plants and in return uses inhabiting space and biochemical metabolites released by plant roots as carbon and energy source. Additionally, there are members of plant growth-promoting rhizobacteria (PGPR) community that is involved in such co-relations (Khan et al. 2020). Members of genera *Bacillus*, *Paenibacillus*, *Burkholderia*, *Azotobacter*, *Rhizobium*, and *Pseudomonas* (Ahmad et al. 2018) inhabit plant rhizosphere where they indulge in diversified mechanism which benefits the plants by solubilization of minerals, production of phytohormones, releasing antagonistic substances that inhibit the growth of pathogenic organisms and many other. In return, they reap the benefits in the form of exudates released by plant roots as their nutrient and energy source (Menéndez and Paço 2020), have termed beneficial bacteria of rhizosphere as plant probiotic bacteria (PPB), and have classified them into two subgroups: (1) rhizobial bacterial endophytes (RBEs) and (2) non-rhizobial bacterial endophytes (NRBEs). Their studies suggested that those symbiotic interactions between plants and microbes are highly complex, and microbial consortia in the form of PPBs have ensured good results in terms of plant benefits. They have also stated improvement in symbiotic effectiveness between RBEs and NRBEs in the form of consortia along with mycorrhiza increased crop yields and reduced the usage of chemical fertilizers.

Another popular mutualism is demonstrated by mycorrhizal fungal species. They are named as arbuscular mycorrhizal fungi (AMF) and belong to subphyla Glomeromycotina. Under symbiotic association with plants, they acquire lipids and carbohydrates from the plants through special structures such as arbuscules



Bacterium "X"	"Y"	○○○○○	□□□□□	▲▲▲▲▲
The Bacterium present in consortium	Another bacterium from the consortium	Substrate (For Figure part A,C,D,E)	Intermediate	Product (For Figure part A,C,D,E)

Fig. 11.1 Interactive mechanisms observed among the members of microbial consortia: The figure deciphers the release of growth factors from one of the members can help in better utilization of the substrate by other member (Section A). Other possibility is of synergistic relationship where both the species are interdependent for their substrate requirements as shown in section B of the figure. Section C explains the favors extended from one member through removal of inhibitors of the reaction, thereby making substrate utilization possible for co-member. Antagonism is also a major interaction observed in microbial communities in which competition for resources can inhibit other organism or else this can divert the organism to produce novel metabolites for the purpose of their survival (Section D). Also, biotransformations can occur as a result of interactions in which the intermediate produced by one microbe can act as starting metabolite for fellow member as shown in Section E

and vesicles. As a return gift, these fungal species are involved in rapid uptake of plant nutrients mainly phosphorous, nitrogen, and potassium, thereby increasing crop yields. Mixture of such species provides better outputs compared to single mycorrhizal species (Paredes et al. 2020).

In addition to the above examples, one more popularized example of symbionts is lichens. They can be considered as tripartite or quadripartite symbionts which comprise of photosynthetic organisms as cyanobacteria or algae, fungus as another member, and a nitrogen-fixing root symbiont which also includes mycorrhiza (Hirsch 2004).

Kouzuma et al. (2015) have reported findings on specific type of mutualism known as "syntrophy," which is referred to as the interspecies interactions in which tropic benefits are procured by both the partners. They have studied the establishment of interspecies communication in syntrophic microbial consortium related with methanogenesis and stated that the rate-limiting step of methanogenesis is the mutualistic interactions between secondary fermentative bacteria and hydrogenotrophic methanogens in which intercellular exchange of reducing equivalents occurs. In this metabolic event, H_2 is generated by propionate-oxidizing bacteria as a part of energy generation through oxidation of propionic acid. Hydrogenotrophic methanogens trap this hydrogen for methane production thereby reducing the product concentration, favoring the reaction to move in forward direction.

11.2.2 Commensalism

Commensalism is an interaction between microbial communities in which one member termed as commensal can have substances released or captured by the host due to special proximity (Bogitsh et al. 2018). In this relationship, host remains unaffected and releases the exudates as a part of its normal metabolic activities. Microbial communities in the biofilms may be governed by such interactions. Use of high-end technologies such as fluorescent in situ hybridization (FISH) and microelectrodes applications has demonstrated the clustering of nitrate-oxidizing bacteria around ammonia-oxidizing bacteria in nitrite-oxidizing zones in the waste water treatment where nitrifying biofilm is involved (Christensen et al. 2002). Hirsch (2004) has suggested that commensalism has resulted in increased bacterial survival in rhizosphere as well as bulk soil.

Kong et al. (2018) demonstrated ecosystem dynamics of commensalism using synthetically designed two strain consortia. They created one strain CmA from *L. lactis* which had full nisin pathway and constitutively expressed tetracycline resistance. It was co-cultured with CmB which contained nisin inducible tetracycline resistance power. Their studies determined that when two strains were grown as mono-cultures in the presence of tetracycline, CmA, because of its constitutive expression of tetR gene, was able to grow; in contrast, CmB could not grow by itself as its resistance was not self-dependent. However, when both the strains were combined, CmB grew well, suggesting that the presence of CmA benefited CmB for its growth. Studies of detailed mechanism conferred that tetracycline resistance was the mechanism of commensalism for CmB which was achieved using nisin released by CmA as a part of its normal metabolism.

Hirsch (2004) has suggested that microbial interactions such as mutualism, commensalism, and parasitism should be considered in continuum. Mutualists like rhizobium species initially flourish in the soil or plant roots as commensals and can form biofilms on inanimate surfaces. This may be for the survival of the bacterium until the suitable host is found.

11.2.3 Antagonism

Antagonism is the mechanism of causing harm to other organisms primarily for the purpose of self-survival or as a product of normal metabolism. It has a substantial contribution for framing the bacterial community by inhibiting one community while opening the doors for other bacterial niches (Long et al. 2013). Competitiveness between different species in the consortia usually occurs due to nutrient depletion and space requirements which result in the synthesis of secondary metabolites, synthesis of enzymes that can combat the situation or activation, and expression of silent genes present in the genome of the species. Under such situations, microbial residents of the same niche excrete such substances that can cause cell damage or oxidative stress in the surroundings. The target species may generate substances that can combat those stressful situations as a part of microbial defense mechanism (Zhang et al. 2018). Hence forth, these mechanisms can provide enormous potential in search of newer secondary metabolites or study of gene expression and regulation or improvement of product yields. It can also be favorable for combating plant pathogens in agricultural practices.

Maiyappan et al. (2010) have studied the effect of microbial consortia Omega on the growth promotion of black gram and found its antagonistic effect on pathogenic fungi like *M. phaseolina*, *S. rolfsii*, *F. oxysporum*, and *R. solani*. Extracellular antagonistic substances secreted by bacteria in combination with plant exudates may be responsible for keeping pathogens in check. Long et al. (2013) conferred the existence of antagonistic interactions between self-sustaining microbial communities in the microbial mats of hypersaline lake and suggested that antagonism which is driven by chemicals is a major contributor in designing of bacterial communities. They supported their hypothesis by co-culturing assay and determined that 57% of bacterial isolates expressed damaging behavior toward one or more species, while 5% of the isolates showed antagonism toward 80% of the isolates.

11.3 Applications of Microbial Consortia

There are number of important areas where unique potential of microbial consortia is exploited for various aspects. Majorly microbial consortia are used for sustainable agricultural practices (Al-Dhabi et al. 2019; Paredes et al. 2020; Maiyappan et al. 2010), bioproduction processes (Canon et al. 2020; Sabra et al. 2010; Ghosh et al. 2016), and bioremediation strategies (Paerl and Pinckney 1996). Furthermore, they are now being explored as a tool to unravel the secrets of coexistence in microbial ecology as well as synthetic biology (Haruta and Yamamoto 2018).

11.3.1 Sustainable Agriculture

In the wave of urbanization, there has been a drastic increase in the usage of chemical fertilizers and chemical pesticides that has raised serious environmental concerns. Also, many of these components have entered the food chain causing several health issues in the people consuming such food. Overall, such harmful chemicals have entered the water bodies and deteriorated soil quality thereby impacting the biodiversity of that particular habitat. Microbial consortia can prove a remedy to such problem providing an eco-friendly alternative (Sekar et al. 2016; Paredes et al. 2020).

Plant growth depends on the availability of various nutrients such as nitrogen, phosphorus, and potassium available in solubilized form as well as growth-promoting hormones, namely, indole acetic acid, gibberellic acid, and others. One of the prime characteristics of microbial consortia is to act synergistically among each other and to secret the components that support plant growth promotion. Plant growth-promoting microorganisms are heterogenous flora encompassing diverse species including bacteria, actinomycetes, fungi, etc., demonstrating cross kingdom interactions. Additionally, those pool of microbes provide immunity to plants by keeping phytopathogenic organisms at bay through various antagonistic mechanisms.

Maiyappan et al. (2010) have isolated, identified, and evaluated the plant growth-promoting efficiency of nine stains of microorganisms that were the members of *Bacillus* sp., *Streptomyces* sp., *Azotobacter* sp., and *Fruateria* sp., developed a consortia Omega, and studied its effect of plant growth of black gram (*Vigna Mungo* L.). Pot culture studies demonstrated significant increase in shoot length of the plant compared to chemical fertilizer-added plants and control plants. Also, the root volume as well as total dry mass was higher in the consortium-fed plants compared to chemical fertilizers.

The potentiality of *Streptomyces* sp. associated with tomato plant roots and the effect of microbial consortia developed from composting the waste were studied by Al-Dhabi et al. (2019). They stated that use of such approach increased the tomato weight up to 15%. The presence of phytohormone such as indole acetic acid and siderophores was also analyzed.

Sekar et al. (2016), Woo and Pepe (2018), and Vishwakarma et al. (2020) have summarized outcomes of various studies carried out in the field of analyzing effects of PGPR and microbial consortia on plant growth and agricultural practices. They have stated that co-inoculation of rhizobia with other microbial species and PGPR proved to be favorable for nodulation as well as below-ground and above-ground development of plant species. Furthermore, such consortia were able to reduce the occurrence of as well as severity of certain plant diseases. Inoculation of AMF (arbuscular mycorrhizal fungi) along with PGPR increased plant yields both in nursery and field studies. They have also suggested that application of microbial cocultures can provide a solution to alleviate plant abiotic stress and enable more growth and productivity of plants under stressful environment such as high salinity, dryness, and rising temperature. This approach can improve the deteriorated soil quality thereby increasing crop production that can combat food shortage in the future.

However, the influence of management practices in agriculture decides the direction of the crop productivity as plants are major selectors of the specific taxa in their phyllosphere. In addition to microbial inoculants, practices such as organic farming, crop rotation, intercropping, automation, and technology implementation are key co-factors that determine the sustainability of agriculture.

11.3.2 Waste Treatment and Pollution Control

A major challenge the world is facing in the present time is rotten fruits of industrialization, in the form of pollutants that has accumulated in soil, water, and air. This is causing serious threat to the sustainability of environment along with risking human health. The processes used currently for reducing toxic compounds involves burning, recycling, land-filling, and pyrolysis. However, such processes are releasing even more toxic and non-degradable compounds in the environment alongside creating difficulty in execution for environmental decontamination (Ahmad et al. 2018).

Microbial communities as weapons of green technology are more efficient in metabolizing chemically complex and toxic metabolites compared to single species. The explanation of this potential of co-cultures lies in the enormous interactions occurring among the multiple species in which metabolite released by one species can be utilized by other co-inhabitant of the community. Hence, this capability of microbial mixed cultures can be utilized in bioremediation of complex polluted sites such as dye polluted water, heavy metal polluted sites, oil spilled sites, and many others (Ahmad et al. 2018; Ghosh et al. 2016). Furthermore, artificial designing of ecological niche is a newer concept emerging in this area where resource specific niche can be sculptured, thereby achieving desirable biotransformations of the target substrates (Shahab et al. 2020).

11.3.2.1 Dyes

Dyes are present in industrial effluents discharged from textile industries. Dyes comprise of recalcitrant compounds, mainly azo dyes that are hard to degrade due to their strong bonding capacity, causing serious concerns. There are certain bacterial species that produces enzymes such as azo-reductase that can act upon azo dyes. Some species produce enzymes that can degrade the intermediate components formed during azo dye reduction reaction. There are number of reports and studies proving potential of different microbial species including fungi, algae, and bacteria for dye degradation and other such xenobiotics excreted in the effluents of paper pulp industries (Ahmad et al. 2018).

11.3.2.2 Organic Domestic Wastes

Domestic waste generated in households also covers a major section of the waste generation. The usual procedure applied for such waste disposal including dumping on landfills which can contaminate the soil of that site in a long run. Household-generated waste is a rich source of organic nutrients, and if treated with microorganisms, they can be transformed into biofertilizers and source of biofuels or animal feed. Microbial communities have physiological diversity that can be manipulated and designed for the customized treatment of such organic wastes which can reduce the treatment time and provide with desired output in waste management.

Sarkar et al. (2011) have developed eleven different consortia which were analyzed for their capacity to produce suitable cocktail of hydrolytic enzymes, namely, amylase, lipase, protease, and cellulase, that can breakdown those components present in kitchen wastes. In their studies, they found effective reduction of kitchen waste in reduced time span, that too without the generation of foul smell.

11.3.3 Bioenergy Generation

Biofuels and bioenergy resources such as bioethanol and biogas are equally important options for sustainable energy generation alongside the renewable energy sources like wind energy, hydro energy, and solar energy. These biological processes depend on the biochemical activities performed by diverse species of microorganisms. As lignocellulosic wastes are major substrate used for biofuel production, monoculture of microbes cannot degrade them efficiently. A single bioreactor equipped with correct combination of microbes can convert complex feedstocks to simpler sugars that can be utilized for the production of bioenergy. For example, when pair of *Bacillus* and *Clostridium* sp. were co-cultured in a bioreactor on lignocellulosic wastes, *Bacillus* sp. produced hydrolytic enzymes that acted upon complex polymeric substrate and released simple sugars which were further utilized by *Clostridium* sp. for efficient generation of hydrogen (Ghosh et al. 2016).

Methanogenic microbes demonstrate a type of mutualistic relationship termed as syntropy, where reducing equivalents such as formate and hydrogen are transferred between syntrophic partners. Microbial species involved in such communication have developed mechanisms at molecular level for the establishment of interspecies partnership. There are evidences that suggest that these transfers can occur in the form of electric current which can be exploited for bioenergy generation (Kouzuma et al. 2015).

11.4 Future Aspects

Deciphering the mysterious potentials of microbial consortia has opened new avenues for its applications in the areas of applied microbiology. Tailor-made designing of microbial co-cultures has enabled the bioprocesses to achieve custom-made desirability; hence continuous attempts are carried out for upgradation of such systems for achieving increased product yields. Engineering of microbial consortia has lot a greater number of future prospects in environmental restoration, in improvement of soil and water quality, as well as in biosystem analysis and industrial biotechnology. Synthetic microbial consortia can be applied in unraveling the intricate mechanisms involved in gene circuits and quorum sensing. Microbial consortia engineering and in silico approach hold huge potential that can reframe ecological niche and can provide novel biomolecules for better environmental sustainability and ecosystem management.

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