Chapter 13
Genetically Modified Microbes as Biofertilizers

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13.1 Introduction to Biotechnology

Biotechnology is a divergent and multidisciplinary field of biology. Biotechnology is basically the utilization of biological substances like microbes or cellular constituents in a controlled manner for the advantage of mankind. In other words, biotechnology is an integrated utilization of biochemistry, microbiology, and engineering knowledge for the utilization of microbes, cultured cells, or tissues to their best (Okeno et al. 2012). In the past, people exploited microbes for production of cheese, bread production, or brewing alcohol; even if the process of fermentation was not tacit, comprehensively human beings have sustained their search for enhancing the natural potential of microbes and making them competent for novel methods. At present, the application of biotechnology is highly complicated. Now, scientists can manipulate living organisms and transfer genetic matter among them, producing transgenic organisms. The present significance of biotechnology is largely in the area of biomedicine and agriculture. Present methods permit the construction of novel and enhanced food. Novel vaccines and antibiotics have been produced against various diseases like AIDS, cancer, and many hereditary diseases. Biotechnology is also used in the area of biofuel production, mining, and pollution control (Fig. 13.1). Genetically modified microbes and plants are utilized to remove toxic chemicals from oil spoil spills or industrial effluents (Chen et al. 2007).

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R. A. Bhat, K. R. Hakeem (eds.), *Bioremediation and Biotechnology, Vol 4*,
https://doi.org/10.1007/978-3-030-48690-7_13

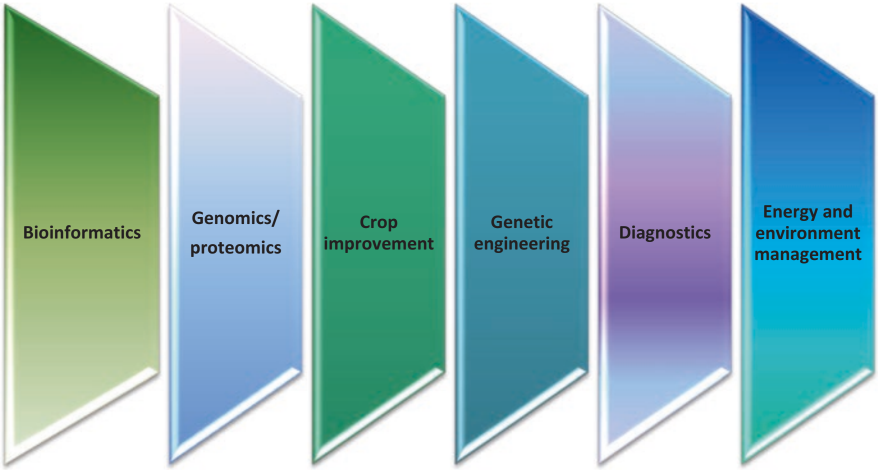

Fig. 13.1 Role of biotechnology for sustainable development

Besides, improved superiority of life there exists countless and exhilarating opportunities in various other fields of biotechnology (e.g., green, red, or white biotechnology).

Green biotechnology is a vital field of contemporary biotechnology. Its foundation is on the crop enhancement and manufacture of new crop products (McAllister et al. 2012). It comprises three major areas which include: plant tissue culture, plant genetic engineering, and plant molecular marker-assisted breeding. Plant tissue culture involves the production of the whole plant or part of it under laboratory conditions. Its main advantage is the quick manufacture of plant materials like citrus fruits, banana, etc. On the other hand, plant genetic engineering involves the introduction of beneficial genes from one living organism to other (Dana et al. 2006). This generates improved varieties of crops with enhanced production (Brookes and Barfoot 2009). In case of plant molecular marker-assisted breeding, molecular markers (specific short sequences of DNA) are accountable for a preferred attribute. Thus, improved properties like disease resistance can be achieved (Horvath et al. 2012).

White biotechnology is concerned with industries. It utilizes enzymes, bacteria, yeast, or molds to produce valuable products. It results in the manufacture of wide range of bioproducts like vitamins, antibiotics, detergents, etc. (Beuno et al. 2016).

Red biotechnology is concerned with medical biotechnology. It involves genetic manipulation of organisms to create antibiotics. Herein, the human body's own tools are utilized to eliminate the pathogens. It is of immense significance in the conventional drug discovery and also aids in improving the potential for cure, anticipation, and analysis of diseases (Becker et al. 2008).

13.2 Biofertilizers

Chemical fertilizers quickly perk up the development and yield of crops and are hence gaining fame around the world. Conversely, extensive use of such fertilizers causes serious ecological problems. Nitrate leaching and contamination of ground water are due to increased exploitation of fertilizers. Inorganic fertilizers like calcium nitrate, ammonium chloride, and sodium nitrate produce greenhouse gases that results in pollution. Elevated levels of greenhouse gases and heavy metal uptake by plants are major causes of environmental damage. Eutrophication of freshwater is also due to chemical fertilizers. Furthermore, chemical fertilizers can eradicate the advantageous microbial or insect community of the soil (Liu and Golden 2002). Fortunately, nature has bestowed the soil with a variety of microbes with specific mechanisms to overcome this challenge. This mechanism besides maintaining soil quality also works in tandem with plants as an element of the ecosystem. Such mechanism is what constitutes "biofertlizers" (Khosro and Yousef 2012). Biofertilizers constitute a central part of green agriculture. Biofertilizers contain proficient strains of microbes, organic products, and departed and rotten parts of plants which supply nutrients to soil. It progressively elevates crop yield by means of enhancing soil fertility. They change the unavailable form of nutrients to the accessible form by escalating the population of microbes in the rhizosphere (Leonardo et al. 2006). Microbes are accountable for delivering soluble nutrients to crops. These are helpful in a variety of ways that include solubilization of plant nutrients (like phosphorus, sulfur, etc.) and fixing of atmospheric nitrogen (Fig. 13.2). They also encourage the formation of growth-promoting phytohormones like cytokinins and auxins. They also defend the plant against various abiotic and biotic stresses (Mitragotri et al. 2014; Chang and Yang 2009).

Biofertilizers aid plants in accessing the nutrient present in its surroundings. The microbes frequently employed as the biofertilizers include *Rhizobium*, *Azotobacter*, *Anbaena* (nitrogen fixers), *Pseudomonas putida*, mycorrhizal fungi, etc. Likewise, phytohormone/auxin-producing bacteria could also be utilized as biofertilizers (Somasegaran and Springer 1994). These microbes enhance growth and development in plants. The grievance from agriculturalists regarding the effectiveness of biofertilizer is their improper storage and the larger time period between field application and production (Youssef and Eissa 2014). This restricts their employment due to compatibility and constancy issues under diverse soil environments. For this reason, improved shelf life is the basis for the popularization of biofertilizers (Adesemoye and Kloepper 2009).

Currently, a variety of marketable biofertilizers are obtainable and a variety of mechanisms have been formulated to guarantee maximum viability of the microbes used in such formulations (Bhattacharyya and Jha 2012). These strategies include: optimization of biofertilizer formulation, usage of thermo-resistant or drought-resistant and genetically modified strains, and employment of liquid biofertilizers.

For dexterity, a carrier substance is utilized as a vehicle for the microbes which are to be used as biofertilizers. Carrier substances include clay, vermiculite, peat, seed, lignite powder, rice bran, charcoal, etc. For enhanced shelf life of biofertilizer

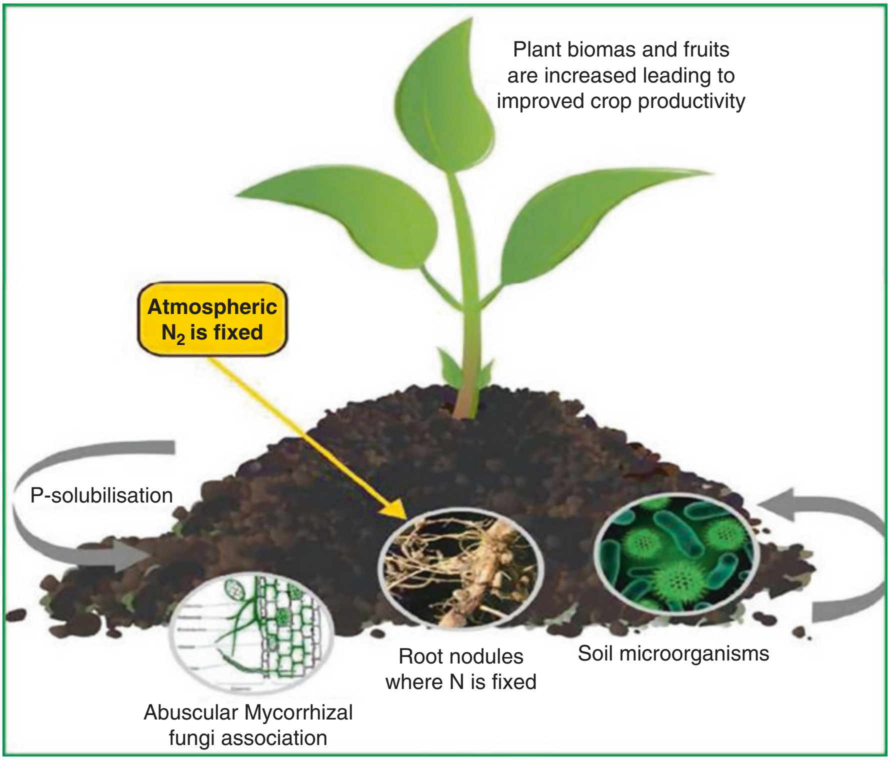

Fig. 13.2 Biofertilizer technology utilizes plant-microbe interactions in influencing plant growth and development. (Raimi et al. Cogent Food & Agriculture 2017)

formulation, a combination of these carriers is employed. Likewise, pre-sterilization of carriers is done to enhance the shelf life of microbes (Wani et al. 2013). Liquid biofertilizer formulation is an important aspect to improve shelf life. These formulations enclose an adequate amount of cell protectants and nutrients that are responsible for the extended shelf life of biofertilizers. Besides, these formulations can endure huge temperature range (Santos et al. 2012).

Biofertilizers got commercialized with the launch of “Nitrogin” by Hiltner and Nobbe. This preparation was for legumes. Later microbial inoculants for legumes were made like “Alnit.” It proved advantageous for the development of non-leguminous plants. These bacteria were recognized to be local ammonifiers. Discovery of *Azotobacter* and *Clostridium* developed a new field for investigating economical bacterial fertilizers (Goswami et al. 2014). The rhizosphere of these plants contains a range of species of soil bacteria that enhance plant growth by numerous ways. Such bacteria are jointly known as plant growth-promoting rhizobacteria (PGPR). One of the ways is through fixing of atmospheric nitrogen which enhances the accessibility of exploitable form of nitrogen in the rhizosphere. They also promote symbiosis between plants and microorganisms (Mfilinge et al. 2014). In general, the benefits of biofertilizers in agriculture can be summed up in Fig. 13.3.

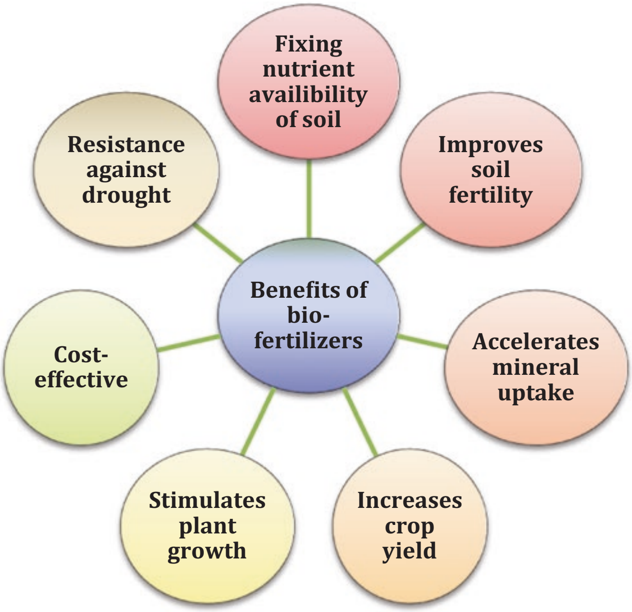

Fig. 13.3 Benefits of biofertilizers

13.3 Categorization of Biofertilizers

Biofertilizers are categorized into various types on the basis of microorganisms they contain (Chun–Li et al. 2014). The different types of biofertilizers are discussed below.

13.3.1 Symbiotic Biofertilizers

Symbiotic microbes infect root tissues and form new structures. In many cases, the application of molecular biology tools allows the discovery of the genes and signals concerned with the positive interaction between the microorganism and the plants (Raja 2013). The main symbiosis relating to agricultural application as biofertilizers is considered below.

13.3.1.1 Rhizobia

Rhizobium is an illustration of a symbiotic association colonizing legume roots and fixes the atmospheric nitrogen. It has capability to fix atmospheric nitrogen in leguminous and non-leguminous plants. The different genus and species inhabiting legumes root nodules are usually referred to as rhizobia. These involve alpha-proteobacterias, such as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mezorrhizobium*, *Azorhizobium*, *Allorhizobium*, *Agrobacterium*, and beta-proteobacteria, such as Burkholderia. The best model describing the interaction between rhizobia and legume roots includes flavonoids/isoflavonoids molecules released by the plants which induce bacterial genes and hence trigger the synthesis of LCO (lipo-chitin-oligosaccharides) molecules, which in turn control infection and nodule growth in the root tissue (Rajaram and Apte 2008). Usually, it pierces the root hair and multiplies there in special root structures called root nodules. The quantity of nitrogen fixed depends on host, strain of *Rhizobium*, and existing environmental conditions. They are very proficient biofertilizers for legumes as far as the magnitude of nitrogen fixation is concerned. The nod, nif, and fix genes control the nodulation and nitrogen fixation by the bacterium (Liu and Golden 2002).

13.3.1.2 Blue Green Algae/Cyanobacteria

BGA (Blue green algae) are the most ancient organisms possibly the first among those that started evolving oxygen. These appear in numerous shapes (single celled, branched, or unbranched with filaments). The majority of them possess special structure called heterocyst whose role if to fix nitrogen. The algae that are frequently applied in fields belong to *Anabaena*, *Nostoc, Scytonema, Tolypothrix*, etc. (Buikema and Haselkon 2001). These are widely used in rice fields. BGA secrete numerous growth-promoting substances like amino acids, vitamins, polysaccharides, sugars, etc. which boost the yield of crops (Schiefer et al. 2002).

13.3.1.3 Mycorrhiza

Mycorrhiza is the best example of the symbiotic association between fungi and plant roots (higher plants). The fungi defend the plants against various stresses and enhance their growth. These fungi colonize the root cortex and mycelia of the plants and help them to obtain nutrients from soil. These fungi are cosmopolitan in soil and are seen in the roots of Thallophytes, Gymnosperms, Pteridophytes, and Angiosperms. Plants, on the other hand, protect fungi from root pathogens and also provide them with carbohydrates, hormones, nutrients, etc. The mycorrhizal plants have better forbearance to poisonous metals, salinity, elevated soil temperatures, and unfavorable pH. Such plants also resist transplantation shocks. They play a significant task by enhancing growth and nutrient uptake in plants (Vessey 2003).

13.3.2 Free-Living Biofertilizers

Since the description of PGPR by Kloepper and Schroth (1978), many different bacteria genera have been described as PGPR: *Pseudomonas, Azospirillum, Azotobacter, Gluconacetobacter, Herbaspirillum, Bacillus, Burkholderia, Erwinia,* *Caulobacter, Azotobacter, Chromobacterium, Serratia, Microccocus, Flavobacterium, Actinobacter, Enterobacter, Arthrobacter, Agrobacterium, Hyphomycrobium*, and fungus such as *Trichoderma*, among others (Gupta 2004).

Many PGPR have been described as endophytic bacteria. It is not clear if the plant growth promotion effects are a consequence of plant-microbe interaction in the external part of the rhizosphere or if an endophytic state is necessary (Hayat et al. 2010). Many different mechanisms have been claimed to be responsible for the plant growth promotion effect after in vitro experiments under controlled conditions. In some cases, the use of appropriate mutants helps in the definition of these mechanisms. But since different mechanisms are always present in a single strain, it is almost impossible to know which are the main mechanisms operating and driving the plant growth promotion. Irrespective of the real mechanisms operating in PGPR with a positive effect in the field, the use of these microorganisms has dramatically increased in recent years and will probably continue to grow because biofertilizers appear as a valuable opportunity for future sustainable agriculture. Many commercial products already exist which are based on *Pseudomonas* or *Azospirillum* strains in the market (Yang et al. 2009). The different mechanisms operating in PGPR can be classified as: N_2 (nitrogen) and P (phosphorus) nutrition effects, and plant root development and fitness mediated by phytohormones (Ahemad and Mohammad 2010).

13.4 Phytohormone-Mediated Mechanism of Plant Growth-Promoting Bacteria (PGPB)

One of the most visible effects on plants after inoculation with PGPB is the huge development – and sometimes changes in the architecture – of the root of the plant. This general improvement of root growth, including root-hair development, is one of the characteristic phenotypes of the interaction plant-PGPB.

It is likely that water and mineral uptake is consequently improved because of the increase in the root system, although the specific mechanism is not completely clear. Changes in hormone balance, enhancement of proton-efflux activity, and modification in a wide range of related enzymatic activities would be part of the mechanisms behind this phenotype (Backman and Sikora 2008).

13.4.1 Cytokinins

The role of cytokinins in the promotion of root development is not clear, but cytokinin-producing PGPB stimulate nodulation in legumes when co-inoculated with rhizobia. Besides, it has been demonstrated recently that there is a Nod factor-independent mechanism for infection and nodulation, possibly mediated by rhizobial cytokinin. This particular area deserves more attention in the future (Riefler et al. 2006; Sokolova et al. 2011).

13.4.2 Auxins

The general root improvement phenotype can be reproduced by replacing phytohormones with PGPB. Auxin-related substances, such as indole acetic acid (IAA), appear to be involved in one of the most important mechanisms regarding the general root development improvement. Nevertheless, bacterial production of IAA in plants has not yet been demonstrated. There are no IAA completely deficient mutants, but IAA attenuated mutants were ineffective as PGPB, compared to parental strains (Aloni et al. 2006; Ahmed and Hasnain 2010).

13.4.3 Ethylene

Ethylene is related to general plant responses when a stress condition appears, even if it is a very low stress situation. When this happens, the plant synthesizes ethylene and stops its growth temporarily. This is because of the regulatory effects of ethylene on different cell functions. 1-aminocyclopropane-1-carboxylate is a precursor of ethylene synthesis. The enzyme ACC deaminase is present in some bacteria which can even use ACC as C (carbon) and N sources (Butterbach et al. 2013). When ACC deaminase is expressed by rhizospheric bacteria, root growth and development is enhanced. It is probably because of the elimination of the inhibitory concentrations of ethylene produced by the plant. This enzyme is not present in every bacteria and its activity is codified by a single gene acdS. The introduction of this gene from *Pseudomona putida* into other bacteria species confers plant growth-promoting functions to the recipient bacteria that are absent in the parental strain. This represents a potential biotechnology-based tool to improve microorganisms to be used as biofertilizers (Reid 1981).

13.4.4 Helper Bacteria

In the studies of plant microbe interaction which induced some kind of plant growth promotion, there are other cases that do not fit into the previous definitions but which can be considered as another kind of biofertilizer. That is the case of bacteria which improve a plant-microbe interaction as a third partner in the interaction. An example can be found in rhizospheric actinomycetes isolated from legumes or actinorhizal nitrogen-fixing nodules which are able to stimulate nodulation, consequently nitrogen fixation in the plant, and finally plant growth. This tripartite plant-microbe interaction is not well known in terms of mechanism. However, it clearly shows that biofertilizers can be improved by the use of more than one microorganism at a time (Egamberdiyeva 2007).

13.5 Significance of Genetically Modified Microbes in Agriculture

Numerous genera of bacteria have great impact on growth and development of plants. Among these are the bacteria that are used as biocontrol as they are able to curb plant diseases. Some other bacteria increase plant growth by increasing the availability of nutrients. Such bacteria are known as plant growth-promoting rhizobacteria (PGPR). These are applied either directly to soil or as seed coating. Nevertheless, to put forth their growth-enhancing effect, huge quantities of the introduced PGPR should stay alive in the rhizosphere (root) and soil. Accordingly, the effectiveness of PGPR is not always enough for marketable usage, and there is a requirement to advance their performance. One of the probable ways is to genetically modify the microbe to ease up their survival (Brown et al. 2015).

Genetically modified organisms symbolize a genetic reserve. These microorganisms may find application as donor or recipient of genes of interest. Microbes play a vital role in various sectors of agriculture, pharmaceutical industries, and environmental management (Cohen et al. 1973). Genes of microbes can be optimized or improved by means of various genetic modifications using Recombinant DNA technology (Tabashnik et al. 2011). Usually, this is dependable on the recognition and selection of the mutants with favorable traits. In numerous cases, the usage of molecular biology tools or recombinant DNA technology allows the discovery of the genes and signals concerned in the advantageous interaction (endophytic, mycorrhizal, and diazotrophic) between the microbe and plant. These symbiotic interactions can assist plant growth and development through nitrogen uptake, siderophore production, phosphate solubilization, etc. (Ruiz et al. 2010; Ritika and Uptal 2014).

Recombinant biotechnology offers an advantage to decrease the employment of synthetic fertilizers. Biofertilizer technology has considerably developed in the market. The nature of multiple mechanisms discovered for PGPR actions intimidate the use of genetically modified organisms as biofertilizers (Tabashnik et al. 2011). Further, the knowledge of microbial ecology and its dynamics will surely enhance the biofertilizer technology (Kakumanu et al. 2012). Microbes are particularly targeted for genetic improvement since they are given huge importance in modern agriculture as they are used as biofertilizers. Biofertilizers represent an alternative to synthetic fertilizers which are facing lots of disparagement due to their negative impact on the ecology and human wellbeing. Thus, there is an important requirement to build up eco-friendly control using existing microbes. Such microbes would offer protection to plants against pathogens and would be economical, reliable, and effective (Pishchik et al. 2002). To obtain this target, better-quality strains are needed. Thus, genetically modified microbes could be used for this purpose. Efforts are in progress to formulate proficient biofertilizers compatible with a broad choice of plants and soil by means of genetically engineered techniques. For example, biofertilizers have been formulated based on nitrogen-fixing rhizobial bacteria occurring naturally in the nodules of leguminous plants. Nevertheless, these microbes are not competent enough to supply nitrogen to non-legumes. In such cases, genetic engineering is of special importance, as it assists in the development of efficient delivery systems. In this way, non-legumes could be grown together with symbiotic rhizobial root nodules devoid of externally applied nitrogen fertilizers (Aloni et al. 2006). The foreign genes used for transforming microbes could be integrated into host genome. For this, the regulatory area of the gene should be modified in promoter or terminators in sites so as to optimize the inserted gene's function within the host. Adding a particular gene which can bestow biocontrol ability could improve the biocontrol ability of microbes lacking such genes (Dash et al. 2016). For example, many rhizobacteria with biocontrol activity produce chitinases. However, few rhizobacteria like *Pseudomonas putidri and Rhizobium melliloti,* both of which are outstanding root colonizers, are deficient in synthesizing chitinase (Bagwan et al. 2010). Incorporation of the chitinase gene into their genome has made them proficient in defending the plant against fungi (Huang et al. 2001).

Nitrogen-fixing property of *Rhizobium* inoculants could be augmented by means of genetic engineering tools. An additional way is by planting the crops that use nitrogen more proficiently. An example of such crops is genetically modified Canola which exhibits a noteworthy decline in the amount of nitrogen fertilizer that is leached into the soil or lost into the atmosphere, and hence it improves the economies of farmers through the enhanced profitability. Moreover, biofertilizers when formulated by means of molecular technologies can improve the cellular pathways of phytohormone production like auxin, cytokinin, etc. which assist in plant growth and development (Nautiyal et al. 2008). Similarly, numerous pseudomonads in the soil manufacture siderophores that can chelate Fe ions, and thus escalate Fe uptake by the plants. The genetically altered strain of *S. melliloti* (RMBPC-2) has added genes that manage the enzyme nitrogenase from plants to bacteria (Boccia and Sarnacchiaro 2015). Likewise, *Trichoderma* species are extensively found in the soil. *Trichoderma harzianum* is an efficient rhizopsphere colonizer and can parasitize disease-causing fungi. Many extracellular enzymes like glucanases, chitinases, and proteases synthesized by *Trichoderma* have been improved by the addition of chitinase genes, especially *S. marcessens* (Awais et al. 2010). Thus, such genetically modified strains could act as efficient biofertilizers and will aid in crop improvement.

13.6 Genetically Modified Organisms in Soil and Their Survival

Microbes introduced in soil face various biotic and abiotic factors that affect their survival rates. High moisture content, hydrogen ion concentration, and clay content have a constructive effect on microbial survival. On the other hand, presence of competing microbes or dry periods and predation by protozoa affect the microbial growth negatively (Castiglioni et al. 2008). Among the microbes that are well adapted to rhizosphere are members of genera *Rhizobium*, *Pseudomonas*, *Bacillus*, *Azotobacter*, *Azospirillium, Agrobacterium,* and *Xanthomonas.* Microbial survival is governed by the interaction between the environment and bacterial physiology. As a result of these interactions, bacteria can switch metabolism to various physiological states. They can either form dwarf cells or become resistant to stress. Besides, they can form exopolysaccharides for defense or can form spores or associations with plants (Oh et al. 2009). The survival rates of these bacteria can be studied by following the wild-type strain. In reality, this extrapolation needs to be applied with few security measures. To begin with, the expression of the inserted gene needs a surplus energy that could lessen their environmental fitness. Besides, the addition can disrupt some unfamiliar functions declining the competiveness of the strains. Lastly, the genetically modified microbes can acclimatize to the prevailing ecological conditions through natural selection. This statement is supported by the fact of evolutionary adjustment or adaptation of bacteria to degrade 2,4-dichlorophenoxyacetic, a herbicide, to acid. This resulted in augmented competitive fitness to usage of succinate as a substrate. Genetically modified microbes have been observed to survive better than the normal wild strains. Nevertheless, improved endurance of genetically modified microbes has been in frequently been observed under field circumstances. Moreover, some modified microbes have been found to outcompete by their parental strains. Besides, it is believed that the occurrence of a number of constitutively expressed marker genes in genetically modified microbes has negative consequence on its survival in competition to wild-type strain (Nelson et al. 2007).

13.7 Environmental Impact of Genetically Modified Microbes

Genetically modified microbes have diverse effect on the environment. It encompasses events like organic substrate, changes in population structure, displacement of species, production of toxic metabolites, changes in population structure, which may lead to disorder in ecological processes (Snow et al. 2005). Minute changes in community symphony are hard or even impracticable to resolve, and their connection to microbial diversity and ecosystem functioning is not quite obvious (Lemaux 2008). Different bacteria perform different functions. Therefore, vanishing of a small number of bacteria would be hard to sense (Wang et al. 2003). Thus, it may be concluded that only severe disturbances might influence the soil microbial communities to the extent that only few functions will be badly affected.

Cultural bacteria are one of the main troubles in microbial ecology. However, various DNA/RNA-based techniques have been used to detect the impact of genetically modified microbes on the microbial community. These techniques do not demand the culture of bacteria. Besides, techniques like gel electrophoresis, restriction fragment length polymorphism, single strain conformation polymorphism, etc. are used to examine shifts in community structures (Okeno et al. 2012; Sandeep et al. 2016).

13.8 Role of Biofertilizer Strains

Genetically modified microbes offer an improved nutrient accessibility to crops, and thus augment development in plants. The most significant biofertilizer are bacteria, like *Rhizobium* and *Azospirillum* that mainly assist in nitrogen fixation. Symbiotic bacteria like *Rhizobium, Sinorhizobium*, and *Bradyrhizobium* form root nodules in leguminous plants and fix nitrogen. These bacteria have been reported to survive in soil for years, in some cases even devoid of definite host (Ngwako 2008). Such microbes have been utilized extensively as plant inoculants to increase crop productivity. Nevertheless, yield enhancement is changeable, and the success of inoculants seems to be reliant on competition with indigenous strains that are generally least effective (Qaim 2009).

13.9 Genetically Modified *Rhizobium* and *Azospirillum* Strains

Plants get all their nutrients from the soil except carbon dioxide which plants obtain from the air. Nature has developed diverse mechanisms to provide nutrients to plants through renewable resources. One of the best examples is nitrogen fixation by leguminous plants (Horvath et al. 2012). Unluckily, not all plants are gifted with such mechanisms. Thus, plant yield greatly depends on the use of chemical fertilizers. Some fertilizers are very mobile and are, therefore, supplied in huge amounts to plants. Many fertilizers are very mobile in the soil and are supplied in greater concentration than required by the plant. The loss of valuable compounds is not only of economic significance, but also leads to grave ecological problems. Besides, it may also accumulate in the soil. Numerous strategies have been designed to enhance nutrient uptake by crops (Newell-McGloughlin 2008). These comprise formulation of plant growth-promoting bacteria and slow release fertilizers (Lavakush et al. 2014).

PGPR can put forth their consequence in both direct and indirect way. For direct way, phytostimulation is the best example (Ma et al. 2003). Many bacteria able to produce plant growth stimulating factors like cytokinins and auxins. This promotes plant growth and aids in enhanced nutrient uptake by plants. Indirect path includes exercise of biocontrol of pathogens and deleterious microbes (Brooks and Barfoot 2009).

13.10 Genetically Modified *Azospirillum* Strains Enhance Nitrogen Uptake

Azospirillum is known for its property of enhancing plant growth through enhancing nitrogen uptake. This is done via production of phytohormones by *Azospirillum* strains (Gonzalez et al. 2015). Nonetheless, the mechanisms by which these bacteria create phytohormones are still not definite and need an improved understanding. To clarify these mechanisms, numerous significant queries should be addressed:

- The formation of GM *Azospirillum* strains with known production levels of Indole-3-acetic acid
- The biochemical and genetic basis of the production of Indole-3- acetic acid
- Testing the result of these genetically modified bacteria on plants and on the environment under the field milieu

Currently, GM strains of *Azospirillum* with such fundamental characteristics are obtainable. Experiments with these strains are mainly focused on nitrogen fixation and impact on residential microbial population and plant growth. These experiments are carried out under lab conditions (green house and growth cabinet). In spite of the improvement of these experiments, broad and vigilant testing under control is needed prior to field release of such strains.

13.11 Genetically Modified Rhizobium Strains with Improved Competitiveness

Legume inoculation with proficient nitrogen fixing bacteria is broadly utilized to augment production in leguminous crops. Such inoculation is not at all times booming because native soil microbes with less nitrogen-fixing ability can outcompete the introduced strains in terms of nodulation initiation. For better use of rhizobial inoculants, their ability to dominate nodulation is must (McAllister et al. 2012). Thus, inoculants strains are modified in such a way that maximum quantities of root nodules are formed. In this backdrop, *Sinorhizobium melliloti* has been genetically modified to enhance nodulation in alfalfa roots. This genetic alteration involves alteration of the expression of *nifA* gene which is accountable for the management of all other nitrogen fixation *(nif)* genes (Bakshi 2003). Thus, wild-type strains were mixed with GM strains, and it was found that the latter occupied most of the nodules of alfalfa roots. The exact means of this augmentation is not implicit, but it is hypothesized that *nifA* regulates the expression of genes other than *nif* cluster that aids in nodule development (Beyer et al. 2002).

The capability of strains of *Rhizobium* to proficiently distinguish the plant root is an important characteristic that adds to their nodule competiveness (Kanchiswamy et al. 2015). It is very significant since the proficient inoculation means lesser dosage of bacterial strains (Overton 2014). In addition, the progress of the inoculation strain toward the root is another important factor governing the competiveness. It has been found that in genetically engineered flagellated *R. leguminosarum* strains, the nodulation is much more compared to flagella deficient non-motile strain. Thus, root attraction is also important for better plant development and growth (Liddycoat et al. 2009; Barrows et al. 2014).

13.12 Effect of Genetically Modified *Rhizobium* on Arbuscular Mycorrhizal Fungi

Mycorrhizal fungi represent the group of fungi that form symbiotic association with the plants. Research has been done to see whether the GM *Rhizobium* strains enhance the nodulation or interfere with the symbiotic association in plants. It has been observed that GM *S. meliloti* strain do not hamper mycorrhiza formation but enhances nodulation. Genetically modified *meliloti* increased arbuscular myccorhizal colonization units and augmented nutrient acquirement capacity of mycorrhizal plants (Papagianni 2004; Van 2007).

13.13 Field Release of Genetically Modified Rhizobium Strains

Numerous *Rhizobium* species have been genetically modified to perk up the nitrogen fixation or to study their survival by utilizing marker genes through field trials. Tn5-marked legumino serum strain persisted for 5 years when introduced into the field. The perseverance of the strain was credited to the type of soil, climate, and the host plant (Huang et al. 2004). The usage of GM *R. meliloti* strain, with supplementary copies of dctABC and nifA genes, had been found to upregulate alfalfa yield under field trials. Nonetheless, at sites with native rhizobial populations, alfalfa productivity did not increase. The outcome of a Tn903-marked *R. meliloti* strain introduced into alfalfa field plots was calculated and it was observed that cell number declined drastically after inoculation (Podile and Kishore 2007; Hussain et al. 2002).

Effect of wild-type strains and genetically modified *S. meliloti* strains was studied on indigenous microbial communities by means of restriction fragment length polymorphism and temperature gradient electrophoresis. Wild-type strains had only limited effects compared to GM strains. Likewise, Luc-marked *S. meliloti* in a field research with *Medicago sativa* was analyzed. The study was observed for 12 weeks. Single-strand cell protein banding patterns revealed shifts confirming the effect of inoculum on native microbial population (Gouse 2012; Scalenghe et al. 2012).

Genetically modified *Rhizobium leguminosarum* strains, marked with HgCb resistance genes (mer genes) and lacZ genes, were inoculated in the rhizosphere of *Pisum sativum*. Three modified strains were utilized. In these strains, nitrogen-transforming ability was monitored. The field experiments revealed that all tested strains colonized the rhizosphere to the same extent. These results specify that even though the presence of the plant had a substantial impact on carbon mineralization in soil, the impact of genetically modified *Rhizobium* strains is almost identical to that of wild-type strain (Ritika and Uptal 2014). *Alcaligenes faecalis,* a non-nodule forming bacterium, has been genetically modified and introduced into rice fields in China, to observe its effect on crop productivity. *A. faecalis* was genetically modified by the insertion of a constitutively expressed nifA regulatory gene (Gray and Smith 2004). Nitrogen fixation got enhanced compared to non-treated fields. Besides, there was a considerable increase in the yield. The effect of this introduction was studied by DGGE of amplified 16S rDNA in a microcosm experiment. These strains survived well in the rhizosphere. The survival of the strain and the augmentation in crop yield denote GM *A. faecalis* is a good candidate for commercial use. Besides, it is ecologically safe strain (Metsheka et al. 2002).

13.14 Conclusion

Biofertilizers represent the central part of green agriculture. These contain proficient strains of microbes, organic products, and departed or rotten parts of plants which provide nutrients to soil. These gradually promote crop yield by increasing soil fertility. It is necessary to identify the positive aspects of biofertilizers so as to apply it to modern agriculture. Usage of biofertilizers containing advantageous microbes improves the crop productivity to a larger extent. Biofertilizers play an important role in maintenance of soil quality. This would in turn guard the environment and would require less expenditure. Besides, biofertilizers when formulated using genetically modified strains can improve the biological pathways of production of plant growth-promoting substances and hence enhance the yield. Genetically modified microbes offer plentiful advantages in this area, as particular metabolic processes could be tackled with extra accuracy and totally novel functions could be introduced in microbes. In spite of the fact that field trials with GM biofertilizers are limited, the preliminary results regarding their use are promising with respect to enhanced performance in modern agriculture. Genetically modified biofertilizers have been introduced with enormous success regarding their activity and survival rates. Till date, non-target effects of genetically modified biofertilizers that have been reported are insignificant and diminutive in comparison to natural variants. Thus, overall genetically modified microbes contribute to an improved nutrient accessibility in plants and aid in plant growth or development.

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