Chapter 9
Strategies to Evaluate Microbial Consortia for Mitigating Abiotic Stress in Plants

Sarita Sharma, Zalak R. Rathod, Ritika Jain, Dweipayan Goswami, and Meenu Saraf

Abstract Abiotic stress is the most significant constraint to agricultural productivity. Crop plants must deal with adverse external pressures caused by environmental conditions through their internal biological systems, leading to a loss in development, growth, and productivity. Plant-associated microbes are crucial to crop yields. Although numerous studies have shown that single bacteria can benefit plants, it is becoming increasingly clear that when a microbial consortium—two or more associating microorganisms are implicated, synergistic or additive results can be predicted. Microbial consortia, which are being assessed as a strategy for applications in a range of fields, must be characterized and managed. In this review, we propose a step-by-step technique for identifying whether the plant growth-promoting microorganisms (PGPMs) included can form viable microbial consortia for future application, and if so, how to establish the ideal combinations. To determine the optimal consortia combinations, different techniques were used, in which diverse PGPMs with host growth-supporting features were explored to evaluate if they could function in cohesion and offer a cumulative effect toward better plant growth promotion. To evaluate the valuable microbial consortia, tests for compatibility, response to external stimuli (pH, temperature), generation time, a unique and rapid plant bioassay, and pot experimentation strategies should be employed. Scanning electron microscopy (SEM) and transmission electron microscope (TEM) methods can be employed to confirm the presence of microbial consortia on the roots of plants. The microbial consortium found in the root microbiome stimulates plant growth by regulating the synthesis of phytohormones, osmolytes, organic acids, increased nutrient intake, and an enhanced antioxidant system, all of which help plants to cope with stress. In this review, we cover the numerous strategies that can be used to develop the most competent consortia and their prospective application in managing abiotic stress.

S. Sharma · Z. R. Rathod · R. Jain · D. Goswami · M. Saraf (✉)
Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India
e-mail: msaraf@gujaratuniversity.ac.in

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_9

Keywords Microbial-consortia · PGPMs · Rapid plant bioassay · SEM · TEM · Abiotic Stress

9.1 Introduction

Since the onset of civilization, agriculture has been the most important source of income (Gouda et al. 2018). Food security has been one of society's primary issues for a long time, and any element that threatens it has been one of society's challenges. With an increasing population rate and an unsustainable traditional agricultural system, farmers and the government are struggling with how to produce enough food to fulfill global demand (Prajapati et al. 2022a, b; Khoshru et al. 2020). According to the FAO, agricultural land covers 38.47% of the world's land area, and while 28.43% of that land is arable, only 3.13% is permanently used for crop production. The issue has deteriorated as 20–25% of land worldwide is degraded each year, with another 5–10 million hectares destroyed each year. The movement of nutrients, energy, and carbon between soil organic matter, the soil environment, the aquatic ecosystem, and the atmosphere has a significant impact on agricultural productivity, water quality, and climate change (Gouda et al. 2018).

Abiotic stresses are major constraints of plant growth and development, which in turn affects crop yield, food quality, and global food security. Under stress conditions, numerous parameters such as biochemistry, molecular biology, and physiology of plants are affected. The use of chemical pesticides and inorganic fertilizers causes environmental pollution and degradation of soil fertility. During the stress period, the plant releases certain exudates that can act as a signaling mechanism to alter or create a healthy rhizosphere soil community (Shaikh et al. 2022; Prajapati et al. 2022a, b).

A well-studied and sustainable alternative for improving plant growth and soil fertility is the application of plant growth-promoting bacteria (PGPB) as biofertilizers, which possess functional traits that regulate the growth, development, and productivity of crops. These growth-promoting effects are due to the improvement of the availability and biosynthesis of several limiting macro- and micronutrients, as well as crop protection against stressful environmental conditions. Plant growth-promoting microorganism (PGPM) is a term that applies to all microorganisms (e.g., bacteria, actinomycetes, fungi, and algae) that have a beneficial effect on plant growth through the action of either direct or indirect mechanisms (e.g., mineral nutrition, ethylene reduction, disease suppression). PGPMs have a significant role in sustainable agriculture. They increase the production of various crops, improve soil fertility, promote diversity and interaction with other beneficial microorganisms, inhibit the growth and infective action of potential pathogens, and generally maintain the sustainability of the systems (Prajapati et al. 2022a, b; Santoyo et al. 2021).

The application of microbial consortia to agricultural fields is an innovative natural approach, which can help plants tolerate different stress conditions and

enhance plant growth as compost is made up of diverse microbial consortia that can function in different temperature segments (Sathiavelu 2021).

9.2 Strategies for the Development of Microbial Consortia/Rhizobacterial Consortia

9.2.1 What Are Microbial Consortia?

Rhizobacteria that stimulate plant growth are a symbiotic association between plants and microbes found in the rhizosphere that boost plant growth (Rochlani et al. 2022). The roots are referred to as rhizomes, and the surrounding environment is referred to as spheres. The rhizosphere is the zone of soil that surrounds a plant's root system. The zone, which is around 1 mm wide, has no defined edges. Rhizobacteria are bacteria found in the rhizosphere that can create an environment for roots (Rochlani et al. 2022; Jha and Saraf 2015). The varied microbial communities of the rhizosphere enable the formation of microorganisms that can stimulate plant growth under abiotic conditions via direct and indirect mechanisms (Rochlani et al. 2022; Shaikh et al. 2022; Saraf et al. 2017).

Currently, agriculture is heavily dependent on mineral fertilizers and inorganic pesticides (inorganic), and the impact of their continuous application is reflected in deteriorating soil health and increased resistance to pests and pathogens (Prajapati et al. 2022a, b). In the past 40 years, usage of nitrogen fertilizers has increased by sevenfold and pesticide usage by threefold. In the future, these trends will continue unabated, as the application threefold of both inorganic fertilizers and pesticides is expected to increase by an additional threefold by 2050, which will cause unprecedented damage to the agroecosystem (Sekar et al. 2016). Engineering the plant rhizomicrobiome is an alternative approach to increasing soil health and enhancing plant productivity (Pindi and Satyanarayana 2012). Microbial interaction in the rhizosphere provides plants with multiple plant growth-promoting traits and different stress-tolerant traits apart from enhancing their own population and function (Sekar et al. 2016; Keswani et al. 2014). The inconsistency in the performance of a single microbial product in field application has emphasized the need for co-inoculation or consortia of the microbial products (Santoyo et al. 2021).

Although numerous studies have shown that single microorganisms can benefit plants, it is becoming increasingly clear that when a microbial consortium (mixed culture)—two or more interacting microorganisms—is involved, additive or synergistic results can be assumed. This is owing, in part, to the fact that multiple species can perform a range of activities in an ecosystem like the rhizosphere. The use of mixed cultures of beneficial microorganisms as soil inoculants is based on the principles of natural ecosystems, which are sustained by the quality and quantity of their inhabitants and specific ecological parameters, i.e., the greater the diversity and number of inhabitants, the higher the order of their interaction and the more

stable the ecosystem (Higa 1994). The mixed culture technique is essentially an attempt to apply these ideas to natural systems such as agricultural soils in order to alter the microbial balance in favor of enhanced plant growth, productivity, and protection (Santoyo et al. 2021; Higa 1994;).

Beneficial plant growth stimulation mechanisms include increased nutrient availability, phytohormone modulation, biocontrol, and biotic and abiotic stress tolerance exerted by various rhizosphere microbial players such as plant growth-promoting bacteria (PGPB) and fungi such as *Trichoderma* and *Mycorrhizae* (Prajapati et al. 2022a, b; Santoyo et al. 2021).

The influence of different PGPR strains on plants has been thoroughly investigated in recent years, leading to the commercialization of a significant number of microbial inoculums (Santos Villalobos et al. 2018; Reed and Glick 2013). The construction of bacterial consortia has received interest as a feasible technique for sustainable food production to improve the beneficial capabilities exhibited by these bacteria. In rare circumstances, a consortium of several strains of the same species can display improved activity and be considered. Due to their coverage of a varied set of plant growth promotion and biological regulatory mechanisms, bacterial consortia have been shown to boost beneficial traits in plants as compared to individual strains (Ju et al. 2019). The adoption of these consortia is a viable technique for improving agricultural crops under drought (Joshi et al. 2020), salinity (Sharma et al. 2022c; Nawaz et al. 2020), heavy metal (Prajapati et al. 2022a, b), nutrient uptake (Rana et al. 2012), pests, and phytopathogenic diseases (Villa-Rodriguez et al. 2019). Furthermore, some bacterial consortiums can fix nitrogen, convert some inaccessible nutrients into assimilable forms, produce phytohormones, and chelate iron, all of which are important in maintaining soil quality and health; these can also mitigate the negative effects of some conventional nonsustainable farming techniques (Shaikh et al. 2022).

Rhizobacterial consortia are classified into two types: simple and complex. The fermentation method or protocol (generation of a large population of bacteria to be later made into an inoculant), in which strains are grown individually or in combination with other species/strains in a suitable medium for all PGPR species (Bashan and Prabhu 2020), is the difference. This is an essential stage because a greater number of species often results in a greater number of interactions between strains, resulting in changes in metabolite secretions. The effectiveness of bacterial consortia in field conditions, on the other hand, is reliant on the type and function of strains utilized, where some elements demand special consideration, such as tolerance to severe climatic conditions, survival, and persistence in the soil after inoculation (Gosal and Kaur 2017; Verbruggen et al. 2013;).

The source of the strain isolation influences the selection of these strains because consortium members must grow in the environmental conditions (soil type, host, and climate) where they will be applied. Additionally, when two or more strains form a rhizobacterial consortium, each strain not only competes with the others functionally for plant growth promotion but also complements the others for soil and/or plant establishment (Sharma et al. 2020; Ney et al. 2018; Morriën 2016; Pandey et al. 2012) (Fig.9.1).

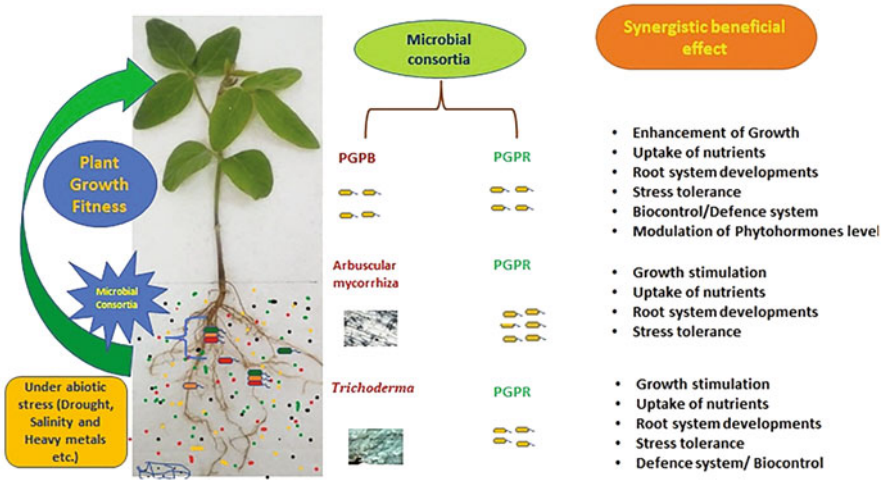

Fig. 9.1 Combining different rhizosphere microorganisms to form microbial consortia can promote plant growth, development, and nutrient uptake while also improving the plant defense system against diseases and enhancing tolerance to various environmental stresses

Though the concept of the consortium is theoretically feasible, developing a consortium is a challenge for researchers due to factors such as the mutual compatibility of microbes, their reliance on one another, and the task of maintaining inoculum potential while not depleting plant resources excessively during mutualism/symbiosis. There is currently no conventional or experimentally confirmed process for screening and choosing promising consortia among a vast number of theoretically feasible consortia. The traditional hit-and-trial strategy yields a vast number of combinations and time-consuming approaches (at least 4 months), which are applied at random, leaving room for scientific improvisation. To address this specific issue, we explored the various methodologies for evaluating rhizobacterial microbial consortia (Fig. 9.2), as outlined below.

9.2.1.1 Step 1: Analysis of Traits of Plant Growth-Promoting Rhizobacteria

Beneficial bacteria and fungi that act as plant growth-promoting microorganisms (PGPMs) can alleviate stress and stimulate plant growth in two ways: indirectly by inducing defense mechanisms against phytopathogens and/or directly by solubilizing mineral nutrients (nitrogen, phosphate, potassium, iron, and so on), producing plant growth-promoting substances (e.g., phytohormones), and secreting specific enzymes (e.g., 1-aminocyclopropane1-carboxylate deaminase).

To prepare possible rhizobacteria consortia, all selected rhizobacteria could evaluate for plant growth-enhancing properties. The following are plant growth-enhancing attributes:

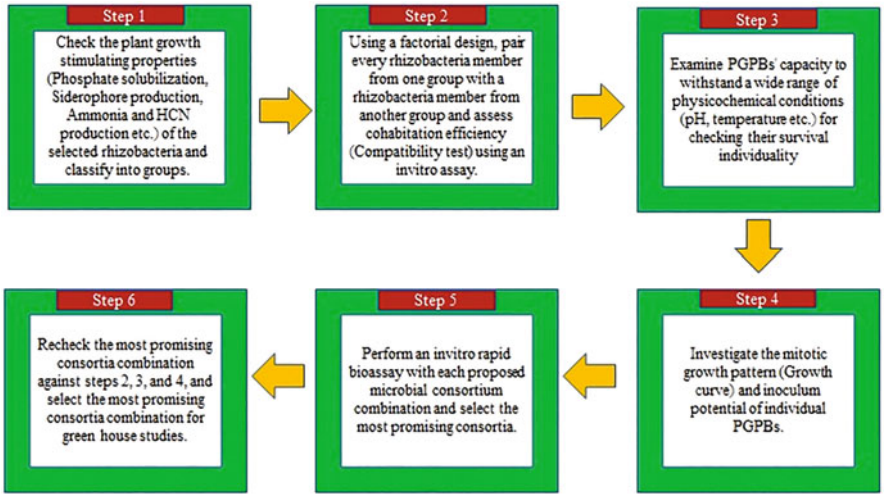

Fig. 9.2 The "strategies" for selecting the most promoting consortia of plant growth-promoting bacteria

(a) Phosphate solubilization: The ability of rhizobacteria to solubilize insoluble phosphates has been investigated using Pikovskaya's medium (Pikovskaya 1948). Each rhizobacterial culture spot could subsequently be inoculated in the center of Pikovskaya's media agar plates with tricalcium phosphate [$Ca_3(PO_4)_2$] as an insoluble phosphate source. Rhizobacteria that can dissolve insoluble phosphates will generate halos. Using the diameter of clearing halo zones, the P solubilization index (PSI) is determined using the formula below (Rathod and Saraf 2021a; Jain et al. 2020).

$$\text{Phosphate solubilization index (PSI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

(b) Siderophore production: Iron [Fe (III)] is required by all organisms as a cofactor for numerous critical metabolic activities. Siderophores are low-molecular-weight organic ligands secreted by soil microorganisms that bind to iron and release it for microbial absorption. Iron shortage in metal-stressed soils can be remedied using siderophores generated by various bacterial genera. Siderophores increase IAA production by chelating hazardous metal species, and IAA has been shown to benefit metal remediating plants. The CAS agar method is used to determine siderophore production (Schwyn and Neilands 1987). In the dark blue medium, the formation of a bright zone with yellowish (hydroxamate), pinkish (catecholate), and whitish (carboxylate) colors indicated the production of siderophore (Saraf et al. 2017).

(c) Hydrogen cyanide and ammonia production: The production of HCN and ammonia is regarded as an indirect plant growth enhancer. A volatile chemical

with antifungal qualities is HCN. In addition to helping the host plant meet its nitrogen needs, ammonia production can help prevent disease invasion. HCN's strong toxicity against phytopathogens makes it a popular biocontrol agent in agricultural systems. However, HCN is also used to chelate metal ions and therefore indirectly contributes to the availability of phosphate (Mahmud et al. 2021). Alström and Burns (1989) found that the synthesis of HCN by rhizobacterial culture could be determined by the color change of filter paper. A change in the color of the filter paper from yellow to light brown, brown, or reddish-brown had been recorded as a weak (+), moderate (++), or strong (+++) production of HCN. NH_3 production could be determined by the method described by Cappuccino and Sherman (1992). The formation of yellow to brown precipitate showed the presence of NH_3 (Trivedi et al. 2018; Jha and Saraf 2011).

(d) Indole acetic acid production (IAA): The synthesis of indole acetic acid was determined using the method described by Bric et al. (1991). The pink color that developed after adding Salkowsky's reagent to cell-free supernatant was spectrophotometrically measured (Shah et al. 2020; Patel et al. 2012).

(e) Biocontrol activity: The agar diffusion method can be used to assess antibacterial and antifungal activity (Sharma et al. 2022b; Thakkar and Saraf 2015).

9.2.1.2 Step 2: Compatibility Efficiency Studies

Individual plant growth-promoting rhizobacteria in the consortia must cultivate in synchrony to exert synergistic effects on plant growth. For that, a paired-wise growth performance study could be conducted in vitro to examine the presence of any antagonism among individual members of the two and more plant growth-boosting rhizobacteria groups. The compatibility efficiency assay has been constructed so that every PGPM member in group I received challenges with every other PGPM member in group II and group III and more. Overnight-grown broth cultures of the relevant PGPMs (one from each group I and II) could be streaked in two halves of nutrient agar. After incubation at optimum temperature, all plates were evaluated for the presence of any zones of inhibition at the colony borders where the two cultures intersected. If the counterpart did not show any zone of inhibition, it suggests the absence of any diffusible toxins or volatile substances that could cause antagonism against each other (Rathod et al. 2020; Rathod and Saraf 2021b; Prasad and Babu 2017). This study provides proof that the tested plant growth-boosting rhizobacterial consortia are growing in a mutually noninhibitory manner, paving the way for further research on microbial consortia.

9.2.1.3 Step 3: Sensitivity to Physical and Chemical Conditions

Temperature responses might have been measured in the 20–45 °C range, while pH can be studied at optimal temperatures in the 5–11 range (with unit interval). In both

trials, an aliquot of overnight-developed plant growth-promoting rhizobacterial cultures has been employed as inoculum. Each experiment was carried out in triplicate to ensure that the results appeared reproducible. Rhizobacteria growth has been assessed spectrophotometrically after overnight incubation at various temperatures and pH levels (Sharma and Saraf 2022).

9.2.1.4 Step 4: PGPR Growth and Mitotic Behavior

Generation times of co-habiting rhizobacteria in microbial consortia would ensure a balance in the relative inoculum density of distinct isolates. If one microbial consortia member develops faster, it may deplete the medium's nutrients and provide unsuitable growth circumstances for the other members of the consortia. The same mitotic growth behavior of PGPR consortia supports co-survival ability and their attractiveness as prospective candidates for consortia creation. At the most optimum temperature and pH conditions, the growth kinetics of each plant growth-promoting rhizobacteria can be studied. Overnight-developed plant growth-promoting rhizobacteria culture was inoculated in triplicate in nutrient broth and incubated in a BOD incubator at 150 rpm. Then, at regular intervals, culture broth aliquot could be collected and a growth curve could be produced with time (in hours) on the x-axis and absorbance (OD measured at 600–610 nm) on the y-axis (Sharma et al. 2021; Jha and Saraf 2012). The generation time can be calculated using standard methods. The generation time is the amount of time it takes for the cells (or population) to divide (Todar 2015).

$$\mathrm{G\ (generation\ time)} = \frac{t(\mathrm{time,\ in\ minute\ or\ hours})}{n\ (\mathrm{number\ of\ generations})}$$

$n = 3.3\mathrm{logb/B}$

$$\mathrm{G\ (generation\ time)} = \frac{t(\mathrm{time,\ in\ minute\ or\ hours})}{3.3 \log b/B}$$

t = time interval in hours or minute.
B = number of bacteria at the beginning of a time interval.
b = number of bacteria at the end of the time interval.
n = number of generations (number of times the cell population doubles during the time interval).

9.2.1.5 Step 5: Design of Microbial Consortia

Microbial consortia combinations can be studied for their plant growth stimulating efficiency using a two- and three-factorial design approach, ensuring that each

consortium comprises one member from group I (perhaps rhizobacteria) with two members from groups II and III (may be endophyte) and may be between different rhizobacteria. Under aseptic conditions, equal amounts of overnight-grown cultures of the various PGPMs (~10^8–10^9 cfu/mL) are mixed together to form consortia combinations, which would then be employed for application in agriculture crop and selecting consortia combinations that show significant increments in vegetative growth parameters as compared with noninoculated crop (Sharma et al. 2022c; Mishra and Sundari 2017; Jha and Saraf 2012).

9.2.1.6 Step 6a: Rapid Plant Bioassay

Mishra and Sundari (2017) created a quick plant bioassay technique that has been utilized to assess the microbial community. It is an in vivo plant growth study in which seeds are surface sterilized according to standard protocol, coated with the corresponding consortia combinations, and placed for germination. Mishra and Sundari (2017) established a novel experimental setup termed the "tube-in-tube" approach in their laboratory using germinated seeds with healthy radicals and plumules. The cap of the sterilized Eppendorf tube (1.5 mL) has been removed, and the bottom could be subsequently cut to create an opening. Germinated seeds were then transferred aseptically into this Eppendorf tube, and the assembly could then be placed in an autoclaved glass test tube (50 mL capacity), giving rise to the term "tube-in-tube" (Fig. 9.3).

It is an in vivo plant growth study in which seeds can surface sterilized as per standard protocol and coated with the respective consortia combinations (detailed in seed germination paragraph) and placed for germination. Germinated seeds with healthy radicals and plumules could be selected and transferred to the novel experimental setup called the "tube-in-tube" method developed by Mishra and Sundari (2017) in their laboratory. The cap of the sterilized Eppendorf tube (1.5 mL) could be removed and bottom cut to make an aperture. Germinated seeds could be transferred aseptically into this Eppendorf, and the assembly could place in an autoclaved glass test tube (50 mL capacity), thus drawing its name "tube-in-tube" method. The test tube may hold 10 mL of half-strength modified Melin–Norkrans medium, free of glucose and malt. The entire "tube-in-tube" system could be then closed with sterile cotton to maintain aseptic conditions and incubated for 10 days at 30 2 °C with no sunshine regulation. To keep the root system in the dark, the bottom portion of the complete test tube rack carrying the setup was wrapped in a black sheet of paper (Fig. 9.2). SEM has been used to visually confirm bacterial attachment with plant roots after harvest. The influence of various PGPR consortia on plant growth may be assessed using four parameters: root length (RL), shoot length (SL), root dry weight (RDW), and shoot dry weight (SDW). The geometrical mean for the different combinations of RL and SL could be determined, and deviations among replicates could be reported as standard deviations (Mishra and Sundari 2017). This rapid plant bioassay technique ("tube-in-tube method") proved to be effective for screening a large set of consortia combinations in a short span of time (Fig.9.3).

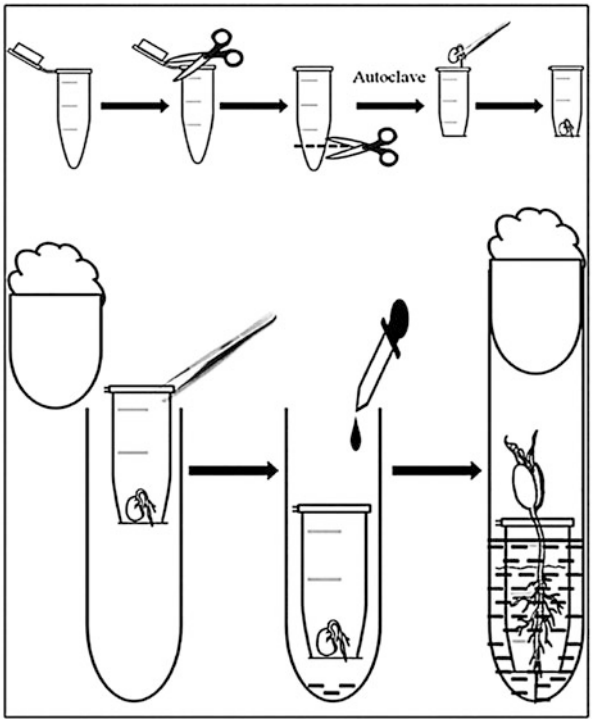

Fig. 9.3 Rapid plant bioassay ("tube-in-tube") approach: (**a**) autoclave Eppendorf (1.5 mL); (**b**, **c**) Eppendorf cap, and bottom removed; (**d**, **e**) Eppendorf germinated seedling; *f*, Eppendorf in an autoclaved test tube with seedling; (**g**) a glass test tube containing medium; (**h**) plugged tube-in-tube system to preserve aseptic state for plant growth (Mishra and Sundari 2017)

9.2.1.7 Step 6b: Pot Experiments

Rhizobacterial consortiums with promising plant growth-boosting properties are evaluated for seed germination. Seeds are surface sterilized with 0.2 percent $HgCl_2$ for 2 min before being washed in sterile distilled water for 10 min. Seedlings for 7–8 h in YEMA/nutrient broth with a pre-screened rhizobacteria consortium combination in log phase containing approximately 10^8–10^9 CFU/mL are held at optimal temperature in a shaker. Control seeds are immersed in a sterile medium. The seeds are then dried aseptically in laminar air flow overnight before being employed in pot experiments. For pot experiments, only sterile soil should be used. Standard protocols could be used to analyze physicochemical parameters. Transfer the sterile soil to pots; the amount of soil used for pot studies is determined by the size of the pots. Standard germination (percentage) of seeds can be counted until no further germination occurs (Rathod et al. 2021; Jha and Saraf 2012). Seedling vigor indices could be determined using the formula proposed by Abdul-Baki and Anderson (1973) as follows: -

$$\text{Seedling Vigour Index (SVI)} = \text{Total Seedling Length (cm)} \times \text{Germination Percentage (\%)}$$

After 1 month, the vegetative growth parameters are assessed to assess the influence of microbial consortia on plant growth in comparison to noninoculated pots. Confirm the presence of microbial consortia on the roots of plants using scanning electron microscopy (SEM) and transmission electron microscope (TEM) techniques.

By applying step-to-step strategies, the best and most manageable number of consortia are thus shortlisted for further field trials to improve productivity in a sustainable manner.

9.3 Microbial Consortia on Plant Roots: Scanning Electron Microscopy (SEM)/Transmission Electron Microscopy (TEM)

Basically, SEM is used in microbiology for analyzing the organism's morphological structure and measurement of size. Nowadays, it is widely used for the observation of microorganisms adhering on plant parts (Root, Shoot, etc.). Trivedi and Saraf (2019) studied endophytes from the *Ricinus communis* plant's stem, leaves, and root. Despite being an excellent tool for investigating ultrastructure, scanning electron microscopy (SEM) is less frequently used than transmission electron microscopy for microbes such as viruses or bacteria. SEM could be used for visual confirmation of bacterial association with plant roots. Olivares et al. (2017) observed the *H. seropedicae* strain HRC54 attached with humic acid plates on the sugarcane leaf surface. Kim and Krcmcr (2005) utilized SEM techniques to detect IAA-producing bacteria viz. *Pseudomonas putida*, *Bacillus megaterium*, *Bradyrhizobium japonicum*, etc., which had been isolated by the IAA screening method based on an in situ membrane assay (Bric et al. 1991) (Fig. 9.4).

Mishra and Sundari (2017) developed the microbial consortia with *Pseudomonas* and diazotrophs applied to *Sorghum bicolor* plant as plant growth-promoting consortium (PGPC). Colonization pattern of primary tomato roots by *Pseudomonas fluorescens* SEM aids in evaluating consortium formation and activity on plants to identify specific microorganisms present in the plant when the consortium can measure multiple microorganisms, as well as their size and structure.

Transmission electron microscopy (TEM) is used for seen internal structure by sending an electron beam across a sample. As a result, an image of the sample's internal structure is created up to 50 million times from its original size. TEM, on the other hand, produces a two-dimensional image. The organism having the ability to accumulate metal has been seen in TEM (Avendaño et al. 2016). Trivedi and Saraf (2019) examined selenium accumulation in endophytic selenobacteria using transmission electron microscopy (TEM) (Fig. 9.5). Sodium selenite had decreased and

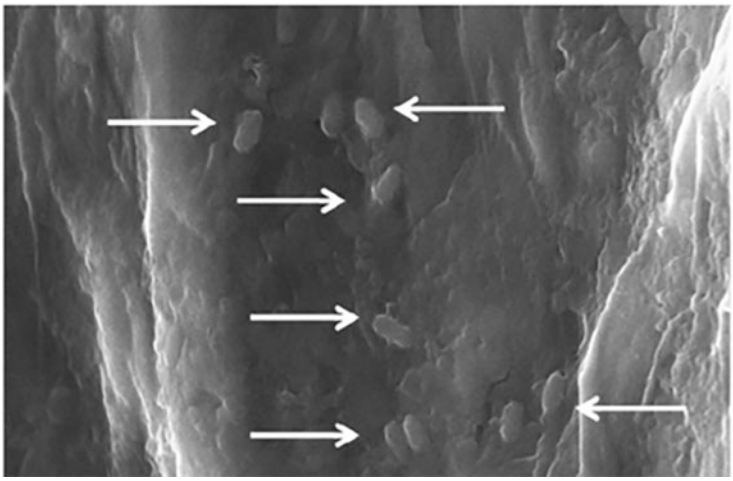

Fig. 9.4 SEM of PGPC association with *Sorghum bicolor* plant roots (Mishra and Sundari 2017)

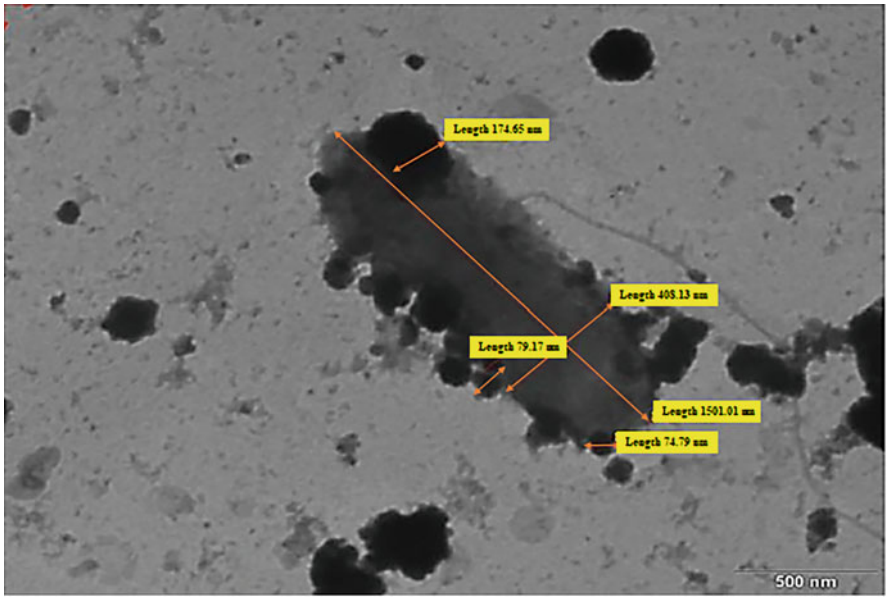

Fig. 9.5 Transmission electron microscopy (TEM) of selenium particle accumulation around endophytic selenobacterial isolates *Parburkholderia megapolitana* sp. *MGT9.* (Trivedi and Saraf 2019)

accumulated in the bacterial cell because of the accumulation of elemental selenium. The bacterial cell revealed red coloring because the reduced form of sodium selenite is red in color. In the presence of selenite, most of the selenium build-up occurred in

the internal cell membrane of bacteria with wavelengths of 174.65 nm, 74.79 nm, and 76.32 nm, according to TEM imaging (Trivedi et al. 2020).

Transmission electron microscopy (TEM) micrographs showed the spherical NPs, which had an average diameter of 12 nm. By mixing it with an aqueous solution of $AgNO_3$, *Argemone mexicana* leaf extract serves as a capping and reducing agent in the creation of AgNPs. Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants were explained by Alabdallah and Hasan (2021). The use of TEM is beneficial in the field of microbiology because it can be used to identify and measure the structure and size of microorganisms that can bind or chelate metals, especially in contaminated sites and waste treatment facilities for dump yards. TEM images can also be used to resolve metal-contaminated fields or accumulate micro and macro metals in crops.

9.4 Role of Microbial Consortia as Efficient Biofertilizer

Microbial inoculants are mixtures that contain live algae, fungus, and bacteria, whether in alone or in a consortium, to boost plant growth and increase agricultural output. Beneficial microbes (algae, fungus, and bacteria alone or in a consortium) in biofertilizers improve soil chemical and biological attributes by fixing nitrogen, cellulolytic activity, iron, or phosphate (Mahmud et al. 2021; Seenivasagan and Babalola 2021). Microbes mostly as biofertilizers accomplish beneficial actions such as phosphorus solubilization, nitrogen fixation, siderophore formation, hydrogen cyanide, and ammonia synthesis, and the production of plant growth chemicals. Because of the presence of these bacteria, plants have antagonistic effects on a variety of phytopathogens (Rochlani et al. 2022; Jha and Saraf 2012). They inhabit the rhizosphere, whether applied to seed, plant surfaces, roots, or soil, and through their biological activity, they improve nutrient bioavailability, boost plant growth, and increase soil microflora. As a result, they are preparations that quickly restore soil fertility (Mahmud et al. 2021; Seenivasagan and Babalola 2021; Jha and Saraf 2015). They are critical elements of integrated nutrient management (INM) strategies for increasing soil productivity and sustainability while also preserving the environment because by being pollution-free, cost-effective, and a source of renewable nutrients to plants to replenish synthetic fertilizers in a sustainable production system (Yadav and Sarkar 2019). According to Panda (2011), the impact of bio-fertilizers on crop improvement ranges from 35% to 65% (Mahmud et al. 2021). The continual application of biofertilizer to the land for 3–4 years can retain fertility due to the efficacy of parental inoculums, which can successfully maintain plant growth and multiplication. They improve the texture, pH, and other characteristics of the soil. Biofertilizers are low-cost, sustainable sources of plant nutrients that are supplemental artificial fertilizers. In comparison to chemical fertilizers, biofertilizers are more environmentally friendly; they can be created from natural sources, are less likely to cause damage, and aid in the development of healthy soil. To some extent, plants are cleansed of chemical fertilizers that are precipitated

(Seenivasagan and Babalola 2021). Depending on their capabilities, such as delivering nutrients to plants and acting as natural pest deterrents, a wide range of microorganisms can be used as biological fertilizers at the industrial level (Rochlani et al. 2022). When considering biofertilizer as a modern agricultural tool, its use is critical as a component of integrated nutrient management, a reduction in the use of hazardous chemicals, a cost-effective source of renewable energy for plants, and a source of renewable energy for plants in sustainable agriculture (Seenivasagan and Babalola 2021).

9.5 Mechanisms as Biofertilizer

Biofertilizers are classified into several categories based on their functional capabilities, such as nitrogen-fixing biofertilizers, phosphate biofertilizers, micronutrient biofertilizers, and plant growth-promoting rhizobacteria, among others. Nitrogen-fixing biofertilizers increase soil nitrogen levels by absorbing atmospheric nitrogen and releasing it to plants. *Azotobacter*, *Nostoc*, *Rhizobium*, and *Azospirillum* are a few examples (Itelima et al. 2018). Phosphate biofertilizers are divided into two types: phosphorous solubilizing biofertilizers (PSB) and phosphorus mobilizing biofertilizers (PMB). PSB dissolves insoluble phosphate from organic and inorganic sources. *Bacillus*, *Pseudomonas*, *Penicillium*, *Aspergillus*, and other bacteria are examples (Etesami et al. 2017). Phosphorus is transferred from the soil to the root cortex via PMB. Arbuscular Mycorrhiza is one example (AM fungi). Micronutrient biofertilizers include silicate and zinc solubilizer bacteria. In soil, these bacteria break down silicates and aluminum silicates. *Bacillus* sp. is one example. Plant growth-promoting rhizobacteria (PGPR) are bacteria that live in the rhizosphere (Upadhyay et al. 2019). The rhizosphere is a thin layer of soil surrounding the roots characterized by high levels of biochemical activities and composed of plants, bacteria, fungi, and soil constituents. They boost plant growth by functioning as bioprotectants, biostimulants, and nutrient enhancers (Fig. 9.6).

The mechanism of action refers to the biological and chemical process by which microorganisms contained in biofertilizers exert their effects on the plant's rhizosphere. Plant growth rhizobacteria can execute a variety of mechanisms that increase plant growth and development, eventually leading to sustainable agriculture methods. Direct mechanisms of these rhizospheric bacteria can increase plant growth by increasing nutrient intake via nitrogen fixation, phosphate solubilization, phytohormone production, and exopolysaccharide production, resulting in sustainable and eco-friendly agri-science perspective. These microorganisms also have an indirect role in plant protection by producing antibiotics, hydrogen cyanide, siderophores, and other biocontrol chemicals (Rochlani et al. 2022; Prajapati et al. 2022a, b; Panchal et al. 2022). Surprisingly, these relationships between plant–root and microbial communities have been labeled as symbiosis. As the former decomposes unavailable nutrients into an available form, the latter benefit from root exudates such as carbohydrates, proteins, sugars, vitamins, mucilage, amino acids, and

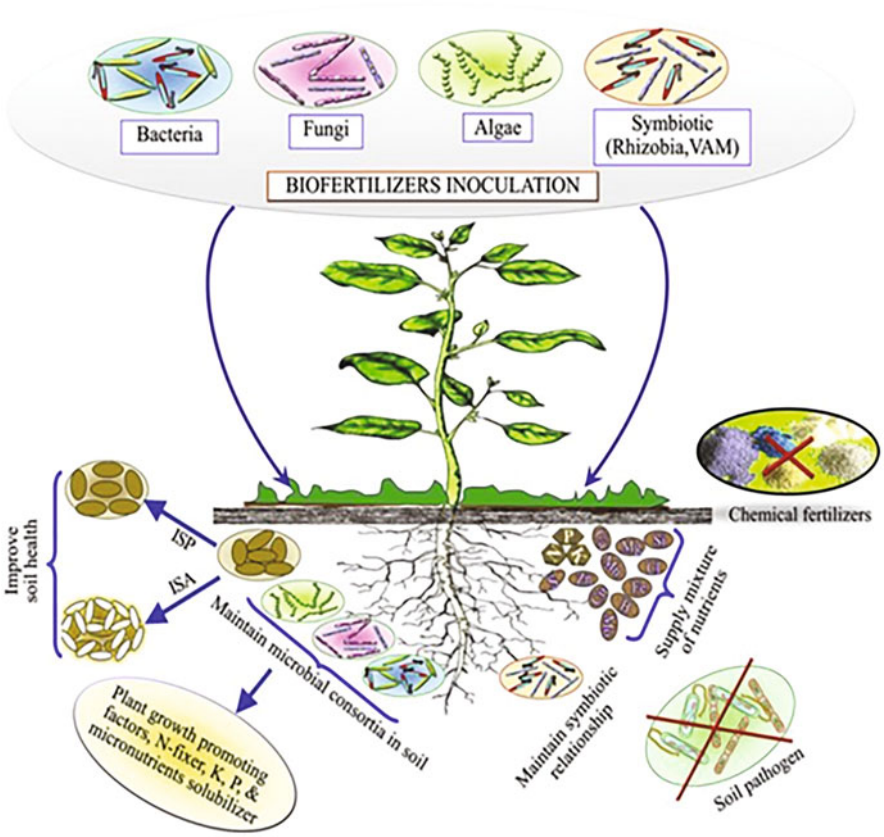

Fig. 9.6 The effect of biofertilizers on plant growth and soil health. [*VAM* vesicular-arbuscular mycorrhiza, *ISA* increased soil aggregation, *ISP* increased soil porosity] (Mahmud et al. 2021). https://www.researchgate.net/figure/Schematic-representation-Influence-of-biofertilizers-on-plant-growth-performance-and_fig1_353131148

organic acids (Vives-Peris et al. 2020), which modify biochemical properties of the rhizosphere by acting as a messenger between the microbes and the plants (Shaikh et al. 2022; Vives-Peris et al. 2020).

9.6 Role of Microbial Consortia to Remediate Abiotic Stress

9.6.1 Abiotic Stress Affecting Crop

There are various types of abiotic stress that affect soil and ultimately crop productivity. These stresses are salinity stress (increase in salts concentration in soil), drought stress (insufficient water availability to plants), heavy metal stress

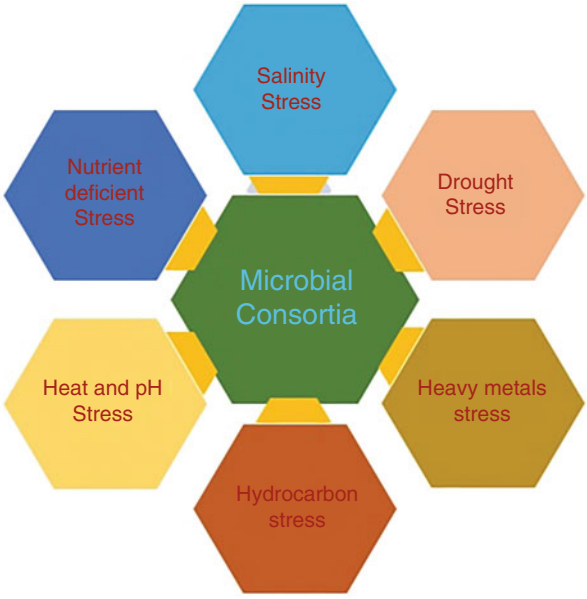

Fig. 9.7 Microbial consortia as biofertilizer to remediate abiotic stress

(excessive harmful metals in soil), temperature stress (very high and very low temperature), and nutrients stress (insufficient nutrients in the soil) (Fig. 9.7). In this review, we describe three major soil stress: drought, salinity, and heavy metals. Drought and salt stress have a complex relationship that affects almost every element of a plant's life. Both these stresses have the most detrimental effects on agriculture (de Oliveira et al. 2013). Stress causes disturbance in photosynthesis, resulting in leaf senescence, the formation of excessive reactive oxygen species (ROS), nutritional deprivation, and the breakdown of cellular organelles and metabolism, all of which result in diminished plant growth (de Oliveira et al. 2013). Another key soil stress is heavy metal stress, which is getting more intense because of a variety of anthropogenic influences (Glick 2010). Unchecked population growth and the industrial revolution are accumulating toxic metals and organic wastes in soil, rendering it unfit for agricultural techniques and detrimental to all living things (Glick 2010).

One of the most common abiotic factors impacting crop plants is water deprivation. Drought stress occurs when the amount of water available in the soil diminishes. Drought produces a variety of harmful consequences on plants that are multifaceted in their effects. From seed germination to maturity and senescence, the plants respond to drought stress at physiological, biochemical, and molecular levels (Tiwari et al. 2017). Because plants need to use groundwater, their root length increases under mild drought stress (Forni et al. 2017), while extremely dry conditions can slow root growth. However, PGPR under stress conditions modifies root architecture and boosts plant nutrient absorption and water drawing ability (Shaikh et al. 2022; Kasim et al. 2013). These rhizobacteria could be able to grow under

stressful conditions and provide a beneficial effect on plants to cope with stressful environments (Jain et al. 2020; Jain and Saraf 2021; Bilal et al. 2018). ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, production of exopolysaccharide (EPS) (Panchal et al. 2022) and volatile organic compounds (VOCs), osmolyte and antioxidant production, enhanced mineral nutrient uptake, phytohormone production, and modulation are among the mechanisms proposed by PGPM to overcome drought stress in plants. The PGPRs are bestowed through these pathways, either singly or jointly, to counteract drought stress in plants (Gontia-Mishra et al. 2020). Microbial consortia of *Pseudomonas putida* NBRIRA + *Bacillus amyloliquefaciens* NBRISN13 mitigates drought stress in chickpea by enhancing physiological parameters such as shoot length, root length, and fresh and dry weight of root and shoot, modulates defense enzymes such as superoxide dismutase, catalase, lipid peroxidase, and enhance soil enzymes activity and microbial diversity in the rhizosphere region under drought stress (Kumar et al. 2016).

Soil salinity is defined a salt level that exceeds the plant's requirements. When the electrical conductivity (EC) in the soil surrounding the root zone reaches 4 dS/m (40 mM NaCl), the soil becomes saline (Egamberdieva et al. 2017). Excessive salt concentrations causing low water availability create drought-like conditions and result in altering the physicochemical features of soil and interfering with nutrient uptake, rendering nutrients unavailable to plants. Salt stress affects plant growth, photosynthetic capacity, CO_2 assimilation, and nitrogen content and leads to ion toxicity, which results in oxidative stress (Liu et al. 2015). However, the combination of compatible yet dissimilar genera of microbes can greatly boost plant growth under saline circumstances and may aid in salinity amelioration (Kapadia et al. 2021). Microbial consortia provide a variety of essential tasks under salinity conditions, including promoting plant growth, acting as osmoprotectants, antioxidants, and biocontrol agents, and reducing stress in the soil. Microbial consortia of four rhizobacteria strains *Bacillus* sp. + *Delftia* sp. + *Enterobacter* sp. + *Achromobacter* sp. helps to overcome the salinity stress in tomato. Consortia alleviate salt stress in tomatoes by increasing plant growth parameters, chlorophyll content, mineral uptake, accumulation, and transportation to a different part of the plant (Kapadia et al. 2021).

Heavy metals (HMs) are described as elements with metallic characteristics and a wide molecular weight range, which includes transition metals. Metal concentrations in soil have risen considerably because of the industrial revolution and human activities (Dabhi et al. 2021; Sharma et al. 2021). Plant metabolism and growth are harmed by the abundant HM in soil, which is absorbed and translocated to numerous organs of plants (Cheng 2003). Excess metals in soil have an adverse effect on soil characteristics and fertility, making it unfit for agricultural uses (Khan et al. 2012). Due to well-known plant growth-enhancing mechanisms such as hormone production (IAA, GA), siderophore generation, nitrogen fixation, and phosphate solubilization, PGPR can be used to help phytoremediation contaminated sites (Ojuederie and Babalola 2017). Heavy metal tolerance and accumulation by plants may be significantly influenced by heavy metal-resistant bacteria living in the rhizosphere. Rhizobacteria isolated from the landfill site and mining areas are able to

tolerate heavy metal stress (Sharma and Saraf 2022). Application of rhizobacteria consortia of *Bacillus cereus* MG257494.1, *Alcaligenes faecalis* MG966440.1, and *Alcaligenes faecalis* MG257493.1 shows tolerance against heavy metals (Cu, Pb, Cd, and Zn), and their application on sorghum mitigate heavy metal stress by increasing the dehydrogenase activity, decreasing metal accumulation in plant parts and soil, also regulating bioaccumulation factor (BAF) of heavy metals (Abou-Aly et al. 2021). Some of the applications of microbial consortia under abiotic stress are listed in Table 9.1.

9.7 Conclusions

Microbial consortium is part of the plant microbiome that interacts synergistically to promote plant growth and health through the production of metabolites with antibiotic activity and by solubilizing nutrients and making them available to the plant, forming nodules to fix nitrogen, and producing plant-growth-stimulating phytohormones or enzymes that degrade ethylene precursors, such as ACC deaminase. This review presents a consortium screening protocol as a step-to-step strategy to develop microbial consortia to construct, evaluate, and shortlist the most potent microbial consortia. The review described a factorial design involving two and more representative groups and has many PGPRs to facilitate the selection of the most auspicious combinations for larger greenhouse trials before developing bio-inoculants. In vivo, rapid plant bioassays are obligatory to evaluate the performances of microbial consortia even when the isolates exhibit similar preference to physiological growth conditions, synergy in co-culture, and high mitotic activity. SEM and TEM help in the evaluation of the development of consortium and their activity on the plant to identify the microorganism, especially present in the plant where the consortium can measure multiple microorganisms, their size, and structure too. Biofertilizers have been used to boost crop production by augmenting the plant's available nutrients through the organic matter decomposition process. Two main reasons necessitate the use of biofertilizers in today's crop production. The first is to increase the use of biofertilizers, which results in the corresponding increase in crop yield, and the second is the long-term use of synthetic fertilizers degrading the soil besides other threats to our health and environment. The efficacy of biofertilizers can be enhanced by sound knowledge and long-time practical experience in a diverse soil type. Application of microbial inoculants, especially consortia, will be one of the solutions to alleviate plant abiotic stress, and enhanced plant growth and productivity under stress conditions have been reported. The directed use of microbial consortia will facilitate the production of plants in a more sustainable way that, eventually, will not depend on agrochemicals.

Table 9.1 Abiotic stress alleviation by microbial consortia

PGPR strain	PGPR traits	Stress	Crop	Effect on plants	References
Consortia of *Aspergillus* sp. S_{11} and S_{17}	Phosphate solubilization and siderophore production	Salinity	Chickpea	Increase in the vegetative parameter such as plant height, no. of lateral roots and leaves, chlorophyll content in chickpea	Urja and Meenu (2010)
Rhizobium tropici CIAT 899 + *P. polymyxa* DSM36		Drought	Common bean	Increase in the production of phytohormones, nodulation rate, nitrogen content, and overall growth of common bean	Figueiredo et al. (2008)
Azospirillum brasilense + *Azotobacter chroococcum*	IAA production and ACC deaminase activity	Heavy metal	Wheat	Consortia of lead-tolerant microbes improve grain yield, proline content, and membrane integrity, while significantly reducing the production of MDA and H_2O_2	Janmohammadi et al. (2013)
Azospirillum + arbuscular mycorrhizal		Drought	Rice	Enhanced stomatal conductance, physiological parameter, and biomass production of rice	Ruíz-Sánchez et al. (2011)
Consortia of four Cr-tolerant PGPR strains RZB-03, RZB-04, BB-A1, and BB-G7		Heavy metal	Mung bean	Increased root length, shoot length, biomass, and chlorophyll content of mung bean	Singh et al. (2010)
Pseudomonas putida NBRIRA + *Bacillus amyloliquefaciens* NBRIS N13	ACC deaminase activity, minerals solubilization, hormone production, biofilm formation, siderophore activity	Drought	Chickpea	Increase in the plant growth parameter, modulates the defense enzymes, soil enzymes, and microbial diversity	Kumar et al. (2016)
*Enterobacter*sp.12+*Enterobacter* sp. 126 + *Serratia* sp. 73	IAA production and ACC deaminase activity	Salinity	Wheat	Increased seedling emergence, shoot and root growth, biomass, and SOD activity	Barra et al. (2016)
Bacillus cereus Y5 + *Bacillus* sp. Y14 + *Bacillus subtilis* Y16		Salinity	Wheat	Improved the gas exchange photosynthetic rate, transpiration rate,	Shahzad et al. (2017)

(continued)

Table 9.1 (continued)

PGPR strain	PGPR traits	Stress	Crop	Effect on plants	References
				stomatal conductance, relative water contents; also enhanced root growth to absorb more soil for nutrient and water uptake, thus increasing the yield of wheat	
Ochrobactrum pseudogrignonense RJ12 + *Pseudomonas* sp. RJ15 + *Bacillus subtilis* RJ46	ACC deaminase activity, IAA, siderophore and HCN producer, phosphate solubilizer	Drought	*Vigna mungo* L. (black gam) and *Pisum sativum* L. (pea)	Inoculation with consortia increase seed germination, root and shoot length, and plant biomass. It also reduced ACC accumulation in plants by downregulating the expression of the ACC-oxidase gene	Saikia et al. (2018)
Bacillus sp. SR-2-1 + *Bacillus* sp. SR-2-1/1	P-solubilization, IAA, and ACC deaminase	Salinity	Potato	Enhance RWC while decreasing antioxidant enzyme activity and MDA content, also regulate Na+/K+ efflux, and higher production of auxin in the rhizosphere improves tuber yield	Tahir et al. (2019)
AM fungi (*Funnelliformis mosseae*, *Claroideoglomus etunicatum*), + *Azotobacter chroococcum*, + *Azospirillium lipoferum*		Drought	Juglans regia L. (walnut.)	Use of consortia decrease the negative effects of drought stress on seedlings by improving growth and nutrient acquisition and increasing the proline, peroxidase activity, phenol, soluble sugar, and starch content	Behrooz et al. (2019)
Acinetobacter pittii + *Acinetobacter oleivorans* + *Acinetobacter calcoaceticus* + *Comamonas testosterone*	Solubilize the insoluble forms of phosphate, potassium, and zinc, and fix N_2 gas	Salinity	Durum wheat	Enhancing the PQ ratio, complete quenching of chlorophyll fluorescence, and the portion of light absorbed by PSII antenna	Yaghoubi Khanghahi et al. (2020)

Pseudomonas putida P45 + *Bacillus amyloliquefaciens* B17; *Pseudomonas putida* P7 + *Paenibacillus favisporus* B30	IAA, gibberellic acid, P-solubilization,	Drought	Sorghum	Improve the seed and Stover yield of kharif sorghum and improve macro- and micronutrients in the soil	Kakde et al. (2020)
B. japonicum USDA 110 + *P. putida* NUU8		Drought	Soybean	Improves growth nutrient uptake and nutrient contents in soybean and soil and also enhance the activities of soil enzymes such as protease and acid and alkaline monophosphoeserase	Jabborova et al. (2021)

Acknowledgments Thanks are due to our DST-FIST Sponsored Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Gujarat, India.

Conflict to Interest The authors declare no conflict of interest.

References

Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria[1]. Science 13(6):630–633. https://doi.org/10.2135/cropsci1973.0011183X001300060013x

Abou-Aly HE, Youssef AM, Tewfike TA, El-Alkshar EA, El-Meihy RM (2021) Reduction of heavy metals bioaccumulation in sorghum and its rhizosphere by heavy metals-tolerant bacterial consortium. Biocatal Agric Biotechnol 31:101911. https://doi.org/10.1016/j.bcab.2021.101911

Alabdallah NM, Hasan MM (2021) Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants. Saudi J Biol Sci 28(10):5631–5639. https://doi.org/10.1016/j.sjbs.2021.05.081

Alström S, Burns RG (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol Fertil Soils 7(3):232–238. https://doi.org/10.1007/BF00709654

Avendaño R, Chaves N, Fuentes P, Sánchez E, Jiménez JI, Chavarría M (2016) Production of selenium nanoparticles in *Pseudomonas putida* KT2440. Sci Rep 6(1):1–9. https://doi.org/10.1038/srep37155

Barra PJ, Inostroza NG, Acuña JJ, Mora ML, Crowley DE, Jorquera MA (2016) Formulation of bacterial consortia from avocado (Persea americana Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Appl Soil Ecol 102:80–91. https://doi.org/10.1016/j.apsoil.2016.02.014

Bashan Y, Prabhu SR, de Bashan LE, Kloepper JW (2020) Disclosure of exact protocols of fermentation, identity of microorganisms within consortia, formation of advanced consortia with microbe-based products. Biol Fertil Soils 56(4):443–445. https://doi.org/10.1007/s00374-020-01464-x

Behrooz A, Vahdati K, Rejali F, Lotfi M, Sarikhani S, Leslie C (2019) Arbuscular mycorrhiza and plant growth-promoting bacteria alleviate drought stress in walnut. HortScience 54(6):1087–1092. https://doi.org/10.21273/HORTSCI13961-19

Bilal S, Shahzad R, Khan AL, Kang SM, Imran QM, Al-Harrasi A et al (2018) Endophytic microbial consortia of phytohormones-producing fungus *Paecilomyces formosus* LHL10 and bacteria *Sphingomonas* sp. LK11 to *Glycine max* L. regulates physio-hormonal changes to attenuate aluminum and zinc stresses. Front Plant Sci 9:1273. https://doi.org/10.3389/fpls.2018.01273

Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57(2):535–538. https://doi.org/10.1128/aem.57.2.535-538.1991

Cappuccino JG, Sherman N (1992) Biochemical activities of microorganisms. Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co., San Francisco, CA, p 76

Cheng S (2003) Effects of heavy metals on plants and resistance mechanisms. Environ Sci Pollut Res 10(4):256–264. https://doi.org/10.1065/espr2002.11.141.2

Dabhi J, Solanki A, Patel R, Sharma S, Saraf MS (2021) Bioremediation of heavy metals: a brand new methodology to sustainable agriculture. Int J Innovat Res Sci Eng Technol 10(6):6031–6049. https://doi.org/10.15680/IJIRSET.2021.1006033

de Oliveira AB, Alencar NLM, Gomes-Filho E (2013) Comparison between the water and salt stress effects on plant growth and development. Respon Organ Water Stress 4:67–94. https://doi.org/10.5772/54223

Egamberdieva D, Davranov K, Wirth S, Hashem A, Abd-Allah EF (2017) Impact of soil salinity on the plant-growth–promoting and biological control abilities of root associated bacteria. Saudi J Biol Sci 24(7):1601–1608. https://doi.org/10.1016/j.sjbs.2017.07.004

Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects a review. J Soil Sci Plant Nutr 17(4):897–911. https://doi.org/10.4067/S0718-95162017000400005

Figueiredo MV, Burity HA, Martinez CR, Chanway CP (2008) Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. Appl Soil Ecol 40(1):182–188. https://doi.org/10.1016/j.apsoil.2008.04.005

Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410(1):335–356. https://doi.org/10.1007/s11104-016-3007-x

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28(3):367–374. https://doi.org/10.1016/j.biotechadv.2010.02.001

Gontia-Mishra I, Sapre S, Deshmukh R, Sikdar S, Tiwari S (2020) Microbe-mediated drought tolerance in plants: current developments and future challenges. In: Plant microbiomes for sustainable agriculture. Springer, Cham, pp 351–379

Gosal SK, Kaur J (2017) Microbial inoculants: a novel approach for better plant microbiome interactions. In: Probiotics in agroecosystem. Springer, Singapore, pp 269–289

Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res 206: 131–140. https://doi.org/10.1016/j.micres.2017.08.016

Higa T (1994) Effective microorganisms: a new dimension for nature farming. In Proceedings of the second international conference on Kyusei nature farming. US Department of Agriculture, Washington, DC, pp 20–22

Itelima JU, Bang WJ, Onyimba IA, Oj E (2018) A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. J Microbiol Biotechnol Rep 2(1):22–28. http://hdl.handle.net/123456789/1999

Jabborova D, Kannepalli A, Davranov K, Narimanov A, Enakiev Y, Syed A et al (2021) Co-inoculation of rhizobacteria promotes growth, yield, and nutrient contents in soybean and improves soil enzymes and nutrients under drought conditions. Sci Rep 11(1):1–9. https://doi.org/10.1038/s41598-021-01337-9

Jain R, Sharma S, Rathod ZR, Shah RK, Lucie KM, Saraf MS (2020) Analyzing the role of phosphate solubilizing bacteria isolated from Rhizospheric soil of Cyamopsis in improving agricultural productivity. Biosci Biotechn Res Commun Spec Issue 13:16–20. https://doi.org/10.21786/bbrc/13.1specialissue/01

Jain R, Saraf M (2021) Exploring the abiotic and biotic stress tolerance potential of rhizobacteria isolated from cyamopsis. J Adv Sci Res 12(03):190–194. https://doi.org/10.55218/JASR.202112327

Janmohammadi M, Bihamta MR, Ghasemzadeh F (2013) Influence of rhizobacteria inoculation and lead stress on the physiological and biochemical attributes of wheat genotypes. Cerc Agron Moldova 46(1):49–67

Jha CK, Saraf M (2011) In vitro evaluation of indigenous plant growth promoting rhizobacteria isolated from *Jatropha curcas* rhizosphere. Int J Genet Eng Biotechnol 2(1):91–100

Jha CK, Saraf M (2012) Evaluation of multispecies plant-growth-promoting consortia for the growth promotion of *Jatropha curcas* L. J Plant Growth Regul 31(4):588–598. https://doi.org/10.1007/s00344-012-9269-5

Jha CK, Saraf M (2015) Plant growth promoting rhizobacteria (PGPR): a review. E3 J Agric Res Dev 5(2):0108–0119. https://doi.org/10.13140/RG.2.1.5171.2164

Joshi B, Chaudhary A, Singh H, Kumar PA (2020) Prospective evaluation of individual and consortia plant growth promoting rhizobacteria for drought stress amelioration in rice (*Oryza sativa* L.). Plant Soil 457(1):225–240. https://doi.org/10.1007/s11104-020-04730-x

Ju W, Liu L, Fang L, Cui Y, Duan C, Wu H (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. Ecotoxicol Environ Saf 167:218–226. https://doi.org/10.1016/j.ecoenv.2018.10.016

Kakde NA, Manjunath M, Hanwate GR, Gourkhede PH (2020) Influence of microbial consortia on yield of kharif sorghum (*Sorghum bicolor* L.) and soil parameters under rainfed conditions. Indian J Dryland Agric Res Dev 35(2):54–56

Kapadia C, Sayyed RZ, El Enshasy HA, Vaidya H, Sharma D, Patel N et al (2021) Halotolerant microbial consortia for sustainable mitigation of salinity stress, growth promotion, and mineral uptake in tomato plants and soil nutrient enrichment. Sustainability 13(15):8369. https://doi.org/10.3390/su13158369

Kasim WA, Osman ME, Omar MN, El-Daim A, Islam A, Bejai S, Meijer J (2013) Control of drought stress in wheat using plant-growth-promoting bacteria. J Plant Growth Regul 32(1):122–130. https://doi.org/10.1007/s00344-012-9283-7

Keswani C, Mishra S, Sarma BK, Singh SP, Singh HB (2014) Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. Appl Microbiol Biotechnol 98(2):533–544. https://doi.org/10.1007/s00253-013-5344-5

Khan MS, Zaidi A, Wani PA (2012) Chromium–plant-growth-promoting rhizobacteria interactions: toxicity and management. In: Toxicity of heavy metals to legumes and bioremediation. Springer, Vienna, pp 67–88

Khoshru B, Moharramnejad S, Gharajeh NH, Asgari Lajayer B, Ghorbanpour M (2020) Plant microbiome and its important in stressful agriculture. In: Plant microbiome paradigm. Springer, Cham, pp 13–48

Kim SJ, Krcmcr RJ (2005) Scanning and transmission electron microscopy of root colonization of *morningglory* (*lpomoea* spp.) seedlings by rhizobacteria. Symbiosis

Kumar M, Mishra S, Dixit V, Kumar M, Agarwal L, Chauhan PS, Nautiyal CS (2016) Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). Plant Signal Behav 11(1):e1071004. https://doi.org/10.1080/15592324.2015.1071004

Liu W, Li RJ, Han TT, Cai W, Fu ZW, Lu YT (2015) Salt stress reduces root meristem size by nitric oxide-mediated modulation of auxin accumulation and signaling in Arabidopsis. Plant Physiol 168(1):343–356. https://doi.org/10.1104/pp.15.00030

Mahmud AA, Upadhyay SK, Srivastava AK, Bhojiya AA (2021) Biofertilizers: a nexus between soil fertility and crop productivity under abiotic stress. Curr Res Environ Sustain 3:100063. https://doi.org/10.1016/j.crsust.2021.100063

Mishra N, Sundari SK (2017) Asix-step-strategy'to evaluate competence of plant growth promoting microbial consortia. Curr Sci 113(1):63–70. http://www.jstor.org/stable/26163908

Morriën E (2016) Understanding soil food web dynamics, how close do we get? Soil Biol Biochem 102:10–13. https://doi.org/10.1016/j.soilbio.2016.06.022

Nawaz A, Shahbaz M, Imran A, Marghoob MU, Imtiaz M, Mubeen F (2020) Potential of salt tolerant PGPR in growth and yield augmentation of wheat (*Triticum aestivum* L.) under saline conditions. Front Microbiol 11:2019. https://doi.org/10.3389/fmicb.2020.02019

Ney L, Franklin D, Mahmud K, Cabrera M, Hancock D, Habteselassie M, Newcomer Q (2018) Examining trophic-level nematode community structure and nitrogen mineralization to assess local effective microorganisms' role in nitrogen availability of swine effluent to forage crops. Appl Soil Ecol 130:209–218. https://doi.org/10.1016/j.apsoil.2018.06.015

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12):1504. https://doi.org/10.3390/ijerph14121504

Olivares FL, Busato JG, de Paula AM, da Silva LL, Aguiar NO, Canellas LP (2017) Plant growth promoting bacteria and humic substances: crop promotion and mechanisms of action. Chem Biol Technol Agric 4(1):1–13. https://doi.org/10.1186/s40538-017-0112-x

Panchal R, Prajapati K, Prajapati M, Sharma S, Saraf MS (2022) Bacterial exopolysaccharides: types, its biosynthesis and their application in different fields. Acta Sci Biotechnol 3(3):1–9

Panda SC (2011) Organic farming for sustainable agriculture, third edn. Kalyani Publisher, New Delhi

Pandey P, Bisht S, Sood A, Aeron A, Sharma GD, & Maheshwari DK (2012). Consortium of plant-growth-promoting bacteria: future perspective in agriculture. Bacteria agrobiol: plant probiotics 185–200

Patel D, Jha CK, Tank N, Saraf M (2012) Growth enhancement of chickpea in saline soils using plant growth-promoting rhizobacteria. J Plant Growth Regul 31(1):53–62. https://doi.org/10.1007/s00344-011-9219-7

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17:362–370

Pindi PK, Satyanarayana SDV (2012) Liquid microbial consortium-a potential tool for sustainable soil health. J Biofertil Biopestici 3:124. https://doi.org/10.4172/2155-6202.1000124

Prajapati K, Prajapati M, Panchal R, Sharma S, Saraf MS (2022a) Application of microorganisms for bioremediation of heavy metals. Acta Sci Environ Sci 1(1):01–09

Prajapati M, Panchal R, Prajapati K, Sharma S, Saraf MS (2022b) Rhizobacterial - plant interaction approaches that enhance plant growth under abiotic stress. Acta Sci Agric 6(5):67–74. https://doi.org/10.31080/ASAG.2022.06.1136

Prasad AA, Babu S (2017) Compatibility of *Azospirillum brasilense* and *Pseudomonas fluorescens* in growth promotion of groundnut (*Arachis hypogea* L.). An Acad Bras Cienc 89:1027–1040. https://doi.org/10.1590/0001-3765201720160617

Rana A, Saharan B, Nain L, Prasanna R, Shivay YS (2012) Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. Soil Sci Plant Nutr 58(5):573–582. https://doi.org/10.1080/00380768.2012.716750

Rathod ZR, Saraf MS (2021a) Effect of endophytes as phosphate solubilizing bacteria on Vigna radiata plant. Vidhya J Gujarat Univ 16(2):38–50. ISSN: 2321-1520

Rathod ZR, Saraf MS (2021b) Effect of endophytic consortium on *Pisum sativum* and *Vigna angularis* plant analyst with FTIR spectroscopy. Acta Sci Microbiol 4(12):129–140. https://doi.org/10.31080/ASMI.2021.04.0969

Rathod ZR, Dharmpal MA, Mishra AY, Jain R, Patel BV, Saraf MS (2020) Compatibility studies on different endophytes of *Citrus limon* antagonistic to bacterial with plant pathogen. Adv Biores 11(3):126–129. https://doi.org/10.15515/abr.0976-4585.11.3.126129

Rathod ZR, Sharma S, Saraf MS (2021) Endophytic bacterial effects on seed as enhancement in germination with mobilization of reserves and microbial disinfection in *Citrus limon* (L) by biopriming under salinity stress. Curr Trends Biomed Eng Biosci 20(2):556033. https://doi.org/10.19080/CTBEB.2021.20.556033

Reed MLE, Glick BR (2013) Applications of plant growth-promoting bacteria for plant and soil systems. Applications of microbial engineering. Taylor and Francis, Enfield, CT, pp 181–229

Rochlani A, Dalwani A, Shaikh NB, Shaikh N, Sharma S, Saraf MS (2022) Plant growth promoting rhizobacteria as biofertilizers: application in agricultural sustainability. Acta Sci Microbiol 5(4): 12–21. https://doi.org/10.31080/ASMI.2022.05.1028

Ruíz-Sánchez M, Armada E, Muñoz Y, de Salamone IEG, Aroca R, Ruíz-Lozano JM, Azcón R (2011) Azospirillum and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. J Plant Physiol 168(10):1031–1037. https://doi.org/10.1016/j.jplph.2010.12.019

Saikia J, Sarma RK, Dhandia R, Yadav A, Bharali R, Gupta VK, Saikia R (2018) Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Sci Rep 8(1):1–16. https://doi.org/10.1038/s41598-018-21921-w

Santos Villalobos SDL, Parra Cota FI, Herrera Sepúlveda A, Valenzuela Aragón B, Estrada Mora JC (2018) Colección de microorganismos edáficos y endófitos nativos para contribuir a la seguridad alimentaria nacional. Rev Mex Cien Agríc 9(1):191–202. https://doi.org/10.29312/remexca.v9i1.858

Santoyo G, Guzmán-Guzmán P, Parra-Cota FI, Santos-Villalobos SDL, Orozco-Mosqueda M, Glick BR (2021) Plant growth stimulation by microbial consortia. Agronomy 11(2):219. https://doi.org/10.3390/agronomy11020219

Saraf MS, Sharma S, Thakkar A, (2017) Production and optimization of siderophore from plant growth promoting rhizobacteria. Scholar Press 1-85. EAN:978-3-330-65139-5

Sathiavelu A (2021) A review on the role of compost microbes in the abiotic stress tolerance in plants. SPAST Abstracts 1(01) https://spast.org/techrep/article/view/1296

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160(1):47–56. https://doi.org/10.1016/0003-2697(87)90612-9

Seenivasagan R, Babalola OO (2021) Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product. Biology 10(11):1111. https://doi.org/10.3390/biology10111111

Sekar J, Raj R, Prabavathy VR (2016) Microbial consortial products for sustainable agriculture: commercialization and regulatory issues in India. In: Agriculturally important microorganisms. Springer, Singapore, pp 107–132

Shah RK, Raval VH, Saraf M (2020) Biosynthesis and purification of indole-3-acetic acid by halotolerant rhizobacteria isolated from little Runn of Kachchh. Biocatal Agric Biotechnol 23: 101435. https://doi.org/10.1016/j.bcab.2019.101435

Shahzad S, Khan MY, Zahir ZA, Asghar HN, Chaudhry UK (2017) Comparative effectiveness of different carriers to improve the efficacy of bacterial consortium for enhancing wheat production under salt affected field conditions. Pak J Bot 49(4):1523–1530

Shaikh NB, Shaikh N, Rochlani A, Dalwani A, Sharma S, Saraf MS (2022) Rhizobacteria that promote plant growth and their impact on root system architecture, root development, and function. Acta Sci Microbiol 5(4):53–62. https://doi.org/10.31080/ASMI.2022.05.1035

Sharma S, Saraf MS (2022) Isolation, screening and biochemical characterizations with multiple traits of heavy metal tolerant rhizobacteria from mining area and landfill site. Adv Biores 13(1): 147–156. https://doi.org/10.15515/abr.0976-4585.13.1.147156

Sharma S, Shah RK, Rathod ZR, Jain R, Lucie KM, Saraf MS (2020) Isolation of heavy metal tolerant rhizobacteria from Zawar mines area, Udaipur, Rajasthan, India. Biosci Biotechnol Res Commun 13(1):233–238. https://doi.org/10.55218/JASR.202112327

Sharma S, Rathod ZR, Saraf MS (2021) Elucidate the influence of heavy metal on bacterial growth isolated from a mining location and a waste dump: using their inducible mechanism. Curr Trends Biomedical Eng Biosci 20(2):556034. https://doi.org/10.19080/CTBEB.2021.20.556034

Sharma S, Rathod ZR, Saraf MS (2022b) Exploring the biotic stress tolerance potential of heavy metal tolerate rhizobacteria isolated from mines area and landfill site. Acta Sci Microbiol 5(2): 31–37. https://doi.org/10.31080/ASMI.2022.05.1000

Sharma S, Rathod ZR, Saraf MS (2022c) Salinity Management in *Glycine max* L. using cytokinin from rhizobacteria isolated from mines and dump sites. Curr Trends Biomedical Eng Biosci 20(5):556047. https://doi.org/10.19080/CTBEB.2022.20.556047

Singh NK, Rai UN, Tewari A, Singh M (2010) Metal accumulation and growth response in *Vigna radiata* L. inoculated with chromate tolerant rhizobacteria and grown on tannery sludge amended soil. Bull Environ Contam Toxicol 84(1):118–124. https://doi.org/10.1007/s00128-009-9875-5

Tahir M, Ahmad I, Shahid M, Shah GM, Farooq ABU, Akram M, Zakir A (2019) Regulation of antioxidant production, ion uptake and productivity in potato (*Solanum tuberosum* L.) plant inoculated with growth promoting salt tolerant *Bacillus strains*. Ecotoxicol Environ Saf 178:33–42. https://doi.org/10.1016/j.ecoenv.2019.04.027

Thakkar A, Saraf M (2015) Role of volatile metabolites from *T. citrinoviride* in biocontrol of phytopathogens. Int J Res Chem Environ 5(1):86–95. http://ijrce.org/issue/jan%202015/12.pdf

Tiwari S, Prasad V, Chauhan PS, Lata C (2017) Bacillus amyloliquefaciens confers tolerance to various abiotic stresses and modulates plant response to phytohormones through

osmoprotectants and gene expression regulation in rice. Front Plant Sci 8:1510. https://doi.org/10.3389/fpls.2017.01510

Todar K (2015) Textbook of bacteriology

Trivedi G, Saraf M (2019) Application of microbial endophytes isolated from plants of semi-arid region, PhD thesis, Gujarat University, Ahmedabad, Gujarat, India

Trivedi G, Patel P, Saraf M (2018) Amelioration of drought tolerant plant growth promoting endophytic bacteria from *Ricinus communis*. Int J Res Adv Technol 6(10):2814–2824

Trivedi G, Patel P, Saraf M (2020) Synergistic effect of endophytic selenobacteria on biofortification and growth of *Glycine max* under drought stress. S Afr J Bot 134:27–35. https://doi.org/10.1016/j.sajb.2019.10.001

Upadhyay SK, Saxena AK, Singh JS, Singh DP (2019) Impact of native ST-PGPR (*Bacillus pumilus*; EU927414) on PGP traits, antioxidants activities, wheat plant growth and yield under salinity. Climat Chang Environ Sustain 7(2):157–168. https://doi.org/10.5958/2320-642X.2019.00021.8

Urja P, Meenu S (2010) Role of single fungal isolates and consortia as plant growth promoters under saline conditions. Res J Biotechnol 5(3):5–9

Verbruggen E, van der Heijden MG, Rillig MC, Kiers ET (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197(4):1104–1109. https://doi.org/10.1111/j.1469-8137.2012.04348.x

Villa-Rodriguez E, Parra-Cota F, Castro-Longoria E, López-Cervantes J, de los Santos-Villalobos S (2019) *Bacillus subtilis* TE3: a promising biological control agent against *Bipolaris sorokiniana*, the causal agent of spot blotch in wheat (*Triticum turgidum* L. subsp. durum). Biol Control 132:135–143. https://doi.org/10.1016/j.biocontrol.2019.02.012

Vives-Peris V, de Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39(1):3–17. https://doi.org/10.1007/s00299-019-02447-5

Yadav KK, Sarkar S (2019) Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. Environ Ecol 37(1):89–93

Yaghoubi Khanghahi M, Strafella S, Crecchio C (2020) Changes in photo-protective energy dissipation of photosystem II in response to beneficial bacteria consortium in durum wheat under drought and salinity stresses. Appl Sci 10(15):5031. https://doi.org/10.3390/app10155031