



Plant growth promoting microbes: Potential link to sustainable agriculture and environment



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ABSTRACT

The host-microbe interaction established through natural evolution in plants sets the basis of plant growth and productivity benefits from a single microbe or a consortium. Select algal, fungal, bacterial (particularly LABs), actinomycetes and yeast groups are potential effective microbes (EMs) in agriculture. As environmental probiotics, EMs help in crop growth and wellbeing by fixing N₂, solubilising K and P, unlocking soil trace elements, secreting exopolysaccharides, transforming organic matter into usable nutrients, enhancing soil water-holding capacity and improving the overall soil health. They also release bioactive compounds like vitamins, hormones and enzymes to stimulate plant growth, secrete biocontrol agents and enhance drought tolerance. As the soil profile and its intrinsic microbial ecology along geographical regions vary, a universal 'one-size-fits-all' effective microbial formulation cannot be envisaged. Although minerals cycling is majorly accomplished by specialised microbes, their activity is positively or adversely impacted by the interacting biotic community (microbe-microbe, microbe-plant or microbe-animal/human) and abiotic constituents. Crops benefit by positive associations with beneficial microbes as mutualism, symbiosis, commensalism, amensalism, photocooperation, etc., and by negative association against the harmful microbes like antagonism. The plant-growth-promoting role of microbes and the scope to formulate EM are the basis on these facts. EMs are applied for crop growth in the form of consortia which usually include phototrophs, lactic acid bacteria, actinomycetes, fermentative fungi, yeasts, etc. along with an effective carrier substrate (prebiotic) as formulations. The rDNA technology in plant growth promoting microbe (PGPM) with enhanced performance is also discussed. The review provides an insight into the dynamics of ecological interactions as the guiding principles of EM formulation with respect to plant growth promoting microbes, and the beneficial roles of effective microbial formulations in crop wellbeing and productivity enhancement.

1. Introduction

With the onset of green revolution in the early 70s through the use of chemical fertilisers and pesticides, the agricultural productivities have appreciably increased to feed the suffering population in recent times. As a result, global agricultural practices are primarily controlled by synthetic chemicals these days. Continuous and indiscriminate use of chemicals poses a negative impact on the soil, the environment, and human health, in the same sequence. Excess use of chemical fertilisers causes soil pollution and gradually deteriorates the soil fertility. Excess accumulation of such chemicals in crops may adversely affect human and animal health through bioaccumulation and biomagnifications

along the food chain. Chemical run-offs from the agricultural fields during flooding further deteriorate the situation.

Plants can use 50% of the total nitrogen fertiliser applied to soil, evaporation loss approaches to 2–20%, 2–10% reaches the ground and surface water while remaining 15–25% react with the soil organic compounds (Savci, 2012). The NH₃ as agricultural run-off damages the adjacent ecosystems and biodiversity compounds, when released into the atmosphere, it oxidises to nitric acid (acid rain) that damages terrestrial vegetation and aquatic lives (Savci, 2012). The soil nitrifying microbes convert NH₃ of nitrogenous fertilisers to NO₃-that reaches the surface and ground waters (Savci, 2012).

Contaminated ground water is associated with various ailments

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including congenital defects, hypertension, goiter, stomach and testicular cancers, etc. (Divya and Belagali, 2012). Excessive NH_3 , in the air and water leads to respiratory ailments, various cancers, pollen hypersensitivity and cardiac disease, while also affecting the degree of pathogenicity of various vector-borne diseases including malaria, cholera and West Nile virus (Hazra, 2016). Excessive nitrogenous fertilisers increase the NO_3^- Content of the plant and leaf above acceptable limits. High NO-contents in the leaf threaten human health when leafy vegetables are consumed. Similarly, fertilisers containing high potassium and sodium change the soil pH, thereby deteriorating the soil structure and texture, Hence, indiscriminate use of such chemicals adversely impacts the ecology and the environment (Divya and Belagali, 2012; Hazra, 2016).

Prolonged indiscriminate use of toxic chemical fertilisers could destroy the beneficial soil microbes thereby disbalancing the microecology. Soil biodiversity needs to be protected from synthetic fertilisers. Reducing soil microbiota badly impacts nutrient cycling, thereby affecting plant growth and crop yield. It also reduces the underground biodiversity that is important in ecosystem sustenance (Calderon et al., 2017). Hence, natural agricultural practices should be practiced as far as practicable to restore soil fertility. For sustainable crop production, organic farming involving no or less chemicals is an alternate agricultural practice to ensure healthy and sustainable productivity. Organic manures improve the physical and chemical properties of the soil while providing nutrients.

PGPM (plant growth promoting microbes) increase the agronomy efficiency by reducing the production cost and environment pollution, as an efficient PGPM reduces the use of chemical fertilisers (Souza et al., 2015). Various microbial communities, viz., fungi, bacteria, actinomycetes and yeasts are used as inoculants and primarily they promote plant growth through nitrogen fixation, phosphate and potassium solubilisation, exopolysaccharide secretion, biocontrol activity, organic matter decomposition, siderophores production, etc. *Rhizobium* sp. and *Bradyrhizobium* sp. are major microbes in symbiotic N_2 fixation (Souza et al., 2015). *Azospirillum* is a free living nitrogen fixer found to enhance growth of non-leguminous crops (Lin et al., 2015). *Pseudomonas putida* and *Pseudomonas fluorescens* groups could stimulate plant growth as biocontrol agents, aiding in N_2 fixation and phosphate solubilisation (Lin et al., 2015; Souza et al., 2015). *Azotobacter* and *Azospirillum* are effective in enhancing production (Rueda et al., 2016). Phosphorus, a key soil nutrient present in complex unavailable form, is made available by phosphate-solubilising microbes that make them avail for plant uptake. A study in similar lines on eggplants was recently reported (Souza et al., 2015) with *Bacillus megaterium* and *Bacillus muclaginosus*. Co-inoculation of two or more organisms may improve yield and growth as compared to mono-septic inoculation as they provide diversified benefits to plants (Souza et al., 2015).

2. Relevance of microecology in agriculture

Farmers in developed nations are shifting their farming practices to organic-based (Yadav et al., 2013). As people become more health conscious, organic farming has increased significantly in the Asia, Africa, Europe, Oceania, Latin America and North America recently. 'Organic' crop consumers are educated, rich, environmentally conscious, and health-sensible. Although it can potentially improve the environment, the quality of food and importantly the sustainability of farming system, conventional organic farming only may not suffice to the ever-increasing food demand.

Serious scientific investigations are being undertaken into the efficient utilisation of agricultural resources to enhance productivity through biological means instead of chemical. Implementing organic systems with the EMs promises to enhance the yield while sustaining the system. As plant nutrients recycling by the saprobes become efficient, the urgency for chemical fertiliser dwindles (Vassilev et al., 2015). Although animal and green manures are extensively used in

conventional agriculture, supplementing with effective microbes helps in crop growth, and yield. Various fungi, bacteria, actinomycete and yeast groups are very effective. Formulated EMs ensure the crop well-being by inducing photosynthesis, performing N_2 -fixation, synthesising growth factors like hormones, vitamins and enzymes, stimulating plant growth, accelerating decomposition of organic materials, producing bioactive substances, making the crop drought tolerant, and controlling soil-borne plant pathogens. Thus, EMs ameliorates soil biodiversity and community composition and aid in sustainable agriculture.

Microbes play a defining role in an ecosystem by balancing the biotic-abiotic interactions through ecological associations with biotic community on one hand and nutrient and minerals cycling of the abiotic components on the other. Soil saprobes decompose organic remains and maintain soil organic carbon above the threshold levels which holds the soil particles together to reduce soil erosion while enhancing its water-holding capacity. Higher soil organic matter revitalises the microbial biodiversity and ensures healthy C:N ratio. Similarly, NH_4^+ and NO_3^- are generated through microbial N_2 -fixation and nitrification. Microbial nitrogenase breaks the strong triple covalent diazo bond to form NH_4^+ while the two-step (nitritation/nitrosification and nitratation/nitratification) nitrification readily oxidises NH_4^+ and NO_3^- . Specialised microbes solubilise the unavailable soil phosphorus by organic acids (for inorganically-bound phosphate or alkaline and acid phosphatases (for organically-bound phosphate). Microbial organic acids act directly or through metal chelation to release soil K^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} etc. and trace elements. Although minerals cycling are majorly accomplished by dedicated microbes, the activity is positively or adversely impacted by the interacting biotic community (microbe-microbe, microbe-plant or microbe-animal/human) and abiotic constituents. Crops benefit positively by associations like mutualism, symbiosis, commensalism, amensalism, photocooperation etc. with beneficial microbes, and negatively by associations like antagonism against the harmful microbes. These facts form the basis of the plant-growth-promoting role of microbes (Table 1).

3. Mechanism in microbial activity to promote plant growth

Microbes are naturally gifted with many special traits that no or very few higher organisms ever possess. These have very extensive use in agricultural practices. The very special ones with physicochemical and biological compatibility are cocultured, formulated and applied together as effective microbes (EM). EMs are plant growth promoting microbes (PGPM) that enhance crop yield through N_2 -fixation, production of siderophores, ACC deaminase and phytohormones activities, phosphorus and minerals solubilisation, soil characteristics enhancement, etc. Besides, microbes can act as biocontrol and biopesticide for plants. EM reportedly improves crop development and productivity by enhancing photosynthesis, synthesising bioactive substances like hormones and enzymes controlling the soil diseases and enhancing the lignin breakdown in soil. Fig. 1 represents the multifarious ways through which microbes directly or indirectly associate themselves with the crops and promote growth.

Direct action involves soil amelioration, production of plant growth substances, and improving soil fertility by mobilising soil mineral components while indirect action includes the production of biocontrol agents that inactivate or kill plant pathogens thereby providing a healthy crop environment (graphical abstract). Indian soil has low organic phosphorus and thus its bioavailability is also low. Phosphate-solubilising microbe (PSM) solubilises the insoluble phosphate PO_4^{3-} and enhances its bioavailability (Sharma et al., 2013). Although bacteria like *Bacillus* and *Pseudomonas*, and fungi like *Aspergillus* and *Penicillium* were considered good phosphate solubilisers, their inconsistent performance under *in situ* conditions could be improved through genetic modification or through coinoculation techniques (Sharma et al., 2013). Plant growth-promoting rhizobacteria (PGPR), plant growth promoting

Table 1
A compilation of the growth-promoting microbes and their effects on plant wellbeing.

| Microbial group | | Mechanism of action | Representative species | Mode of Inhabitation | Beneficial or detrimental (\pm) | Type of association | Reference(s) |
|-----------------------------------|--|---|--|--|-------------------------------------|--|---|
| Bacteria and Fungi | P-solubilising microbe (PSM) | P-solubilisation | <i>Bacillus</i> and <i>Pseudomonas</i> (bacteria) <i>Aspergillus</i> and <i>Penicillium</i> (fungi) | Soil/rhizosphere | + | Symbiotic association | Sharma et al. (2013) |
| Bacteria and Fungi | PGPR and AMF | N-fixation | <i>Pseudomonas reactans</i> , <i>Chryseobacterium humi</i> , <i>Rhizopagus irregularis</i> | Rhizosphere/soil | + | Symbiotic association | Moreira et al. (2016) |
| Bacteria | PGPR | N-fixation | <i>Rhizobium</i> , <i>Sinorhizobium</i> , <i>Bradyrhizobium</i> , <i>Azorhizobium</i> , <i>Mesorhizobium</i> , <i>Allorhizobium</i> <i>Azospirillum</i> , <i>Enterobacter</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> | Rhizosphere soil | + | Symbiotic N ₂ -fixers Free-living N ₂ -fixers | Hayat et al. (2010) |
| Bacteria | PGPR Isolates | Biocontrol agent, plant growth substances, Antagonistic activity against phytopathogen | <i>Pseudomonas fluorescens</i> , <i>Bacillus subtilis</i> | Rhizosphere soil | - | Symbiotic association | Sivasakthi et al. (2014) |
| Bacteria | PGPR | - | <i>P. fluorescens</i> , <i>B. subtilis</i> , <i>P. putida</i> | soil | + | Symbiotic association | Del et al. (2017) |
| Bacteria | PGPR | Antagonistic activity, IAA, GA3 | <i>Bacillus amyloliquefaciens</i> | | - | - | Yuan et al. (2013) |
| Bacteria | Microbial pest control agent, plant growth promotion | Root growth development, biocontrol agent | <i>Bacillus subtilis</i> , <i>Azospirillum brasilense</i> Sp245, <i>Rhizobacterium</i> (PGPR) | Rhizosphere | +/- | Symbiotic association | Felici et al. (2008) |
| Fungi and bacteria | AMF and PGPB | Sugar and vitamin production, sweetness to tomato | <i>Pseudomonas</i> , AM fungi | soil | + | Symbiotic association | Bona et al. (2017) |
| Fungi | AM fungi | Phosphatase activity | <i>Glomus fasciculatum</i> , <i>Glomus fasciculatum</i> , <i>Glomus mossae</i> , <i>Gigaspora margarita</i> , <i>Acaulospora laevis</i> | Soil | + | Symbiotic association | Usharani et al. (2014) |
| Actinomycetes | Endophytic actinomycetes | Antimicrobial activity against phytopathogen | <i>Streptomyces</i> , <i>Streptosporangium</i> , <i>Microbispora</i> , <i>Streptoyerticillium</i> , <i>sacchromonospora</i> , <i>Nocardia</i> | <i>Azadirachta indica</i> A. juss (stem, root, leaf) | - | Symbiotic association | Verma et al. (2009) |
| Bacteria and actinomycetes | PGP agents | ACC deaminase (stress-buster) and IAA, N ₂ fixing, PO ₄ ³⁻ solubilising, siderophore producing | <i>Microbispora</i> sp., <i>Streptomyces</i> sp. | Soil | +/- | Symbiotic association | Souza et al. (2015), Glick (2014) |
| Bacteria | Halotolerant endophyte | Salt tolerance | <i>Bacillus flexus</i> , <i>Enterobacter</i> sp. UPMR18 | Halophyte <i>Limonium sinense</i> (PGPR) | - | Symbiotic association | Wang et al. (2017), Habib et al. (2016) |
| Bacteria, fungi and actinomycetes | PGPR (PGPB), fungi, actinomycetes | Soil conditioner, plant pathogen suppressor, biofertiliser, plant straightener, phytostimulator, biopesticide | <i>Azospirillum</i> , <i>Rhizobium</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Serratia</i> , <i>Stenotrophomonas</i> , <i>Streptomyces</i> , <i>Coniothyrium</i> , <i>Ampelomyces</i> , <i>Trichoderma</i> | soil | +/- | Symbiotic association | Berg (2009) |
| Bacteria and fungi | PGPB and Endophytic fungi | Biocontrol agent against pathogen, pest | <i>P. aeruginosa</i> , <i>Trichoderma viride</i> | Soil and rhizosphere | - | Symbiotic association | Afzal et al. (2013) |
| Fungi | - | suppress fungal infections | <i>Trichoderma harzianum</i> | <i>Trichoderma</i> -enriched compost extracts | - | Symbiotic association | Siddiqui et al. (2008) |
| Bacteria and Fungi | AM fungi and PGPB | Stimulate plant growth, drought tolerance, IAA production | <i>Pseudomonas putida</i> , <i>Bacillus megaterium</i> , AM fungi (<i>Glomus coronatum</i> , <i>Glomus constrictum</i> or <i>Glomus claroideum</i>) | Abiotic (water)-stress condition | +/- | Symbiotic association | Marulanda et al. (2009) |

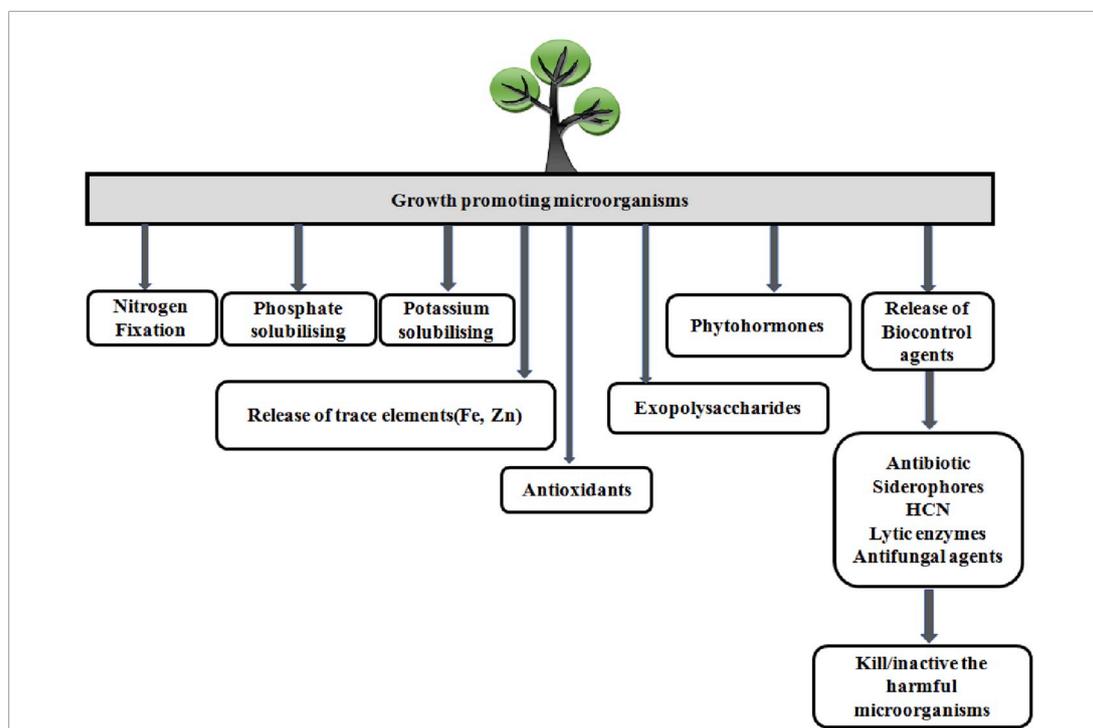


Fig. 1. The various means through which plant growth is promoted by effective microorganisms.

bacteria (PGPB) and vesicular-arbuscular mycorrhizae (AMF) fungi are the subgroups of plant growth promoting microorganisms (PGPM). Plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas reactans*, *Chryseobacterium humi* and also the VAM such as *Rhizophagus irregularis* also improve soil productivity and plant growth (Moreira et al., 2016). EMs could be applied along with manure, chemical fertilisers, organic biofertilisers and molasses. Microbial consortium consisting of PGPR could replace fertilisers and pesticides. *In lieu* of chemical fertilisers for cropping, these decrease environmental pollution and reduce input costs without compromising on the yield. PGPR provide a conducive environment for plant–microbe interaction. Mutually benefitting N_2 -fixing partners, genera including *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium*, *Allorhizobium* aid in plant growth and yield. Genera like *Azospirillum*, *Enterobacter*, *Klebsiella* and *Pseudomonas* are associated N_2 -fixers that attach to the rhizosphere, proficiently colonise the root surfaces, and fix N_2 (as NH_3) in plants (Hayat et al., 2010). PGPB such as *Pseudomonas fluorescens* and *Bacillus subtilis* induce PGPR isolates to produce plant growth-promoting substances (Sivasakthi et al., 2014). PGPR isolates *P. fluorescens*, *B. subtilis* and *P. putida* could potentially improve the productivity of cultivars (Del et al., 2017). *Bacillus amyloliquefaciens* protected banana from *Fusarium oxysporum* and promoted plant growth (Yuan et al., 2013). Single and coinoculated *Bacillus subtilis* and *Azospirillum brasiliense* were effective on *Lycopersicon esculentum* (Felici et al., 2008). VAM and PGPB (*Pseudomonas*) improve nutritional value and quality of tomato by positively affecting the flowering, fruiting, and sugar and vitamin contents in tomato (Bona et al., 2017). Arbuscular mycorrhiza *Glomus fasciculatum* was effective for *Zea mays* (Usharani et al., 2014). Fungi produced alkaline and acid phosphatases that solubilised phosphorus and made it available to the plant (Mohammadi, 2012). Soil actinomycetes can produce an array of antibiotics and extracellular enzymes. Numerous actinomycetes strains have been found to protect plants against diseases (Verma et al., 2009). The antagonism of PGPR isolates against phytopathogens has been reported by Sivasakthi et al. (2014).

Phytohormone ethylene regulates growth, senescence and stress in plant at low concentrations (Souza et al., 2015), and an increased

concentration adversely affects the plant root growth. Ethylene in the plant is produced from ACC (1-aminocyclopropane-1-decarboxylate). ACC deaminase breaks down ACC thereby preventing ethylene production. ACC deaminase producing microbes such as actinomycetes can uptake ACC, metabolise it to α -ketoglutarate and NH_3 , and thereby decrease the ethylene concentration in plant (Souza et al., 2015; Glick, 2014; Kruasuwan and Thamchaipenet, 2016). Actinomycetes *Microbispora* sp. and *Streptomyces* sp. produced stress-buster ACC deaminase (1-aminocyclopropane-1-decarboxylate deaminase) and plant growth hormone IAA (indole-3-acetic acid) (Glick, 2014). These actinomycetes individually or as coinoculants reportedly fixed.

N_2 , solubilised PO_4^{2-} , and produced siderophore for growth in sugarcane (Kruasuwan and Thamchaipenet, 2016). Inoculated microbes produced various plant growth promoting hormones like gibberellic acid, indole acetic acid, ethylene and cytokines (Hayat et al., 2010).

Soil salinity, being a challenge to crop growth and wellbeing, negatively impacts its production (Qin et al., 2017) particularly in coastal belts. Single microbial culture may suffer from competitive and aggressive effects of indigenous soil microflora and their number may soon decline. A *Bacillus flexus* strain improved salt tolerance in crops (Wang et al., 2017). *Enterobacter* sp. UPMR18 helped in okra plant growth by enhancing salt tolerance and its inoculation bettered the germination percentage, growth parameters, and chlorophyll content (Habib et al., 2016). A mixed culture (EM) improves and maintains the soil physical and chemical properties, thereby shifting the equilibrium and enhancing crop growth, yield and health (Bhattacharyya and Jha, 2012). Bacteria like *Bacillus*, *Pseudomonas*, *Streptomyces*, *Serratia* and *Stenotrophomonas*, and fungi like *Coniothyrium*, *Ampelomyces* and *Trichoderma* are model organisms with proven positive effect on crop health (Berg, 2009).

EM might initiate symbiotic associations in the rhizosphere (Kruasuwan and Thamchaipenet, 2016). Amino acids, carbohydrates, active enzymes and organic acids are secreted by plant roots. These secretions are used by the effective microorganisms as nutrients. In return, the microorganisms secrete amino acids, variety of vitamins, nucleic acids and hormones in the plant. Soils with a huge population of antibiotics-producing organisms such as, *Penicillium*, *Trichoderma*,

Aspergillus and *Streptomyces*, are called disease suppressive soils (Singh et al., 2017). The 'zymogenic soils' rich with zymogenic organisms like yeasts, lactic acid bacteria and increased population of fermenting fungi like *Rhizopus* and *Aspergillus* possess very good physical characteristics with the enhanced water holding ability (Souza et al., 2015; Singh et al., 2017).

4. Effect of abiotic stress in crops

A stress can be single, multiple and/or combined (Pandey et al., 2017). Combined stress refers to some degree of or a complete overlap between various stresses. The co-occurrence of drought and heat stress during the summer is an example of combined stress and stand in most cases. Other such combined stress instances could be salinity and heat stress (in plants growing in arid and semi-arid conditions), and cold and high light stress (in plants growing in the Mediterranean). Abiotic stresses like drought, water logging, heat and light intensity, and salinity affect plant growth and development significantly. Factors like acidity, alkalinity, contaminating pollutants, harmful emissions (like ozone, SO₂ and nitrogen oxides) adversely affect plant development and negatively impact the productivity. Acidic condition may limit the availability of soil nutrients, thereby hampering the physiological pattern of growth and development (Pandey et al., 2017). Excessively alkaline or salty water can result in a nutrient imbalance and poor plant growth. Premature exposure to salinity leads to ion toxicity within the cell followed by the disturbance in the osmotic balance when the stress continues for a longer duration. The ion toxicity and osmotic imbalance together would reduce the plant growth. Typically, crops encounter an increased number of abiotic and biotic stresses that affect plant growth and yield adversely. Such stress conditions could also directly affect the plant-microbes interaction, including the phytopathogens. Stress factors facilitate the occurrence and spread of insects, weeds and phytopathogens equally (Wu et al., 2017; Pandey et al., 2017).

Drought stress occurs when the soil and atmospheric humidity is low and the ambient air temperature is high. This leads to an imbalance between the evapotranspiration flux and water intake from the soil (Lipiec et al., 2013). As several weeds exhibit enhanced water use efficiency than the crops, drought enhances the competitive interaction of weeds on crops. A combined stress of drought and cold in *Vitis vinifera* (grape) growing in North China has been reported by Su et al. (2015). Increased soil and air temperatures beyond the threshold levels permanently harm plant growth and development. The crops grow well within a limited temperature range, too high and low temperature results in abnormal development and reduced production. The optimum temperature range for plant growth, particularly in the tropics is between 25 and 28 °C. The cold and increased temperature may lead to crop loss (Meena et al., 2017). Such adverse conditions also contribute to the onset of drought-prone areas, and consequently affecting crop growth and productivity (Zhao et al., 2017). Water stress adversely affects plant metabolism, including photosynthesis. Prolonged water stress decreases the leaf water potential and stomatal opening, reduces the leaf size and seed numbers, obstructs root growth, and delays flowering and fruiting in the crop (Meena et al., 2017). The combined stress of ozone and cold in *Triticum aestivum* (the winter wheat) is reported by Pandey et al. (2017).

Plants vary from each other in terms of salt tolerance and are classified as glycophytes and halophytes, the halophytes being salt lovers. Halophytes, accounting for up to 1% of the global flora, can tolerate a salinity concentration up to 1 M NaCl (Etesami and Beattie, 2018), and survive in salt-contaminated environments naturally. These are found mainly in arid, semi arid land and high salinity wetlands along the tropical and sub-tropical coasts. Halophytes could be obligate that needs some amount of salt for survival, or facultative that can grow even in freshwater conditions (Etesami and Beattie, 2018). In terms of water availability, the halophytes can be hydro-halophytes and xero-halophytes. The former could grow in aquatic environment or on

wet soil while the latter inhabits the dry soil. Halophytes are considered extremophiles due to their ability to flourish in high salinity conditions (Su et al., 2015). Contrarily, the glycophytes are salt sensitive plants and cannot tolerate increased salinity (Kumari et al., 2015).

Exposure to increased or decreased light intensities negatively affects the physiological processes and the plant growth. Excess light persuades phytooxidation in crops, leading to the production of highly reactive oxygen intermediates which in turn adversely affect the biomolecules and enzymes (Su et al., 2015), thereby reducing the productivity.

5. Microbes in crop stress management

Plants are impacted by abiotic and biotic stress equally, and sometimes inversely. For instance, the effect of drought and salinity leads to either resistance or susceptibility of plants to *Puccinia* sp. (that causes rust), *Verticillium* sp. (that causes verticillium wilt), *Fusarium* sp. (that causes fusarium wilt), *Pythium* sp. (that causes root rot) and *Erysiphe* sp. (that causes powdery mildew) (Pandey et al., 2017). Strains of *P. aeruginosa* alone or in combination with *Trichoderma viride* (endophytic bacterium and fungus, respectively) exhibited significant biocontrol against okra root infecting (root-knot) nematodes and *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Meloidogyne javanica*, positively impacting plant growth by improving its height, fresh shoot weight and root length (Afzal et al., 2013). Foliar application of fungicidal *Trichoderma harzianum* suppressed fungal infections (Siddiqui et al., 2008). Positive association in *Pseudomonas putida* or *Bacillus megaterium* and AM fungi (*Glomus coronatum*, *Glomus constrictum* or *Glomus claroideum*) induced plant development and drought forbearance (Marulanda et al., 2009).

Beneficial microbial consortia could facilitate crop wellbeing and yield when judiciously formulated and allowed to colonise the target crops. Similar to the human probiotics in food or non-human probiotics in the formulated feed of farmed animal, these would serve as beneficial colonisers in the ecosystem (popularly referred as the environmental probiotics, or the EM). Establishment of an EM formulation is often restricted by definite soil properties and the native microbial population. As opined often, the impact of EM on the crop yield is usually not obvious, or even lower in some instance, especially in the first application. Single application during only a single season is frequently tough to ensure that the EM predominates, and thus the indiscernible benefit. Thus, a continual periodic application of EM is suggestible. As plants depend on the mobilisation of vital nutrients (viz., P, S, Fe, K and Zn) and N₂-fixation for healthy growth, EMs manage these along with the biotic and abiotic stresses to ensure healthy growth (Marshall and Levy, 2011; Condor et al., 2007). Fig. 2 depicts the various benefits against biotic and abiotic stresses derived by a crop from EM formulations.

6. Microbes as potential EM candidates

EM formulation is a microbial consortium sourced from nature (or may include genetically engineered microbe of native strains), and is applied to ensure the essential microbial diversity in the soil that is useful in agricultural production. Microbes associated with the plants accomplish preliminary importance as EM candidates. In light of the above discussions, however, not all the beneficial microbes can be potential EM candidates.

EM formulations usually include phototrophs, lactic acid bacteria, actinomycetes, fermentative fungi and yeasts, etc. (Table 2). Few of the representative genera are, lactic acid bacteria (e.g., *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*), photosynthetic bacteria (e.g., *Rhodospseudomonas palustris*, *Rhodobacter sphaeroides*), yeasts (e.g., *Saccharomyces cerevisiae*, *Candida utilis*), actinomycetes (e.g., *Streptomyces albus*, *Streptomyces griseus*) and fungi (e.g., *Aspergillus oryzae*, *Mucor hiemalis*), the formulation being topped with organic 'prebiotic' base (liquid carrier substrate) as excipient. The resulting biological material enhances growth patterns, nutrient uptake and grain yield as

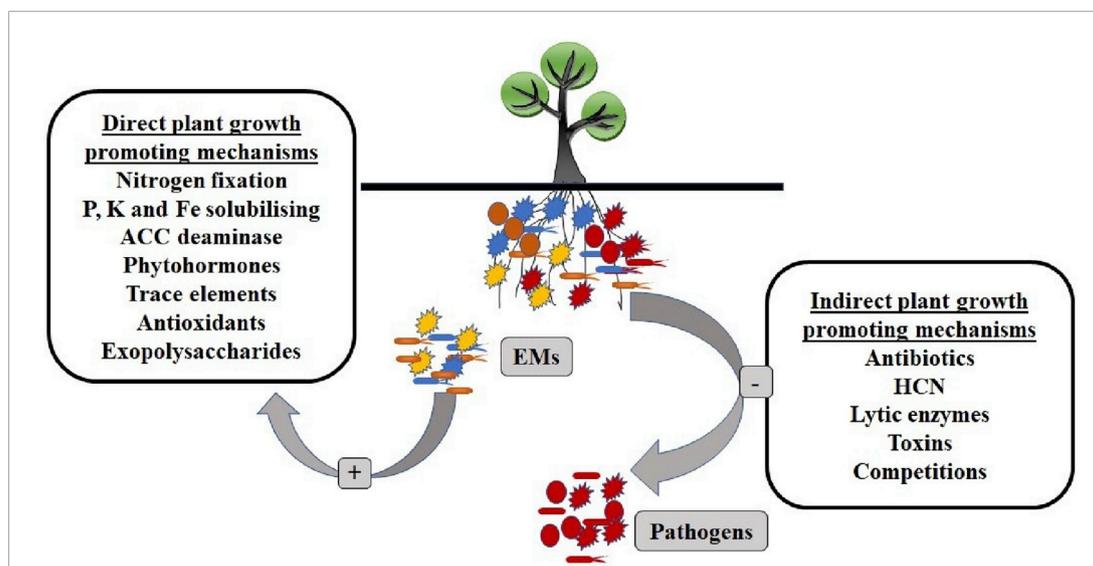


Fig. 2. Direct and indirect beneficial effects of effective microorganisms on plant wellbeing.

compared to chemical fertilisers (Fig. 3). Reports on such beneficial roles of EM on crops, such as sweet corn (Mayer et al., 2010), lettuce (Condor et al., 2007), bananas (Condor et al., 2007), tomato (Olle and William, 2013), carrot (Olle and William, 2013), pea (Olle and William, 2013) and, beet (Olle and William, 2013), tobacco (Su et al., 2017) and cucumbers (Kang et al., 2015) are abound.

The formulated EM is applied either foliar or to the soil by mixing with appropriate proportions of organic manure prior to the application of chemical fertilisers, if any. Besides, EM solution can be used to ferment organic wastes first, and then the resulting EM-enriched compost could be applied to the field (Table 2).

6.1. Phototrophic bacteria

These are autotrophs that use sunlight as an energy source to synthesise sugars, amino acids, nucleic acids, and bioactive substances etc., and hence are considered as independent and self-supporting. Among various ranges of solar radiation, infrared band of 700–1200 nm is useful for such organism as also for the crops (Conder et al., 2007; Olle and William, 2013). Such microbes are widely applied in farming to enhance plant growth and crop quality.

Rhodospseudomonas palustris is a useful strain that helps plants fight against viral pathogens (Fig. 3). It produces phytohormones like indole-3-acetic acid and 5-aminolevulinic acid, thereby stimulating the growth and germination in tobacco (Su et al., 2017). Plants inoculated with this strain confirm elevated immune response against subsequent TMV infection (Su et al., 2017). In combination with other microbes, phototrophs are found to be effective in plant growth. Phycomyces such as vesicular-arbuscular mycorrhiza (VAM) symbiotically help in plant growth (Condor et al., 2007), and their activity is boosted due to their alleviated accessibility to nitrogenous compounds (amino acids) formed by phototrophs (like *Rhodospseudomonas* sp.), an unique instance of a biological agent having dual functions of crop growth and disease management (Su et al., 2017). Inoculation of phototrophs like *R. sphaeroides* along with *S. cerevisiae* (yeast) and *L. plantarum* (lactic acid bacteria) increased the shoot fresh weight, shoot length, shoot dry weight, root length, plant hormones, chlorophyll content and 17 amino acids (Kang et al., 2015).

Cyanobacteria (blue-green algae) are the largest cluster of photosynthetic prokaryotes existing in diverse ecology, globally. They are found in almost every terrestrial and aquatic habitat, from oceans, freshwater, damp soil, temporarily moistened rocks in deserts, bare rock

and soil, to even Antarctic rocks (Mo et al., 2015). These N₂-fixing cyanobacteria are promising biofertilisers capable of improving soil physicochemical characteristics like water-holding capacity and mineral nutrient status, significantly boosting crop growth (Bhattacharjee and Dey, 2014; Chittapun et al., 2018). These bioinoculants are increasingly being applied to improve soil fertility and environmental quality (Singh et al., 2016a). Heterocystous *Anabaena*, *Cylindrospermum*, *Nostoc*, *Calothrix*, *Hapalosiphon*, *Scytonema*, *Tolypothrix*, *Aulosira* and *Westiellopsis* fix molecular nitrogen (N₂) as NH₃ for the plant, and release amino acids like alanine, aspartic acid, glutamic acid, vitamin B₁₂ and auxin-like substances that benefit the crops (Issa et al., 2014). *Anabaena vaginalis* and *Nostoc calcicola* are useful as biofertilisers for vegetable crops. Beneficial effects of cyanobacteria in rice, wheat, soybean, tomato, oat, wheat, sugarcane, maize, radish, muskmelon, cotton, lettuce, bean and chili etc. are reported often. Applied in combination with chemical fertilisers, *Nostoc carneum* reportedly enhanced the growth of rice seedling and root, increased the grain number and weight (Chittapun et al., 2018). Application of *Nostoc entophyllum* and *Oscillatoria angustissima* with various doses of chemical fertilisers stimulated growth, seed germination and photosynthesis in peas (Osman et al., 2010).

6.2. Lactic acid bacteria

Lactic acid bacteria (LAB) have a long history of use in food processing preventing microbial pathogens like *Salmonella*, *E. coli*, etc. (Gomez et al., 2016). These omnipresent microbes produce lactic acid, a strong disinfectant, and are beneficial in crop and livestock farming. Lactic acid inhibits destructive microbes and aids in faster degradation of lignin and cellulose. LAB is capable of suppressing *Fusarium* wilt propagation. *Fusarium* sp. causes disease, and also increases the growth of harmful nematodes during continuous cropping (Olle and William, 2013; Lee et al., 2011). *Lactobacillus* sp. produces nitric oxide (NO) (Yarullina et al., 2014), and controls nematodes (Lee et al., 2011). LAB control tomato wilt caused by *Ralstonia solanacearum* (Konappa et al., 2016). *Lactobacillus* sp. KLF01 is an antagonist against *Ralstonia solanacearum* (Limanska et al., 2013). *L. plantarum* stimulates plant growth in nutritionally compromised ecological niche (Shrestha et al., 2014). A consortium of *L. plantarum*, *Sphaeroides*, *Rhodobacter* and *Saccharomyces cerevisiae* promoted growth in cucumber (Kang et al., 2015).

Table 2
Various microbial consortia as potential candidates for EM formulation.

| Microbial group | Representative species | Role in the formulated EM | Reference(s) |
|----------------------|--|--|--|
| Photosynthetic | <i>Rhodospseudomonas palustris</i> , <i>Rhodobacter spaeroides</i> | Produce amino acids, nucleic acids and bioactive substances, sugars; aids in photosynthesis | Condor et al. (2007), Olle and Williams (2013), Su et al., 2017, Kang et al. (2015) |
| | <i>Anabaena</i> , <i>Cylindrospermum</i> , <i>Hapalosiphon</i> , <i>Aulosira</i> , <i>Nostoc</i> , <i>Calothrix</i> , <i>Scytonema</i> , <i>Tolypothrix</i> , <i>Westiellopsis</i> (heterocystous) | Photosynthesises, fixes N ₂ through heterocyst and provides amino acids to plants | Mo et al. (2015), Bhattacharjee and Dey (2014), Chittapun et al. (2018), Singh et al. (2016a), Issa et al. (2014), Osman et al. (2010) |
| Lactic acid bacteria | <i>Lactobacillus plantarum</i> , <i>L. casei</i> , <i>Streptococcus lactis</i> | Suppresses and reduces pathogens and enhances rapid decomposition of organic matter | Olle and Williams (2013), Kang et al. (2015), Gomez et al. (2016), Lee et al. (2011), Yarullina et al. (2014), Konappa et al. (2016), Limanska et al. (2013), Shrestha et al. (2014) |
| Actinomycetes | <i>Streptomyces griseus</i> , <i>S. albus</i> , <i>S. antibioticus</i> , <i>S. champavatii</i> | Produce antibacterials, inhibits harmful bacteria and fungi and enhances phospholipids decomposition | Qin et al. (2017), Lamont et al. (2017), Nguyen et al. (2012), Singh et al. (2016b), Sreevidya et al. (2016) |
| Fermentative fungi | <i>Aspergillus oryzae</i> , <i>Mucor heimalis</i> , <i>Penicillium</i> sp., <i>Trichoderma harzianum</i> | Decompose organic matters, synthesises amino acids and glucose from carbohydrates and controls odour | Sequeira (2016), Verma et al. (2007), Panda (2013) |
| Yeasts | <i>Saccharomyces cerevisiae</i> , <i>Candida utilis</i> , <i>Candida tropicalis</i> | Breaks down dead plant tissues and encourages root growth | Agamy et al. (2013) |

6.3. Actinomycetes

Actinomycetes coexist with photosynthetic bacteria and improve soil quality through antimicrobial activity (Lamont et al., 2017). Such antimicrobials inhibit pathogenic fungi and bacteria (Fig. 3). Actinomycetes, the missing link between bacteria and fungi, synthesise antimicrobial compounds from amino acids released by photosynthetic bacteria. Lytic enzymes like chitinase, β -1,3-glucanase, lipase and protease of *Streptomyces griseus* protect pepper plants against *Phytophthora capsici* by degrading the fungal cell wall (chitin) and cellular components like lipids and proteins (Nguyen et al., 2012). *Solanum lycopersicon* is protected against *Rhizoctonia solani* by *Streptomyces* sp., viz., *S. griseus*, *S. albus*, *S. antibioticus* and *S. champavatii* (Singh et al., 2016b). Apart from biocontrol role, actinomycetes help in promoting crop growth. *Streptomyces* enhanced nodule number, shoot weight and yield in chickpea and increased the total N, available P and organic C contents of soil (Sreevidya et al., 2016). *Actinobacteria* is a recommended inoculant in salt-inundated cropping (Qin et al., 2017).

6.4. Fermentative fungi

Although fungi are ill-famed as prominent plant pathogens, some inhibit pathogenic fungi thereby enhancing plant growth. 'Disease suppressive soils' are rich in antibiotics from *Penicillium*, *Trichoderma*, *Aspergillus*, etc. *Aspergillus* and *Penicillium* decompose organic materials rapidly to esters, alcohol and antimicrobial substances. These reduce malodor and prevent invasion of harmful maggots and insects (Sequeira, 2016). *Trichoderma* sp. has wide use as an antagonist against several pests as it produces a wide range of enzymes like cellulases, hemicellulases, proteases and β -1,3-glucanase (Verma et al., 2007).

Plant-root associated VAM may harbor N₂-fixing *Rhizobium* and *Azotobacter* (Panda, 2013). Besides the mouldy forms, the unicellular yeasts perform several ecological functions including their role in the detritus food chain, synthesising antimicrobial and other substances useful in plant growth and thus, ensuring the overall plant wellbeing. Bioactive compounds like enzymes and hormones from yeasts enhance cell division in the root system (Agamy et al., 2013). *Candida tropicalis* mobilised phosphate (PO₄³⁻) from insoluble tricalcium phosphate through phytase and polyamine, and stimulated rice seedling (Amprayn et al., 2012).

7. Biochemical interactions

The growth and wellbeing of crops depend on the soil nutrient status, particularly the macronutrients N, P and K.

Soil is often associated with an inadequate macronutrients profile and requires allochthonous organic and/or inorganic inputs. Usually, nitrogen is supplied through manure and fertilisers, phosphorus through superphosphates, organic manure, bone meal, and the potassium through organic manure and fertilisers. Besides the overall growth effect, nitrogen is important in chlorophyll, enzymes and proteins, phosphorus in phosphoproteins, phospholipids, ATP/ADP formation and root growth, and potassium in promoting enzyme activity and enhancing protein synthesis (Zaghloul et al., 2015).

Excess nutrients may damage crops by inhibiting their growth and reducing the yield. A suggested N, P and K proportion is 40 kg N₂ (ammonium sulphate), 30 kg P₂O₅ (calcium superphosphate) and 50 kg K₂O (potassium sulphate) (Uchida, 2000). The N, P and K accumulation and crop yields were higher under NPK as compared to the PK fertilisers, the order of the effect being NPK > NP > NK > PK (Yousaf et al., 2017). N, P and K proportion of 46% urea (H₂NCONH₂), 16% superphosphate (P₂O₅) and 60% muriate of potash (K₂O) increased the chlorophyll, the root protein and carbohydrate, and overall growth and biochemical contents of the root of *Asparagus racemosus* (Vijay et al., 2009).

Nitrogen is the basic building block of living organisms and, so also, is a constituent of photosynthetic pigment chlorophyll. Due to the difficult-to-break highly stable triple covalent bond in molecular nitrogen, plants cannot utilise atmospheric nitrogen (N₂) directly. Instead, they obtain nitrogen in the form of ammonium (NH₄⁺) and nitrate (NO₃⁻) from the soil through N₂-fixing and nitrifying microorganisms respectively. N₂-fixation is carried out by free-living (like Cyanobacteria, *Azotobacter* and *Azospirillum*) and symbiotic (like *Rhizobium* and *Bradyrhizobium*) microbes. The nitrogenase enzyme in these specialised microbes breaks down the strong triple covalent bond in the atmospheric nitrogen reducing it to ammonia.

Being highly energy consuming, this conversion requires 16 ATP molecules. The NH₃ thus generated is utilised by plants as ammonium that enters the Krebs cycle. Nitrogenase being O₂ sensitive, N₂-fixation occurs in anaerobic or microaerophilic conditions. On the other hand, the NH₄⁺/NH₃ in the soil formed through ammonification by the saprobes are readily oxidised to NO₃⁻ through a two-step nitrification as below, ensuring a soil rich in nitrate.

The NO₂⁻ concentration may be 10 times higher than NH₄⁺ concentration in well-aerated highly warm soils. Among all autotrophic

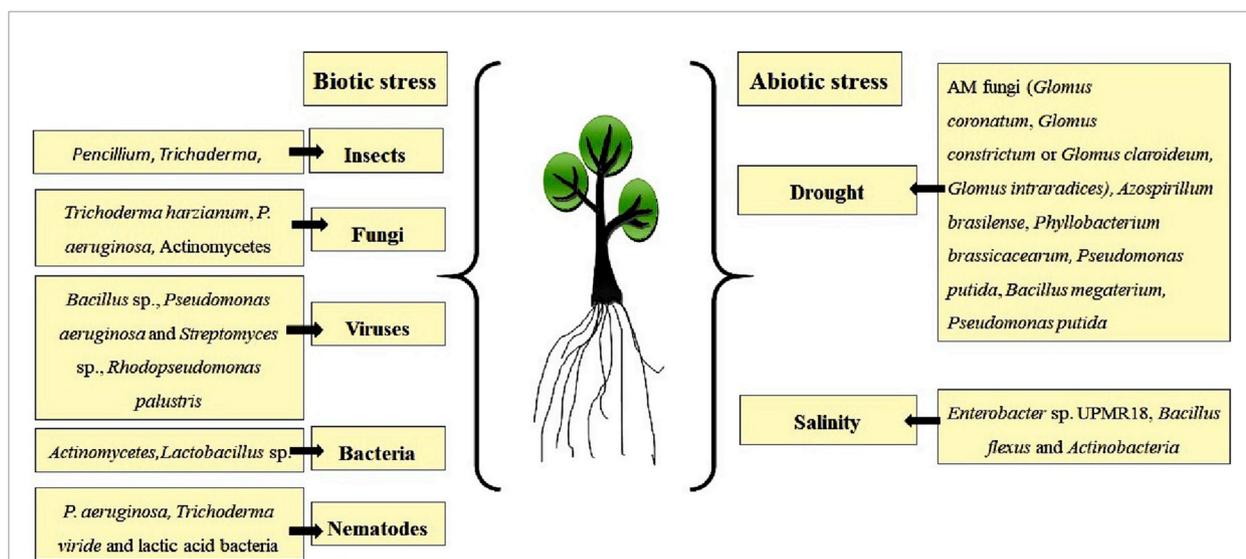


Fig. 3. Microbial groups effective in alleviating biotic and abiotic stresses in crops.

NH₄⁺-oxidisers associated with the first step (Nitritation or nitrification), *Nitrosomonas* is a frequently identified genus, along with other genera including *Nitrosobacter*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*. Similarly, *Nitrobacter* associated with the second step (nitration or nitrification) is frequently identified, although others, including *Nitromonas*, *Nitrospina*, *Nitrococcus* and *Nitrospira* are also encountered.

Uptake of phosphorus in plants is mainly in soluble forms. The majority of the soil phosphate is organically- and/or inorganically-bound insoluble form. The inorganically-bound phosphate is solubilised by organic (*viz.*, glucanic, oxalic, citric, malic and lactic, etc.) acids from specialised soil microbes (Khan et al., 2014). The organic acids decrease the soil pH leading to the release of the phosphate by substitution of H⁺ with cations, *e.g.*, Ca²⁺. Phosphate-solubilising bacteria include *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Azotobacter* and *Arthobacter sp.* etc., and the fungal counterparts are *Aspergillus sp.* (*A. niger*, *A. awamori* and *A. flavus*), *Penicillium*, and the VAM to certain extent. Unlike inorganically-bound one, the organically-bound phosphate is enzymatically solubilised by extracellular (alkaline and acid phosphatases) enzymes from specialised microbes. Out of the two enzymes, acid phosphatase is commonly encountered in fungi (*e.g.*, *Colletotrichum graminicola* and *Aspergillus niger*, for instance) in releasing phosphate from phosphate inositol phytic acid, C₆H₁₈O₂₄P₆ (Khan et al., 2014).

Similarly, potassium (K) is solubilised based on the same biochemical principle of organic acid dissolution by soil microbes. K⁺ from K⁻ bearing minerals is released to the soil by exchanging with H⁺ of organic acid (Meena et al., 2016). Thus, besides displacing phosphate, organic acids also make K⁺, Mg²⁺, Ca²⁺ and Mn²⁺ and trace elements available to the plant similarly. Some organic compounds facilitate the availability of trace elements to plants through enhanced metal chelation (Sebastian and Prasad, 2015).

Carbon is plenty in terrestrial ecosystems, and the soil microbial saprobes play a vital role in its cycling. The flora and fauna residues undergo decomposition by these decomposers that help maintain high soil organic carbon (SOC) levels. Thus, often than not, the carbon reserve in the soil for the plants is always above the threshold levels. Soil carbon holds the soil particles together, which further helps in enhancing the soil water-holding capacity while reducing soil erosion. SOC often binds to synthetic chemicals to minimise leaching through the soil profile and making them available for plants while reducing the toxicity (Rice, 2005). Higher soil organic matter (SOM) levels in soil to enhance the microbial biodiversity and promote root-penetrating AM fungal growth to facilitate the movement of plant nutrients, thereby

stimulate crop growth and yields.

While enhancing the abiotic constituents through external inputs, the biotic community also plays critical roles in their geochemical cycling and availability. For instance, administration of phosphate solubilising bacteria along with NPK reportedly benefitted yardlong bean (*Vigna unguiculata*) and rice (*Oryza sativa*) through enhanced NPK uptake (Duarah et al., 2011). In light of the above discussions, more than the organisms themselves, an EM researcher obviously would be interested in the biochemical changes they undertake. Thus, the interdependence of biogeochemical cycles in nature is also interesting and pertinent to understand from the point of view of the benefits of EM.

8. Ecological associations

As discussed above, soil mineral cycling is majorly accomplished by specialised microbes, and their activities are positively or adversely impacted by the interacting biotic community and abiotic constituents. In nature, various living entities of the microbial world are dynamically associated. These associations are, microbe-microbe, microbe-plant or microbe-animal/human, wherein one entity is the host and the other is the guest. Plants are in constant interaction with numerous soil microbes (*viz.*, bacteria, actinomycetes, fungi, algae etc.) contributing to crop growth. These interactions are categorised as mutualism, symbiosis, commensalism, amensalism, photocooperation, parasitism, etc.

Mutualism is an association in which both partner organisms benefit from each other. An example of such a microbe-plant (symbiotic) association is *Rhizobium*-leguminous plant where the microbe partner is protected from environmental stresses by leguminous plant and the latter readily acquires the available nitrogen fixed by the former. Another glaring example is the obligate association of green or blue green (cyanobacteria) algae with ascomycete fungus in nature to form lichen. Here, the algal partner provides nutrients and oxygen through photosynthesis to the fungal partner while the latter protects the former from abiotic stress by its hyphae (Selim and Zayed, 2017). Similarly, the actinomycete *Frankia* forms a symbiotic association with the non-leguminous plants *Alnus*, *Casuarina*, fixing nitrogen for them while obtaining organic nutrients in turn. Mycorrhizae associate with plant roots for carbohydrates, while in return helps the plant to absorb water, N, P and other soil nutrients better by increasing the surface area of the root. Its extended internal endomycorrhizal symbiosis helps plant resist different environmental stresses while enhancing the soil structure by forming the essential hydro-stable aggregates (Selim and Zayed, 2017).

An example of fungal commensal (where one partner benefits while

the other maintains neutrality) is *Phanerochaete* that degrade lignin and other recalcitrant organics to be used by others. These specialised fungi produce extracellular enzymes to convert the cellulose and lignin to help improve and strengthen the nutritional status and microbial activities in the soil thereby ensuring plant growth and health. Such commensal associations are quite common and elaborative in the detritus food chain (the decomposers group, [Selim and Zayed, 2017](#)).

Another common association with nature is antagonism where one organism directly or indirectly prohibits the activities of the other. A well-known industrial application of this is in the antibiotic production. *Bacillus*, *Pseudomonas fluorescens* and *Streptomyces* sp. in soil produce antibacterial and antifungal antibiotics that prevent the growth of plant pathogens. *Thiobacillus* sp. reduces the soil pH up to two that inhibit the growth of pH-sensitive phytopathogens. The O₂ produced by algae partner in lichen prevent the anaerobic microbes from colonising. Fungi occasionally produce cyanide at a concentration that is toxic to other microbes ([Selim and Zayed, 2017](#)).

Just because a microorganism is beneficial to the soil, environment and plant, it could not be considered as a potential participant in the formulation of EM consortium. For instance, could the two (N₂-fixing and nitrifying) microbial groups useful in nitrogen cycle be good candidates for EM formulation? The answer is yes for the former and no for the latter. This is because of the excruciatingly long generation time of the latter on one hand and the relatively small role nitrification plays in the nitrogen cycle on the other. Based on these various understandings, this review attempts to provide here a gist of the various qualifying criteria for an identified potential microorganism as a good EM participant as:

1. They should be environment friendly, ideally non-invading ([Condor et al., 2007](#)).
2. They are easily available in the natural environment, in most type of soils ([Condor et al., 2007](#); [Marshall and Levy, 2011](#)).
3. The microbes must be able to coexist together and be mutually beneficial ([Condor et al., 2007](#); [Marshall and Levy, 2011](#)).
4. Their growth condition requirements should be broad-based ([Condor et al., 2007](#); [Saidia et al., 2010](#); [Marshall and Levy, 2011](#)).
5. They should be easy to culture in lab conditions ([Condor et al., 2007](#); [Saidia et al., 2010](#); [Marshall and Levy, 2011](#)).
6. Their generation time should be short ([Condor et al., 2007](#); [Saidia et al., 2010](#)).
7. They could be mass-cultured on cheap C- and N- sources, such as organically-rich wastes ([Marshall and Levy, 2011](#); [Condor et al., 2007](#)).
8. Their stress-tolerance levels should be high ([Marshall and Levy, 2011](#); [Condor et al., 2007](#); [Saidia et al., 2010](#)).
9. Ideally, they should be forming resting/latent forms, such as spores ([Marshall and Levy, 2011](#); [Condor et al., 2007](#); [Saidia et al., 2010](#)).
10. They should be safe (non-pathogenic) to the human handlers ([Condor et al., 2007](#); [Saidia et al., 2010](#)).

9. Role of prebiotics in formulated EM

Prebiotic, the liquid organic carrier material, is the food ingredient or nutrient-base that sustain the plant growth promoting microbes (PGPM). Prebiotics are carrier substrates of EM that support their viability for a certain period (at least till a declared period of 'shelf-life'), and increase their sustainability in adverse conditions. Prebiotic is sourced either from natural sources, or it can also be synthesised, the cost and availability being the two important factors for choice. The characteristics of a good carrier include its substantial availability, moisture absorption capacity, ease of processing, free of lump-forming materials, ease of sterilising (e.g., autoclaving, gamma-irradiation), cost effectiveness, and good pH buffering capacity.

9.1. Carriers/prebiotics

Liquid formulations employ carrier materials like molasses, humic acid and landfill leachates etc. ([Saidia et al., 2010](#); [Reddy and Saravanan, 2013](#)). Molasses is a carbohydrate-rich liquid carrier obtained in the form of residual syrup from processing of sugar beet and sugarcane. Molasses has been used in EM-1, EM-A, EM-5 and EM-FPE liquid formulations. EM-1 is brownish liquid, containing water, molasses, and a mixture of microbes (LAB, yeast, and phototrophs). EM-1 acts as a core product (component) to produce other EM products ([Saidia et al., 2010](#)). EM-A is an activated EM-1, produced by mixing EM-1 with more molasses and water and fermenting for about 3–5 days until the pH attained 4.0. For instance, 22 gallons of activated EM (EM-A) was produced from a gallon of EM-1 in the proportion of 1:1:20:EM-1:water: molasses. To prepare EM-5, 100 ml of molasses was added to 600 ml of water, followed by 100 ml vinegar, 100 ml distilled spirit and 100 ml EM-1. EM-5 is applied directly to plants to prevent pest infection. They could be applied by spraying on the crop prior to the incidence of pests. EM-FPE or FPE is prepared by adding 2–3 kg chopped fresh grasses or weeds, 14-L water, and enough molasses to 420 ml EM-1 and fermenting for up to 10–14 days. After fermentation, the liquid is extracted and used as liquid-formulated fertiliser, often diluted with water before application. It is applied by watering into the soil or by spraying.

Humic acid is a popular carrier for a number of EMs. Primarily produced by biodegrading lignin and humin-rich dead organic material, it enhances soil fertility, stimulates microbial activity, enhances seed germination, breaks down compact soils that enhance water penetration and better root growth, increases fertiliser retention, enhances P uptake, increases bioavailability of micronutrients through chelation and increases the organic content of soil ([Reddy and Saravanan, 2013](#)). Seaweed liquid fertiliser (SLF) and humic acid (HA) at different concentrations have been effective for the growth of *Abelmoschus esculentus*. EM was maximally effective with 0.4% HA and 8% SLF (8.5:0.5) combination ([Prakash et al., 2018](#)). In soil, humic substance such as K humate acts as 'soil conditioners' to enhance the growth of beneficial microbes, deactivate toxic metals, improve soil structure and texture, increase nutrient and micronutrient uptake, make plants resistant to abiotic stress, including salinity, and, as a result, increase growth, yield and product quality of crops ([Lyons and Genc, 2016](#)). Nourish-L is a humic acid containing natural carbon from marine animal and plant sources. It also contains lignin, a high-quality nutrient for beneficial fungi and bacteria, which strengthens plant cell walls and enhances nutrient absorption and water-binding capacities.

Landfill leachate is rich in organic matter and nitrogen contents and is used as a biomanure. It's a heterogeneous mixture of refractory organic compounds, inorganic contaminants, heavy metals, humic and fulvic acids, and high nitrogen contents effective for microbial and plant growth. Its significantly higher carbon and nitrogen levels also stimulate the growth of useful microbes. Thus, this is also a potential prebiotic.

[Daly and Stewart \(1999\)](#) reported about the application of the EM formulation (bacteria, yeast, fungi and actinomycetes) as probiotic with molasses the prebiotic. Foliar application of EM (photosynthetic and lactic acid bacteria, yeast and actinomycetes) improved nodulation and yields in pea ([Javaid, 2006](#)). Foliar application of EM along with NPK supplement enhanced the nodule numbers (217%), and nodule biomass (167%) in pea ([Javaid, 2006](#)). EM containing *Bradyrhizobium japonicum* along with NPK supplement enhanced the grain yield by 48% ([Javaid, 2009](#)), while the grain yield in pea increased by 126% through NPK-supplemented foliar application and 145% through green manure-supplemented foliar application. Organics-supplemented EM application enhanced the yields and nodulation in bush bean, especially in soil with low C:N ratio ([Olle and Williams, 2013](#)). They also discussed that the yields of spinach and costmary increased respectively by 10.4–24.8 and 19.4–32.9% with EM and EM-fermented compost treatments in greenhouse condition. Autumn yields increased respectively by 23.5–57.9 and 38.8–47.2% in Chinese cabbage and radish ([Olle and](#)

Williams, 2013).

10. Synthetic biology and EM

Recombinant DNA technology and mutagenesis have been employed to produce recombinant plant growth promoting microbes (PGPM). PGPM produces a wide range of secondary compounds like siderophores, antibiotics, volatile metabolites, enzymes, etc. Recombinant DNA technology and mutagenesis are a widespread technique to transform wild microbial strains to recombinant ones with acquired active/noble properties.

Pseudomonas, including both pathogenic and saprophytic species, are cosmopolitan in distribution, and are environmentally significant. *Pseudomonas fluorescens* produces siderophore under Fe-limiting conditions and inhibit spore germination and growth of phytopathogen *Fusarium solani*. The mutant strain obtained through *N*-methyl-*N*-nitro-*N*-nitrosoguanidie (NTG) mutagenesis of *Pseudomonas fluorescens* produce 2.3 times more siderophore (Lim et al., 2002). This mutant enhanced plant growth 1.5 times compared to its wild counterpart. Study of the outer membrane of the mutant strain revealed the presence of receptors for Fe- and heterologous siderophores (Lim et al., 2002). *Pseudomonas fluorescens* a root coloniser produces 2,4-diacetylphloroglucinol (DAPG) which suppresses take-all (root rot) in wheat. The strain was engineered by transforming with a seven-gene operon to synthesise phenazine-1-carboxylic acid (PCA) to prevent *Rhizoctonia* root rot. The recombinant strain produced antifungal metabolites DAPG and PCA. The strain could effectively suppress *Rhizoctonia* root rot at a dose of only 10² CFUs, one to two orders of lower magnitude dose compared to the wild (Huang et al., 2004).

Root colonisation of pseudomonads was examined by manipulating a 6.5 kb region involved in flagellar filament synthesis (necessary for motility) (Capdevila et al., 2004). The region was consisted of genes such as *fliC*, *flaG*, *fliD*, *fliS*, *fliT* and *fleQ* and a part of *feS* gene. The manipulated process generated several non motile (affected in *fliC*, *fliS*, *fleQ*) and reduce motile (affected in *fliT*) mutants. These mutants were completely displaced from the root tip, suggesting the wild type motility is necessary for root colonisation. A mutant affected in *flaG* was obtained, possessed no flagella, but retained the motility and colonisation properties as wild type. In rich medium or absence of iron limitation, the mutant strain showed higher motility, suggesting the possibility of improving motility and root colonisation by gene manipulation (Capdevila et al., 2004).

Pseudomonas fluorescens can suppress plant pathogen *Pythium utimum*. Using genetic engineering approach, using mutagenesis and sequencing methods, the genes involved in the biocontrol activity of *Pseudomonas fluorescens* against *Pythium utimum* were determined. The genes involved in the biocontrol activity included a *gcd* gene encoding glucose dehydrogenase, genes encoding its coenzyme pyruroloquinole quinone (PQQ) and two genes *sup5* and *sup6* appearing to be organised in a putative operon (Kremmydas et al., 2013).

There is a word of caution. The genetic engineering approach may act negatively in promoting plant growth. In such cases, the wild strain is preferred over the engineered strain. For example, 2,4-diacetylphloroglucinol (DAPG) acts as a signal by which the beneficial Pseudomonads stimulate plant-beneficial activities of *Azospirillum*. On roots, the DAPG-producing *Pseudomonas fluorescens* F113 strain enhanced the colonisation (cell motility, biofilm formation, and poly- β -hydroxybutyrate production) of *Azospirillum brasilense* Sp245-Rif in the root and phytostimulation (auxin production) which in turn stimulated plant growth (Combes et al., 2011). In contrast, the mutant strain did not have a similar effect on *A. brasilense*.

Agrobacterium tumefaciens causes crown-gall disease in a wide range of crops by transferring a part of its DNA (T-DNA), into the plant cell (Barton et al., 2018). pathogenic *Agrobacterium tumefaciens* strains are susceptible to the bacteriocin from *A. radiobacter* K84 (agrocin 84). Additionally, *Agrobacterium radiobacter* K84 prevents pathogenesis by

competing for nutrients (competitive exclusion). This prompted *A. radiobacter* K84 as the first commercial biological control agent against crown gall disease (Barton et al., 2018; Penyalver et al., 2009). *Medicago sativa* (alfalfa) exposed to genetically modified *Sinorhizobium* (*Rhizobium*) *meliloti* expressing the *Klebsiella pneumoniae* *nif A* gene showed better root nodulation compared to the wild *S. meliloti* (Dicenzo et al., 2018). Galleguillos et al. (2000) reported that the recombinant *S. meliloti* enhanced the plant biomass as compared to the wild-type strain. Bean plants inoculated with haemoglobin-containing *Rhizobium etli* had 68% more nitrogenase activity than wild *Rhizobium etli* inoculation in greenhouse conditions. It led to 25–30% and 16% increase in nitrogen contents respectively in the leaves and the seeds (Glick, 2012).

11. Conclusion

Effective microbes fix atmospheric nitrogen, supplement the plants with phosphorus, potassium, and other soil nutrients, thereby enhancing plant growth and yield. Besides solubilising phosphorous and potassium, their release of trace elements, secretion of antioxidants, exopolysaccharides, bioactive compounds (vitamins, hormones and enzymes) stimulate crop growth and productivity. Biocontrol metabolites of some of these protect the crops from harmful microbes as well as various abiotic stresses like salinity and drought. A formulated EM is primarily a combination of PSB, LAB, fermentative fungi, yeast and actinomycetes, etc. sourced from microbial type culture collection centres worldwide, or preferably from nature.

The prebiotics (carrier substrate) base in the formulation, acting as a nutrient-base for the microbial consortium, is usually molasses, humic acid and/or landfill leachate. Similar to the probiotic (candidate microbes in the EM), the prebiotic must also fulfil certain criteria for the purpose, though. As understood and experienced, owing to the variations in agro-climatic environment between regions, there cannot be a universal EM for global application. A suggestible approach for commercial exploitation of this microbial technology, therefore, could be to formulate a base (prebiotic) that could be fortified with region-specific microbial consortium (probiotic) later before field applications.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101326>.

Conflicts of interest

The authors declare no competing and/or conflict of interests.

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