ANNQUEST DECEMBER 2018
ISSN: 2321-3043
www.stannsannquest.com

Plant Growth Promoting Rhizobacteria (PGPR): Ecofriendly alternatives for sustainable agriculture

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I. INTRODUCTION

Increased public concern about environmental problems caused either directly or indirectly by the use of fertilizers, pesticides, herbicides, and fungicides, has prompted researchers to consider alternatives to these established chemical strategies for facilitating plant growth in agriculture, horticulture, and silviculture. Ideally, replacements for the chemicals that are currently in widespread use should not only enhance plant growth, but should also inhibit plant pathogens. One potential alternative may be the use of plant growth-promoting bacteria (Brown, 1974; Davison, 1988). These plantbeneficial bacteria can bind to either roots (rhizosphere bacteria), leaves (phyllosphere bacteria), or they may exist within plant tissues (bacterial endophytes).

The highest concentrations of these microorganisms typically exists around the roots, in the rhizosphere, most probably due to the high levels of nutrients exuded from the roots of many plants that can be utilized by bacteria to support their growth. A large number of plant growth-promoting bacteria have been isolated to date, each with one or more traits that might, under the appropriate conditions, enhance plant growth. Some of these bacteria may directly influence plant growth, e.g., by synthesizing plant hormones or facilitating uptake of nutrients from the soil. Others exert their beneficial effects indirectly via biological control, whereby they limit the growth of phytopathogens that would otherwise inhibit plant growth (Glick, 1995).

Rhizosphere:

Region of contact between root and soil where soil is affected by roots is designated as"rhizosphere" (Hiltner, 1904). Broadly, there are three separate, but interacting, componentsrecognized in the rhizosphere. These are the rhizosphere (soil), the rhizoplane, and root itself.The rhizosphere is the zone of soil influenced by roots through the release of substrates thataffect microbial activity. The rhizoplane is the surface, including the strongly adhering soilparticles.Several microorganisms are able to promote the plant growth. Several microbial productseither directly promote growth or indirectly protect them from diseases, have been marketed(Lugtenberg, *et al*., 2004).

Plant Growth-Promoting Rhizobacteria (PGPR):

Root colonizing bacteria (rhizobacteria) that exert beneficial effecton plant development via direct or indirect mechanisms have been defined as plant growthpromoting rhizobacteria (PGPR) (Nelson, 2004). The concept of plant growth promotingrhizobacteria is now well established, both for growth promotion and biocontrol. Plantgrowthpromoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978)to describe soil bacteria that colonize the roots of plants following inoculation onto seed andthey enhance plant growth.Plant growth promoting rhizobacteria (PGPRs) have been originally defined as root-colonizing bacteria (rhizobacteria) that cause either plant growth promotion or biological control of plant diseases (Kloepper and Schroth, 1978). PGPR's are known to control a wide range of phytopathogens like fungi, bacteria, viruses, nematodes, etc., they are known to control these pathogens by biocontrol mechanisms which may be by competition or antagonisms, however, the most studied phenomenon is the induction of systemic resistance by these bacteria in the host plant thereby containing the invading pathogens (Ramamoorthy*et al*., 2001). Plant growth-promoting bacteria control the damage to plants from phytopathogens by a number of different mechanisms including: out-competing the phytopathogen, byphysical displacement of the phytopathogens, secretion of the siderophores to prevent pathogens in the immediate vicinity from proliferating, synthesis of antibiotics, synthesis of variety of small molecules that can inhibit phytopathogen growth, production of enzymes that inhibit the phytopathogen and stimulation of the systemic resistance of the plants (Mishra *et al*., 2011).

Beneficial rhizosphere organisms are generally classified into two broad groups based on their primary effects, i.e., their most well known beneficial effect on the plant:

(i) Microorganisms with direct effects on plant growth promotion [plant growth promoting microorganisms (PGPM)] and

(ii) Biological control agents (BCA), that indirectly assists with plant productivity through the control of plant pathogens.

Interactions between biocontrol plant growth-promoting rhizobacteria (PGPR), plants, pathogens and soil in Fig.1.

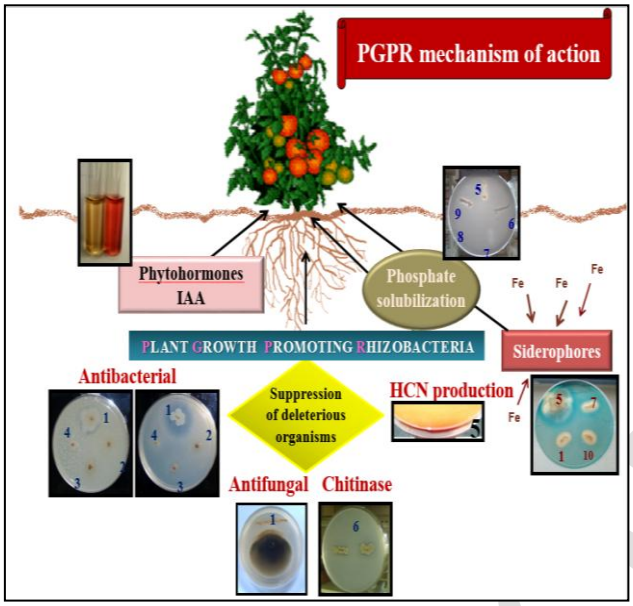

Fig.1. Interactions between biocontrol plant growth-promoting rhizobacteria (PGPR), plants, pathogens and soil.

The beneficial effects of these rhizobacteria on plant growth can be direct or indirect PGPR that promote the plant growth directly include:

- Biofertilizers
- Rhizoremediaters
- Phytostimulators
- Stress controllers

Mechanisms of biological control by which rhizobacteria can promote plant growth indirectly is by reducing the level of disease, include antibiosis, induction of systemic resistance, and competition for nutrients and niches.

Direct plant growth promotion:

Direct plant-growth-promoting rhizobacteria enhance plant growth in the absence of pathogen.

A. Biofertilizers:

Bio-fertilizers are the preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms used for application to seed or composting areas with the objective of increasing the numbers of such micro-organisms and accelerating those microbial processes which augment the availability of nutrients that can be easily assimilated by plants.

Nitrogen is the most abundant element in our atmosphere.Nitrogen fixation is one process by which molecular nitrogen is reduced to form ammonia. This complex process is carried out by nitrogen-fixing bacteria present in the soil.

B. Symbiotic nitrogen fixation:

N_2 fixing bacteria such as *Rhizobium* and *Bradyrhizobium* can form nodules on roots of leguminous plants. In which they convert N_2 into ammonia, which in contrast to N_2 can be used by the plant as a nitrogen source (Schwintzer and Tjepkema,1990).

Application of inoculants in agriculture has resulted in notable increases in crop yields, especially in cereals, where *Azotobacterchroococcum*and*Azospirillumbrasilense*have been very important. These two species include strains capable of releasing substances such as vitamins and plant growth regulators which have a direct influence on plant growth.

C. Phosphate solubilizing PGPR:

After nitrogen, phosphorus is the second most limiting nutrient for plants. However phosphorus reserves, although abundant are not available in forms suitable for plants. Many bacteria from different genera were capable of solubilizing phosphate from either organic or inorganic bound phosphates, thereby facilitating plant growth (Vassilev*et al*., 2006).These include members of genus *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aerobacter, Flavobacterium, Chryseobacterium*and *Erwinia.*Bacteria use two mechanisms to solubilize phosphate:

1. Releasing organic acids that mobilize phosphorus by means of ionic interactions with the cations of the phosphate salt and

2. By releasing phosphatases responsible for releasing phosphate groups bound to organic matter.

D. Siderophore producing PGPR:

Plants have developed two strategies for efficient iron absorption. The first consists of releasing organic compounds capable of chelating iron, thus rendering it soluble. The second strategy consists of absorbing the complex formed by the organic compound and Fe^{+3}, where the iron is reduced inside the plant and readily absorbed (Whipps, 2001).

Siderophore producingrhizobacteria improve plant health at various levels:

They improve iron nutrition, inhibit growth of the other microorganisms with release of their antibiotic molecule and hinder the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron-siderophore complex.

E. Production of HCN:

The cyanide ion is exhaled as HCN and metabolized to a lesser degree in to othercompounds. HCN first inhibits the electron transport and the energy supply to the cell isdisrupted leading to the death of the organisms. It inhibits proper functioning of enzymes andnatural receptors reversible mechanism of inhibition and it also known toinhibit the action of cytochrome oxidase (Gehring*et al*., 1993). HCN is produced by manyrhizobacteria and is postulated to play a role in biological control of pathogens (Defago*et al*.,1990). HCN produced by bacterial isolates reacts with picric acid in presence of Na_2CO_3, results in the color change of the filter paperkept, from deep yellow to reddish brown and later to dark brown. In the case negative test, the filter paper remains unchanged after the growth of bacteria. Cyanogenesis from glycine results in the production of HCN, which is volatile nature.

Tryptophan-dependent pathways of bacterial indole-3-acetic acid (IAA):

Synthesis Auxin regulates almost every aspect of plant growth and development in various biological processes In the plant, indole-3-acetic acid (IAA) can be derived from either of two tryptophan-independent pathways, which may utilize indole-3-glycerol phosphate or indole as a precursor, or four tryptophan-dependent pathways. The bioactive form of IAA is believed to be free IAA (Salisbury, 1994).

Similar to plant IAA production, microorganisms also possess several different IAA biosynthetic pathways. The metabolic routes are classified in terms of their intermediates as the indole-3-acetamide (IAM), IPyA, indole-3-acetonitrile, andtryptamine pathways. One major route, the IAM pathway, is employed mostly by pathogenic bacteria. First, oxidative decarboxylation of tryptophan leading to indole-3-acetamide is catalyzed by IaaM (tryptophan 2-monooxygenase). The conversion of indole-3-acetamide to IAA is catalyzed by IaaH (indole-3 acetamide hydrolase) (L-tryptophan3IAM3 IAA). Another common pathway, the IPyA pathway is the major IAA biosynthetic pathway used by plant growth-promoting bacteria including *Pseudomonas putida*GR12-2. Although the role of IAA biosynthesis by microorganisms is not fully understood, IAA provides bacteria with a mechanism to influence plant growth by supplementing the host plant's endogenous pool of auxin

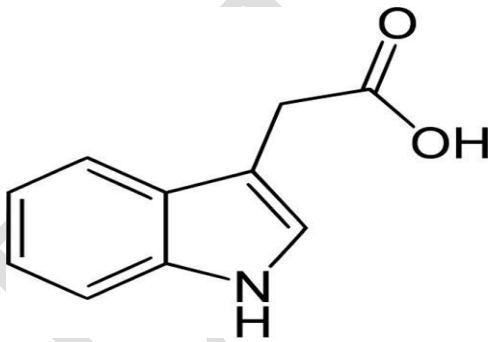

Fig.2. Chemical Structure of Indole-acetic acid

Siderophore production:

Iron is essential element for all living cells. It is difficult to take up iron into cells due to its poor solubility. Microorganisms have to develop methods to solubilize and uptake of mineral iron. For the supply of iron, certain microbes produce iron chelater called siderophore, outside the cell. The siderophore is chelated gets ferric ions . This complex is recognized by a specific receptor on the outer cell membrane and is transported into the periplasm. The complex is then stored and ferric ions are utilized as need arises ((Hofte, 1993).

Continued worldwide industrialization has led to extensive environmental problems. Soils are getting contaminated with heavy metals. Metal-resistant siderophore producing bacteria are able to grow on metal contaminated sites and play an important role in successful survival and growth of plants.

Chitinolytic activity:

Cell wall of most of the fungal phytopathogens contains considerable amounts of chitin, glucans (β-1,3 and β-1,6) and mannoproteins. Figure below shows the structure of fungal cell wall. The chitin, glucan and glycoprotein components are extensively cross-linked together to form a complex network, which forms the structural basis of the cell wall. Although, the chitin content in fungal cell wall is less compared to glucan and glycoproteins but it is the main constituents that provides rigidity to the cell wall. Moreover,

since chitin is not present in either plants or mammals, chitin synthases are attractive targets for the development of antifungal agents. Therefore, Chitinases are considered as the major cell wall degrading enzyme produced by microorganisms. Chitinases (EC 3.2.1.14) are glycosyl hydrolases that catalyze the hydrolytic degradation of chitin, an insoluble linear β-1,4-linked homopolymer of N-acetyl-glucosamine (Das et al.,2010).

Many PGPR produces chitinases, which are major mycolytic enzymes active against a number of phytopathogenic fungi. Fungal cell wall of many phytopathogens have considerable amount of chitin in their cell wall. Fungal cell wall degradation by action of chitinase is one of the major mechanisms to overcome fungal attack on plants.

Challenges with PGPR

One of the challenges of using PGPR is natural variation. It is difficult to predict how an organism may respond when placed in the field (compared to the controlled environment of a laboratory). For example an organism may perform well in the lab, but not have the ability to compete with existing organisms when put in the field.

Another challenge is that PGPR are living organisms. They must be able to be propagated artificially and produced in a manner to optimize their viability and biological activity until field application. Like *Rhizobia*, PGPR bacteria will not live forever in soil, and over time growers will need to re-inoculate seeds to bring back populations. Some PGPR need to be re-inoculated every season. This can be seen as a benefit since it shows that the amount of naturally occurring bacteria quickly goes back to normal after a season.

Applications:

PGPR can be used in a variety of ways when plant growths required. Especially in agriculture, horticulture, forestry and environmental restoration.

Applications of PGPR in Agriculture:

Use of PGPR has been reported to increase the crop yields by 50-70%. PGPR may act by

1. Increasing germination rates
2. Increasing root growth
3. Increasing yield including grain size, leaf area
4. Increasing chlorophyll, magnesium, nitrogen and protein contents
5. Increasing hydraulic activity, that is fluid movement within the plant
6. Tolerance to drought and low temperature
7. Delaying leaf senescence and disease resistance.

REFERENCES

[1] Das SN, Sarma PVSRN, Neeraja C, Malati N, Podile AR (2010). Members of *Gammaproteobacteria*and *Bacilli* represent the culturable diversity of chitinolytic bacteria in chitin-enriched soils. *World J. Microbiol. Biotechnol*. **26**: 1875–188.

[2] Defago, G. and Haas, D. (1990). *Pseudomonads* as antagonists of soil borne plant pathogens: modes of action and genetic analysis. Soil Biochemistry. 6: 249-291.

[3] Gehring, P.J., Mohan, R.J. and Watamare, P.G. (1993). Solvents, fumigants and relatedcompounds. In: Handbook of Pesticide Toxiocology, Vol. 2, Eds., Hayes, W.J. andLaws, E.R., Academic Press, Inc., San Diego, California, pp.646-649.

[4] Hiltner, L. (1904). UberneuereErfahrungen und Probleme auf demGebiet derBodenbakteriologie und unterbesondererBerücksichtigung der Gründüngung undBrachte. Arbeiten der DeutschenLandwirtschaftlichenGesellschaft, 98: pp 5978.

[5] Hofte, M. (1993). Classes of microbial siderophores. In: Iron Chelation in Plants and Soil

[6] Microorganisms (Borton, L.L. and Hemmingings, B.C., San Diego, Eds.) VCHPress.

[7] Kloepper, J.W. and Schorth, M.N. (1978). Plant growth promoting rhizobacteria on radishes in proceedings of the fourth international conference on plant pathogenic bacteria, Vol 2, INRA, Angers (Ed., Gibert-clareyTours) pp 879-882.

[8] Lugtenberg, B.J.J., Bloemberg, G.V., Bolwerk, A., vandenBroek, D., CazorlaLopez, F., ChinAWoeng, T.F.C., Eijkemans, K., Kamilova, F.D., Kuiper, I., Mulders, I.H., van Rij, E.T., deWeert, S. (2004). Biocontrol of pathogens. In: Biology of plant microbes interactions, (eds) Tikhonovich I., Lugtenberg, B.J.J., Provorov, N. vol 4 th International Society for Molecular Plant Microbe Interactions, St Paul, Minnnesota. USA. pp 305309. ISBN: 095462536

[9] Mishra,R.K., Prakash,O., Tiwari, A.K., Pandey,A., AlamM., and Dikshit A. (2011). Culture filtrate antibiosis of Plant growth promoting rhizobacteria PGPRs against phytopathogens infecting medicinal and aromatic plants. International Journal of Research in Biological Sciences. 1 (4): 45-51.

[10] Nelson, L.M. (2004). Plant growth promoting rhizobacteria (PGPR): Prospectsfor new inoculants. Online. Crop Management doi: 10.1094/CM2004030105RV.

[11] Ramamoorthy, V., Viswanathan, R., Raghuchander, T., Prakasam, V. and Samiyappan, R. (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. Crop Protection 20: 1-11.

[12] Salisbury, F.B. (1994). The Role of Plant Hormones, in Plant Environment Interactions. Ed.R.E. Wilkinson, Marcel Dekker, New York, USA, pp.39-81.

[13] Schwintzer R., and Tjepkema J.D. (1990). The Biology of Frankia and Actinorrhizal Plants. Academic Press Inc. San Diego, USA, 99.

[14] Van Loon, L.C., Bakker, P.A.H.M., and Pieterse, C.M.J. (1998). Systemic resistence induced by rhizosphere bacteria. Annual Review of Phytopathology. 36: 453-483.

[15] Whipps J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany, 52 (1):487-511.