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Environmental Effects and Antagonism of Rhizobacteria from Chickpea Rhizospheric Soil

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ABSTRACT: Plant growth promoting rhizobacteria (PGPR), are associated with roots, found in the rhizosphere and can directly or indirectly enhance the plant growth. In this study soil was collected from rhizosphere of chickpea fields of different areas of Rawalpindi division of Pakistan. PGPR were isolated, screened and characterized. Eight isolates of rhizobacteria (RHA, RPG, RFJ, RC, RTR, RT and RK) were isolated from Rawalpindi division and were characterized. The antagonistic activity of these PGPR isolates against root infecting fungi (*Fusarium oxysporum* and *Verticillium* spp.,) was done and production of indole acetic acid (IAA), siderophore and P-solubilization was evaluated. The isolates RHA, RPG, RFJ, RC, RRD and RT were found to be positive in producing siderophore, IAA and P-solubilization. Furthermore, most of the isolates showed antifungal activity against *Fusarium oxysporum*, and *Verticillium* spp. The rhizobacterial isolates RHA, RPG, RFJ, RC, RRD, RTR, RT and RK were used as bio-inoculants that might be beneficial for chickpea cultivation as the rhizobacterial isolates possessed the plant growth promoting characters i.e. siderophore, IAA production, phosphate solubilization. In in vitro tests, Pseudomonas sp. and Bacillus spp. inhibited the mycelial growth of the fungal root pathogens. The isolates (RHA and RPG) also significantly increased (60-70%) seed germination, shoot length, root length of the chickpea. The incidence of fungi was reduced by the colonization of RHA and RPG which enhanced the seedling vigor index and seed germination. The observations revealed that isolates RHA and RPG is quite effective to reduce the fungal root infection in greenhouse, and also increases seed yields significantly. These rhizobacterial isolates appear to be efficient yield increasing as well as effective biocontrol agent against fungal root pathogen. The antagonistic activity against Fusarium oxysporum f. sp. ciceris was determined for 40 chickpea rhizobacteria. Twenty eight isolates showed antagonistic activity against test fungus ranging from 18.2 to 41.8%. Characterization of the antagonistic attributes showed that all the antagonistic isolates produced diffusible and volatile antifungal metabolites in terms of growth inhibition, maximum being with the isolates 39P (77.8%) and 15B (64.2%), respectively. Nineteen of the isolates showed catechol and hydroxamate type siderophore production. All the isolates produced ammonia and twelve showed HCN production. On the basis of their antagonistic and PGP functionality traits, five isolates (2B, 7B, 28P, 34P and 38P) were selected for glass house studies on two chickpea varieties (JG-62 and GPF-2). Isolates 28P, 34P and 38P were found to be most promising for wilt control and plant growth promotion. Isolate 38P reduced the wilt incidence to 44.6% which was at par with fungicide treatment (55.5%) and had a significant edge over negative control (85%) in the chickpea variety JG-62. Similar trend of wilt incidence was observed in GPF-2 variety. Green house experiments on two varieties of chickpea JG-62 and GPF-2 showed that seed treatment with plant growth promoting rhizobacteria (PGPR) + Mesorhizobia had a synergistic effect in terms of disease control and growth promotion as compared to use of single bioinoculants, thus positively influencing plant microbe interaction. Sixty-nine rhizobacteria isolated from chickpea rhizosphere were screened for their antagonistic potential against *Fusarium oxysporum* f. sp. *ciceris* under in vitro condition. Of these, 30 isolates inhibited growth of the pathogen and 13 potent antagonists were assessed for their functionality traits. Among the antagonistic traits exhibited by antagonists, production of diffusible antimetabolites insured its inhibitory effect on growth of test fungus as well as spore germination on solid media. Inhibition of fungus biomass in broth-based dual culture was revealed by 11 antagonists, while metabolic extracts of 5 isolates reduced the radial proliferation on solid media. Implication of biocidal volatiles in antagonism was demonstrated by 11 isolates; 3 produced cyanogenic volatiles, while all were ammonia producers. Investigation for hydrolytic activity demonstrated production of chitinase by 4 isolates and β-1,4-glucanase by 11, while all were protease and amylase producers. Revealing the dual potential of bioantagonists, all the isolates tested positive for IAA and salicylic acid production, 7 for siderophore production while 10 were P-solubilizers and 8 Zn-solubilizers. Under glass house condition, bacterization of chickpea seeds with potential isolate Ps14c alone and in consortium with *Mesorhizobium ciceris* reduced wilt incidence in pots (soil amended with *F. oxysporum*) to 48 and 28%, respectively, as compared to uninoculated control (74%) and also recorded appreciable increase in growth parameters.[1]

I. INTRODUCTION

Conventional agricultural practices often rely on synthetic fertilizers and pesticides which have immense and adverse effects on humans, animals and environments. To minimize these effects, scientists world over are now deeply engaged in finding alternative approached for crop production which are less dependent on chemical inputs. One such approach is the use of rhizospheric bacteria as vital components of soil fertility and plant growth promotion (PGP) through their direct and indirect processes in plant rhizospheres. Among the most studied rhizobacteria are the Bacilli, particularly for production of antibiotics, enzymes and siderophores all of which are important aspects of PGP. Despite this, little information is available especially on their potentiality in crop production and their direct application only involves a few species, leaving a majority of these important rhizobacteria unexploited. Plant growth promoting rhizobacteria (PGPR) shows an important role in the sustainable agriculture industry. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. In addition, PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere and beyond in bulk soil, which indirectly boosts plant growth rate. There are many bacteria species that act as PGPR, described in the literature as successful for improving plant growth. However, there is a gap between the mode of action (mechanism) of the PGPR for plant growth and the role of the PGPR as biofertilizer—thus the importance of nano-encapsulation technology in improving the efficacy of PGPR. Hence, this review bridges the gap mentioned and summarizes the mechanism of PGPR as a biofertilizer for agricultural sustainability.[2,3]

Agriculture is one of the human activities that contributes most to the increasing amount of chemical pollutants via excessive use of synthetic chemical fertilizers and pesticides, which cause further environmental damage with potential risks to human health. Nitrous oxide (N_2O) is an example of chemical pollutant produced by excessive use of nitrogen fertilizer and is a major source of greenhouse gases causing global warming. Moreover, 74% of total U.S. N_2O emissions in 2013 were accounted for by agricultural soil management, the largest single source [1]. Apart from that, nitrogen fertilizers reduce biological nitrogen fixation in the soil. Farmers apply a high concentration of nitrogen fertilizers in the form of ammonium nitrate to fertilize their soil to grow crops. Due to the influx of ammonium, plants no longer need the symbiotic microbes to provide ammonium and this leads to the degree of symbiosis being diminished. Furthermore, nitrifying bacteria also take advantage of this excess ammonium and utilize it to produce nitrate. This high amount of nitrate is then utilized by denitrifying bacteria to produce N_2O and excess nitrate leaches into the groundwater [2]. As a result, increased microbial processes of nitrification and denitrification increase the natural production of N_2O. Denitrification is the step whereby nitrogen oxides are reduced by microorganisms to gaseous products and released back into the atmosphere and nitrification is a two-step process of ammonium (NH_4) being converted to nitrate (NO_3) by soil bacteria [3].

Rhizospheric soils of crop plants have more flora and fauna due to availability of more organic compound, macronutrient and micronutrient. Rhizobacteria that exert beneficial effects on plant growth and development are referred to as Plant Growth Promoting Rhizobacteria (PGPR). Plant growth promoting rhizobacteria is a group of free living soil bacteria, which have ability to promote growth and yield of crop plant by direct and indirect mechanism. PGPR is generally two type, one is colonies inside plant cells that called intracellular PGPR (iPGPR) and other colonies out side plant in rhizosphere that called extracellular PGPR (ePGPR). This review generally focused on direct and indirect mechanism of PGPR. Direct mechanism of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment. The indirect mechanism of plant growth occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host. The search for PGPR and investigation of their mode of action are increasing at a rapid use as commercial biofertilizers. [4]

II. DISCUSSION

The use of beneficial soil microorganisms as agricultural inputs for improved crop production requires selection of rhizosphere-competent microorganisms with plant growth-promoting attributes. A collection of 563 bacteria originating from the roots of pea, lentil, and chickpea grown in Saskatchewan was screened for several plant growth-promoting traits, for suppression of legume fungal pathogens, and for plant growth promotion. Siderophore production was detected in 427 isolates (76%), amino-cyclopropane-1-carboxylic acid (ACC) deaminase activity in 29 isolates (5%), and indole production in 38 isolates (7%). Twenty-six isolates (5%) suppressed the growth of *Pythium* sp. strain p88-p3, 40 isolates (7%) suppressed the growth of *Fusarium avenaceum*, and 53 isolates (9%) suppressed the growth of *Rhizoctonia solani* CKP7. Seventeen isolates (3%) promoted canola root elongation in a growth pouch assay, and of these, 4 isolates promoted the growth of lentil and one isolate promoted the growth of pea. Fatty acid profile analysis and 16S rRNA sequencing of smaller subsets of the isolates that were positive for the plant growth-promotion traits tested showed that 39%–42% were members of the *Pseudomonadaceae* and 36%–42% of the *Enterobacteriaceae* families. Several of these isolates may have potential for development as biofertilizers or biopesticides for western Canadian legume crops.[5]

Soil microbial populations are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural systems and natural ecosystems. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low-input biotechnology, to help sustainable, environmentally-friendly, agro-technological practices. Much research is addressed at improving understanding of the diversity, dynamics, and significance of rhizosphere microbial populations and their co-operative activities. An analysis of the co-operative microbial activities known to affect plant development is the general aim of this review. In particular, this article summarizes and discusses significant aspects of this general topic, including (i) the analysis of the key activities carried out by the diverse trophic and functional groups of micro-organisms involved in co-operative rhizosphere interactions; (ii) a critical discussion of the direct microbe–microbe interactions which results in processes benefiting sustainable agro-ecosystem development; and (iii) beneficial microbial interactions involving arbuscular mycorrhiza, the omnipresent fungus–plant beneficial symbiosis. The trends of this thematic area will be outlined, from molecular biology and ecophysiological issues to the biotechnological developments for integrated management, to indicate where research is needed in the future. Pathogen suppression by antagonistic micro-organisms can result from one or more mechanisms depending on the antagonist involved. Direct effects on the pathogen include competition for colonization or infection sites, competition for carbon and nitrogen sources as nutrients and signals, competition for iron through the production of iron-chelating compounds or siderophores, inhibition of the pathogen by antimicrobial compounds such as antibiotics and HCN, degradation of pathogen germination factors or pathogenicity factors, and parasitism. These effects can be accompanied by indirect mechanisms, including improvement of plant nutrition and damage compensation, changes in root system anatomy, microbial changes in the rhizosphere, and activation of plant defence mechanisms, leading to enhanced plant resistance. An effective biocontrol agent often acts through the combination of several different mechanisms[7]

III. RESULTS

Rhizosphere- The bacteriologist and agronomist Lorenz Hiltner were first defined the term rhizosphere in 1904 [3] as a thin layer of soil where interactions between absorbent roots and microorganisms take place [9]. Rhizobacteria or rhizospheric bacteria are a specific community of soil bacteria that have the ability to colonize the rhizospheric soil, with the potential to reside in contact with plant roots at various stages of development and growth. Bacteria meeting this definition belong to different genera and species, of which the most studied are *Pseudomonas*, *Azospirillum, Agrobacterium* and *Bacillus*. Plant Growth Promoting Rhizobacteria (PGPR) is a rhizobacteria that has the ability to enhance plant growth directly or indirectly through the colonization of root systems. Biological control, also known as "biocontrol", is the deliberate use of the biological capabilities (natural mechanisms of action and/or interactions) of a beneficial species to reduce the development of another harmful species. The need for natural, ecologically sustainable, environment-friendly and non-toxic alternatives to chemicals is increasingly being sought, and is leading to the consideration of using PGPRs as biocontrol agents because of the strength that they give to the rhizosphere to deal with threats that target plant roots, in addition to contributing to restoring biodiversity in agro-ecosystems [8].

The modes of action of PGPRs as a biocontrol agent depend mainly on the microorganism used and the type of plant pathogen to which is applied [11]. In general, the main modes of biocontrol attributed to PGPRs to reduce soil-borne diseases .[12] Antagonism Beneficial rhizobacteria that can secrete substances that inhibit the growth of phytopathogenic microorganisms are called antagonistic bacteria. Therefore, antagonism is the ability of one germ to inhibit the growth of another germ when they are in the same micro-biotope [7]. Similarly, it is expressed in the laboratory when they are grown together in the same Petri dish [7], and it often linked to the phenomenon of antibiosis [11]. Antibiotic production is one of the mechanisms used by PGPRs in the prevention of phytopathogenic attacks and in the suppression of biotic diseases [6]. Regarding the use of PGPRs as a biocontrol tool, both genera Paenibacillus spp and Bacillus are frequently documented [6]. Competition Competition for space, nutrients or other environmental factors that become limiting to microbial growth is a biological mechanism used by PGPRs to repel or eliminate plant pathogens [5]. An effective competitive agent must be an intense colonizer capable of immediately and efficiently exploiting nutrients present at low concentrations in the soil or stopping their uptaken by other microorganisms [9]. For example, some strains can synthesize extracellular enzymes that led to use organic compounds as a source of energy and/or to degrade phytotoxins [3]. However, in some cases, a reduction in disease may be associated with significant root colonization by PGPRs, which reduces the number of habitable sites for plant pathogenic microorganisms and consequently their growth [7]. The density and intensity of rhizobacteria activity influenced this interaction between beneficial bacteria and phytopathogens [5].. Biofilm formation Biofilms are structurally complex aggregates of microbial cells attached to a surface and surrounded by an extracellular polymer matrix [7]. PGPRs have a very strong capacity to attach to the plant root system when they form a biofilm [2]. Biofilms have the power to provide significant protection against external aggression and stress, as they act as a protective barrier that prevents the penetration of plant pathogens, releasing a wide range of enzymes, and reducing microbial competition [8]. The best studied examples of PGPRs that form biofilms. . Hydrolytic enzymes The synthesis of hydrolytic enzymes is one of the essential biocontrol mechanisms used by PGPRs against telluric plant pathogens [5]. These strains play a major role in decomposing organic matter in ecosystems and thus protecting plants from environmental stresses [5]. PGPRs can produce certain enzymes, such as amylase [3], chitinase [1], phosphatase [5], protease [6], urease [8], cellulase and lipase [5]. Improvement of stress resistance The action of PGPRs can improve plant's resistance against pathogens. It is mainly due to two signalling pathways ✓The Acquired Systemic Resistance (ASR) whose signal molecule is salicylic acid. It acts by increasing the production of salicylic acid during a microbial infection at the site of contamination as well as in the whole plant. In some plant/pathogen models, salicylic acid, brought exogenously by fluorescent Pseudomonas, conferred protection against pathogens [42]. ✓Induced Systemic Resistance (ISR): Some PGPRs can stimulate the induced response mechanisms in the plant and lead the whole plant to a state of resistance called Induced Systemic Resistance (ISR)[12]

IV. CONCLUSION

The potential of PGPRs in biocontrol is well established, and their use is proving to be a promising strategy for chemical pesticides. In the present review, the phytoprotective effects of certain PGPRs suggest the possibility of the direct inclusion of these microorganisms in programs for the prevention and control of microbial infections of plants, particularly in agriculture.

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