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Research Review

Mechanism of PGPR Concerning Nanobiotechnological Advances Acting as a Suppressor for Phytopathogens

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Abstract

New eco-friendly techniques should be used to fulfill the demand for foodstuffs of the day-by-day increasing population. So that we can overcome negative impacts on the environment as well as on land because of the overuse of chemical fertilizers and pesticides for enhancing crop productivity and increasing yield. It leads to hyperaccumulation of chemicals, soil degradation, soil compaction reduction, reduction in soil organic matter, leaching problems, and soil becoming infertile. In addition to this climatic change is one of the major problems. To overcome this PGPR growth-promoting bacteria can use direct and indirect mechanisms which lead to the acquisition of resources and also helps in the regulation of plant hormone levels and also assist in suppressing the inhibitory effects of pathogens on plant growth and development. It also acts as a biocontrol agent (BCA) to soil-borne pathogens and ISR. This can be achieved by the ability of PGPR to synthesize compounds such as Siderophore production, and the synthesis of lytic enzymes which hydrolyze the phytopathogen's cell wall and suppress negative effects on crops, Antibiotic production is one of the important antagonist's mechanisms against plant pathogens and also acts ISR to certain pathways for inducing systemic resistance and stress detecting markers which includes ACC deaminase and it produces various stress markers enzymes. Indirect mechanisms also cope with some nanobiotechnological aspects to improve crop productivity by creating certain genetic manipulation, thus suppressing detrimental effects on crop productivity.

***Key words*:** Hyperaccumulation, PGPR, Indirect mechanism, Resource acquisition, BCA, Lytic enzymes, Antagonism, ISR, ACC deaminase, Nanobiotechnological advances

In this period of climate change and resource limitation, challenges to crop production in terms of abiotic stress, nutritional deficiency, and disease are considerable. Managing these challenges with conventional agrochemicals is no longer practical as they will only produce significant negative impacts on both the environment and human health. Hence, sustainable and innovative approaches are essential to successfully counteract the adverse impacts of climate stress and lower yields. The excessive use of fertilizers and pesticides has distorted soil composition, fertility, and integrity with non-desirable environmental and ecological consequences. A strategy was designed to prepare a nanostructured slow-release fertilizer system that delivers nutrients and plant growth-promoting rhizobacteria simultaneously. Plant growth-promoting rhizobacteria (PGPR) are heterogeneous root-associated beneficial bacteria that are known for their ability to enhance plant growth by either direct or indirect Phyto stimulatory mechanisms. Direct mechanisms involve those related to the mobilization of important nutrients such as phosphorous, zinc, sulfur, and iron, and for promoting non-symbiotic nitrogen fixation along with the production of various phytohormones like indole acetic acid [1]. Indirectly, produces the deleterious effects of phytopathogens and protects the plant against biotic and abiotic stress conditions. However, the variability in the performance of PGPR under varied climates, weather parameters, and soil characteristics is a major difficulty in exploring its field efficacy. PGPR formulations are applied as suspensions to seeds, root surfaces, or directly to the soil. It is difficult for a single microbial inoculant to perform consistently under varying agro-climatic conditions and stresses; therefore, recent trends in PGPR applications adopt multiple inocula. Microbial consortia have proven to have higher efficiency than the application of a single species. Their survival and colonization, however, depend on the recipient environment's physical, chemical, and biological nature. Declining microbial diversity and numbers within the consortia can result in inefficient colonization of the rhizosphere of the host plant. Microbial consortia can be prepared in liquid,

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organic, inorganic, polymeric, and encapsulated formulations for wider use [2]. The carrier of the consortia can provide the necessary microenvironment to ensure the survival of organisms and also act as a niche for security against soil predators. Peat, coal, clay, waste plant materials, vermiculite, and the residues of Azolla are commonly employed for PGPR applications. Maintenance of adequate growth conditions over time in terms of nutrition and climate are major hurdles in transferring the developed consortia from the lab to the field. Failure to maintain the desired environs can considerably affect microbial counts, which in turn can adversely affect field results. Hence, introducing innovative and effective methods for the field delivery of PGPR is important. The application of nanotechnology in the agricultural sector has gained immense attention due to its ability to enhance biotic and abiotic stress tolerance, disease detection, and prevention along with refined nutrient absorption. Nanomaterials can improve the nutrient utilization efficiency of plants when compared to conventional approaches. Nanoparticles (NPs) can boost plant metabolism through their defined physicochemical properties and enhancing crop yield and providing nutrients to the soil [3]. Nanoparticles can generally be classified as carbon nanoparticles, metal nanoparticles, organic, and semiconductor nanoparticles. Among these, silver, titanium, zinc oxide, silica, carbon, boron, and zeolite nanoparticles have been reported to have plant growth-promoting effects. Nano-fertilizers are more effective than conventional chemical fertilizers as they do not cause problems with leaching and nutrient loss following application and only minimal amounts are required which thereby reduces the risk of soil and water pollution. Nanotechnology-based plant viral disease detection kits and Nano biosensors are gaining popularity by their improved efficacy in the detection of various viral diseases Nano biosensors can be used to detect even minute levels of fertilizers, herbicides, pesticides, insecticides, pathogens, moisture, and soil pH, thus supporting sustainable agriculture for enhanced crop production. The rhizosphere is the zone surrounding the plant root and contains abundant plant growth-promoting microorganisms. Plants secrete various exudates into rhizosphere soil which attracts microorganisms. The exudates and microbial communities also can produce various nano-size minerals in the soil that have not yet been fully studied. Reports are available on the plant growth promotion activity exhibited by nanoparticles in combination with various PGPR organisms. PGPR and nanotechnological applications can make the agriculture sector more powerful than conventional technologies used for crop improvement. By developing a conjugative approach of both NP and PGPR, there is immense potential to improve both yields and disease resistance of plants.

The functional classification of PGPR is into two direct and indirect activities. Direct activity helps in nutrient cycling and photo stimulation by releasing growth regulators and hormones. Indirect activity includes systemic resistance to biotic and protection against abiotic stress. Phytohormone includes IAA, cytokinins, gibberellins (GA), and siderophore which helps in phytohormone production and substantial increase in the growth and yield of plants. There are mainly auxins, gibberellins, and cytokinin this IAA and auxin help in cell elongation, cell division, tissue differentiation, and apical dominance. Cytokinin influences physiological and developmental processes by microorganisms like *Pseudomonas, Azospirillum*, and *Bacillus* and helps in cell division, root development, and shoot inhibition. The most abundant cytokinins are adenine-type and work by two different pathways namely the tRNA pathway and the adenosine monophosphate (AMP)pathway. Gibberellins (GAs) effects are not exactly known but used in seed germination out of 136 GAs only 4 from PGPR, GA1, GA3, GA4, and GA20 are known to form. Bacteria synthesizing GA are *P. pumilus, B. licheniformis*. Siderophore production is act both directly as well as indirectly. Its iron nutrition attracts iron towards the rhizosphere and indirectly act by inhibiting the growth of other microorganism and hindering the growth of pathogens by limiting the iron available for the pathogens (fungi), *Pseudomonas fluorescence* and *Pseudomonas aeruginosa* are siderophore-producing microorganism. Chitinase and glucanase control soil-borne pathogens and thus act as biocontrol agents by producing cell-wall degrading enzymes which include B-1-3-glucanase, chitinase, cellulase, and Protease. Directly inhibitory effect on hyphal growth of fungal pathogens. Another mechanism includes Antibiotic production which serves as a microbial antagonist means inhibition against plant pathogens and is an alternate solution for chemical pesticide and mainly *Bacillus* and *Pseudomonas* species are showing inhibitory effect at lower concentration and *Bacillus subtilis* acts as antibacterial and antifungal antibiotics which includes volatile and non-volatile antibiotics. Another mechanism includes ISR which mainly deals with the protection of plants from diseases is mainly achieved by ISR increasing in level of basal resistance to several pathogens simultaneously ex. *Pseudomonas* strain undergoes different pathways which include jasmonate or ethylene pathways thus including host-parasite defense response. Different kinds of negative factors such as stress level may lead to problems to identify or suppress environmental stress certain stress markers for temperature, cold, drought, salinity, alkalinity, UV radiation, and pathogen infection. High salinity leads to an increase in oxidative stress and generation of reactive oxygen species (ROS) certain ROS scavengers enzymes and stress markers enzymes produced by *B. cereus* AR156, peroxidase (POX), superoxide dismutase (POD), Catalase (CAT). Next is the production of ACC Deaminase is 1-aminocyclopropane -1-carboxylic acid (ACC) precursor of phytohormone ethylene. increase dramatically under abiotic stresses it has a detrimental effect on the plant *Achromobacter piechaudii ARV8*- tomato plants. Under natural environmental conditions, successful plant growth and development and high crop yields depend on the genetic constitution of the crop species, suitable weather conditions, and soil components, including the availability of nutrients; the absence of growth inhibitory substances, such as salt; the presence of certain beneficial microorganisms; and the absence of pathogenic ones (called phytopathogens, from Phyto, meaning plant). Some beneficial indigenous soil bacteria and fungi act directly by providing a plant growth-enhancing product, and others act indirectly. The latter organisms inhibit the growth of pathogenic soil microorganisms, thereby preventing them from hindering plant growth. The indirect promotion of plant growth occurs when plant growth-promoting bacteria prevent the deleterious effects of phytopathogenic organisms, either fungi or bacteria, i.e., they act as biocontrol agents. This activity is called antibiosis, and it either depletes a scarce resource required by the pathogen or produces a compound that impedes the growth of the phytopathogenic organism [4]. Nanoscience has become one of the most promising fields of research with a greater impact on the economy and environmental health. The materials of 100 nm in at least one dimension, are likely to result in the production of a huge number of new nano-products in the coming years. In addition, nanotechnology is also likely to influence agricultural research, especially in the conversion of agricultural and food wastes to energy and other useful products through enzymatic nano-bio-processing. Disease prevention

and treatment of plants using various nanomaterials and reproductive science and technology. Such nanoparticles, however, have been found highly resistant to degradation and persist in soil or water bodies. Nanomaterials for example carbon nanotubes, iron-based nanoparticles, silver and copper, zinc, and titanium oxide nanoparticles have been reported to cause biologically undesirable toxic effects on both deleterious (DRMOs) and beneficial rhizosphere microorganisms including *Escherichia coli, Bacillus subtilis, Streptococcus aureus, Pseudomonas chlororaphis, Pseudomonas putida,* and *Campylobacter jejuni.*

Mechanism of PGPR

Siderophore production

Siderophore-producing PGPR acts in both direct and indirect mechanisms. It acts directly by iron-chelating molecules to the rhizosphere and improves iron nutrition by attracting iron toward the rhizosphere. And indirectly by inhibiting the growth of other microorganisms and hindering the growth of pathogens by limiting the iron available for pathogens. Siderophore-producing PGPR helps in crop nutrition and phytopathogen suppression. There are various ways by which one can enhance the yield and productivity of crops. The use of chemical-based fertilizers mainly N, P, K based fertilizers are considered the quickest and surest way of boosting crop production but chemicals used to increase yield may lead to lost soil fertility and make soil infertile for further use. Thus, the concept of integrated plant disease management (IPDM) has emphasized increasing soil productivity and fertility through plant growth promotion or phytopathogen suppression [5].

PGPR are plant growth-promoting rhizobacteria in close vicinity of plant roots that also play a vital role in plant growth for increasing crop productivity, it can be achieved by various direct and indirect mechanisms [6]. The addition of bioinoculants for sustainable soil helps in increasing soil organic matter and microbial population mainly rhizobacteria. PGPR works in both manners for growth promotion and disease suppression [7]. PGPR has many effects which include positive, negative, and neutral effects on the growth of plants [8]. Rhizosphere has accumulated by a diverse group of microorganisms which is beneficial for not only growth promotion but also the indirect manner by growth stimulation [9]. The major function is the inhibition of plant pathogens infecting in an indirect manner [10-11]. PGPR is classified according to direct and indirect mechanisms into two groups as biocontrol agents (BCAs) and antibiotic production are some indirect mechanisms [12].

PGPR is a term that can synonymously use with VIB (yield-yielding bacteria) which can work in both directions as well as indirect manner. It serves as a direct mechanism by phytohormones production, nitrogen fixation, and nutrient solubilization [13] and indirectly by suppressing plant pathogen phytopathogen infestations in cash crops. Common species which include *Azotobacter*, *Azospirillum* sp, *Pseudomonas*, *Acetenobacter*, *Alcaligens*, *Klebsiella*, *Enterobacter*, *Xanthomonas*, *Bacillus* sp. are known PGPR [14].

Direct and indirect mechanism of siderophore producing PGPR

Direct PGPR enhances plant growth in the absence of pathogens. By this, soil bacterial species in the plant rhizosphere which grow in, on, or around plant tissues stimulate plant growth by a plethora of mechanisms. In addition to providing mechanical support and facilitating water and nutrient uptake, microbial activity in the rhizosphere affects rooting patterns and the supply. Direct PGPR enhances plant growth in the absence of pathogens. And also acts indirectly by inhibiting the growth of other microorganisms and hindering the growth of pathogens by limiting the iron available for pathogens

Direct mechanism of PGPR

Siderophores are low molecular weight and are ferric-specific ligands specially produced by microbes as scavenging agents to cope with iron stress [15]. Synthesis of siderophore not only windrows iron deficiency by converting insoluble form into soluble but also by maintaining the adequate concentration of siderophore [16]. Certain aerobic and anaerobic bacteria play important role in siderophore production and they are very well known as iron chelators which include *Agrobacterium tumefaciens*, *Pseudomonas* species mainly *P. Fluroscens*, *P. aeruginosa*, *P. putida*, *Bacillus megaterium*, *Arthrobacter*, *Rhizobium Azotobacter chrococcum*, *Azotobacter uinelandii*. Siderophore is classified as Bacterial, and Fungal in origin which includes hydroxamate types such as ferritin, aerobactin, and ferrioxamine. Catecholate or carboxylates type includes enterochelin, aerobactin, and paratactic. Peptide type, mycobacteria, citrate hydroxamate. Other fungal origin includes ferrichrome, coprogens, rhodotorulic, and fusarinines. Siderophores are produced by both bacterial and fungal species which include Agrobactin by *Agrobacterium tumefaciens*, *ferrochrome* by *Penicillium paruum*, Asperchrome A, B, and C by *Aspergillus achraceus* and Azotochelin by *A. vinelandii* and providing by *Pseudomonas*, *Rhodotorulic acid* produces *Rhodotorula piliminae.* The function of siderophore is to store iron in cells and provide iron nutrition to plants and invades parasites to help in removing toxic metals from polluted soil. Iron is a most important element in the growth, metabolism, and survival of the majority of cell types and iron uptake by siderophores converts the insoluble form into a soluble form [17-18].

Iron chelation

Iron is essential for the growth and metabolism of major types of plants and it is the most important nutrient like NPK and the fourth most abundant nutrient present in the soil. They are present in insoluble form and it is unassecible to plants so they must convert into the soluble form like their ferric and ferrous form. Sometimes in aerobic environments, these soluble forms become oxidized into their oxidized form like ferric oxide and oxyhydroxides which are insoluble in nature and lead to immobilization and also cause massive mineral deposition which pollutes the soil. It leads to hyperaccumulation and contaminates the soil.

Significance of iron chelation

Iron play's an important role in the growth and metabolic activities of microorganisms, plants, and animals [19]. Iron is important electron transporter because it acts as the electron acceptor or donor at multiple steps of electron transport. The examples are cytochrome C, Cytochrome C oxidase 1 (Cox 1) and succinate dehydrogenase are all iron-dependent enzymes of ETC. Iron act as a cofactor of enzymes. If it is not present in the proper amount or deficiency may lead to growth inhibition, a decrease in RNA and DNA synthesis, and leads to certain physiochemical changes in cellular structure. Iron required for various important life processes and metabolic reactions is governed by iron which includes the TCA cycle, ETC, oxidative phosphorylation, nitrogen fixation, aromatic biosynthesis, photosynthesis, and certain important role in the regulation and biosynthesis and certain important role in the

regulation and biosynthesis and certain important role in regulation and biosynthesis of porphyrins, toxins, vitamins, antibiotics, cytochromes, pigments, siderophores. Iron storage proteins like ferritin in animals and bacterioferritin in microorganisms were also discovered. Iron storage proteins like ferritin in animals and bacterioferritin are between 12 and 24 ppm and there is a correlation between iron availability in soil and plants. Management of soils and fertilization to maintain optimum range of iron and maintain soil pH by addition of organic matter. The competitive ability of microorganisms for sequestering iron by siderophore and make it available for plants to suppress iron deficiency [20].

Iron uptake and assimilation of iron by plants are achieved by uptaking iron in its solubilization iron (Fe^{3+}) from iron oxidized and hydroxide. Mechanisms of iron uptake are more diverse than siderophore-mediated iron uptake [21] different systems are involved in the conservation of insoluble iron into its soluble usable form.

Acidification of rhizosphere

This type of mechanism is observed in non-graminaceous and all dicots, graminaceous include naturally growing or cultivated herbaceous flowering plants examples sunflowers, vegetables, pulses, strawberries, and dicotyledons including roses, oaks, sunflowers, and mustards. Acidification of the rhizosphere increases the solubility of iron by reduction of Fe^{3+} and Fe^{2+}·this reduction leads to the uptake of iron-starved plants [22].

Photosiderophores

In graminaceous monocots, it includes secretions of iron chelating substances which are phytosiderophores, and uptake of Fe^{3+} phytosiderophores and uptake of Fe^{3+} phytosiderophores by PGPR as nutrition role or phytopathogen suppression some are as following Fpv A has receptor ferripyroveredin, Fpt A for Pseudobactin 38 receptor, Pup A for Pseudobactin BN7 and BN 8 receptor, Fhu E for ferrioxiamine E [23-24]. Some IROMP means iron-regulated outer membrane proteins. Microorganisms produce siderophores for the uptake of iron by plants which help in the conversion of insoluble iron into a soluble form. This siderophore-producing bacterial strain possesses iron-regulated outer membrane proteins in their outer cell surface and transports ferric ions to membranes [25].

Significance of using siderophore-based PGPR as BCAs

Certain chemical-based biocontrol agents are available for suppressing plant pathogens but chemical-based agents may lead to hyperaccumulation of metals and crops facing certain difficulties during production and lead to infertile land thus using microbial-based biocontrol agents will neutralize the harmful effects caused by chemical agents this siderophore based biocontrol agents have a relatively wide spectrum of activity with high, consistent and reliable efficacy and bioproducts also must have a shelf life and has environmental and toxicological safety and it seems to be effective products for the application of biocontrol products may lead to an easy, possible way of protection of crops and are enhancing products as compared to chemical agents. biotechnological exploitation of using siderophore in rhizosphere region may lead to the maximum availability of iron to plants and helps in invading pathogens by various mechanisms. Certain techniques such as "Siderotyping" which is helpful in the classification of *Pseudomonads* based on siderophores [26]. Certain types of metals are recovered from mines with the help of siderophores and certain siderophores have a high affinity towards siderophores and Fe^{3+} specific certain conditions such as acidic, heavy metal polluted, or fertilizers-affected soil, other metal ions may be more abundant than Fe^{3+} will bind to siderophores and chelates the ions and which then gather by plants for growth and development and also for suppressing phytopathogens. chelating iron forms soluble Fe-siderophore complex and the addition of this to plants that suppress phytopathogens generally fungi, which are incapable of absorbing chelated iron complex and are deprived of available iron in the soil.

Indirect mechanism of PGPR

It includes phytopathogens suppression and acts as a controlling agent of plants pathogen known as a biocontrol agent of various plant diseases like the rotting of wheat, potato, and peanuts [27]. Antagonistic action against phytopathogens among various types of siderophores. Hydroxamate type of siderophore showing activities against *F. oxysporum*, *F. solani* [28]. Phytopathogens specifically play role in inhibiting bacterial and fungal strains and one of the important key roles of PGPR is fluorescent *Pseudomonas* exhibits antagonistic action against several phytopathogens [29-30]. PGPR synthesizes siderophores against phytopathogens and this enhances biocontrol agents more than those chemical fungicides. It is superior and shows in vitro phytopathogens suppression activity of siderophore genic preparations of Ni- and Mn- resistant *Alcaligenes sp*. which are more beneficial than that of chemical fungicides this are mainly used in the supernatant form and act against *Aspergillus niger, A. flavus, Fusarium oxysporum, Alternaria alternata*.

Mechanism of phytopathogen suppression

Phytopathogen suppression is an important mechanism for determining the enhancement and implementation of biocontrol agents by direct and indirect mechanisms.

Competition for iron

The competition mechanism is work by competing with biocontrol agents thus suppressing phytopathogens by colonizing at plant roots thus it is an important mechanism by which Fe suppresses the pathogens. Under Fe stress bacteria will produce siderophores like pyoverdine and pseudobactin which have an affinity for fungal siderophores [31].

Antibiosis

This mechanism works on the principle of antibiotic production which includes various antibiotics such as pyrrolnitrin, pyoluteorin, tropolone, phycocyanin, and 2,4-diacetylphloroglucinol which suppress the various pathogens by induction of fungistatic and inhibit spore germination and lysis of fungal mycelia. Certain bacterial-based antibiotics which are produced by *P. fluorescens* act as disease control agents by controlling crown gall in dicots produce by again 84, which inhibits *A. tumifaciens* [32].

Predation and parasitism

Predation is a phenomenon that deals with the preying of one on another by attacking the pathogens and parasitism includes living on another host organism which certain siderophores producers which are Mycoparasites such as *Coniothyrium minitans* which are proven as good biocontrol agents and controlling diseases caused by *Sclerotinia sp.* and other sclerotia-forming fungi [33].

Induced systemic resistance

Some PGPR are showing some indirect mechanisms which induced resistance against certain forms of pathogens and induce resistance [34] which includes PGPR such as

fluorescent *Pseudomonas* which help resistance to certain broad-spectrum phytopathogens [35]. Also, help in the induction of systemic resistance and leads to changes in plant physiology which help in resistance against phytopathogens [36].

Production of lytic enzymes

Introduction lytic enzymes

It is one of the broad varieties of microbial biocontrol agents which are stated as lytic enzymes. PGPR has the capability of indirectly enhancing plant growth through the production of bioactive compounds and suppressing phytopathogens by producing biochemicals which include lytic enzymes and peculiar species synthesizing lytic enzymes which include cellulases, glucanases, proteases, and chitinases. Many polymeric compounds like cellulose, hemicellulose, chitin, and protein, which are generally present in phytopathogens' cell wall composition, can be hydrolyzed by lytic enzymes produced by various microorganisms. Microbes can directly suppress the growth and activities of pathogens by secreting lytic enzymes, this lytic enzyme will lyse the fungal cell wall [37]. This lytic enzyme will either digest the enzymes of intercalates or deforms components of the fungal cell wall of fungal pathogens. And it is an environmentally friendly mechanism to control soil-borne pathogens [38]. These enzymes decompose nonliving organic matter and plant residues to obtain carbon nutrition one example of lytic enzymes produced by Myxobacteria is effective in suppressing fungal plant pathogens [39]. Antagonistic bacteria are effective in reducing the mycelial network of *Sclerotium rolfsii* by expressing chitinase important bacteria including *Serratia marcescens* [40]. Glucanase is produced by *Lysobacter* and it directly contributes to the parasitization of phytopathogens and reduces biotic stress [41]. The vast range of phytopathogens causes various types of diseases by infecting the whole or specific parts of plants. And their effects range from mild symptoms to catastrophic effects which may lead to the depletion of the food supply. To neutralize its harmful effects traditional methods are to use chemical fertilizers to minimize the pathogenic effects but continuous use of chemical fertilizers may lead to the development of resistance which causes the loss of productivity thud there is a need to find out modern and promising methods that of chemical fertilizers.

Mechanism of lytic enzymes

The biocontrol activity of PGPR includes various mechanisms namely Niche competition which deals with excluding the growth of phytopathogens from soil or host tissue. Mycoparasitism includes the lysis of fungal pathogen, the production of antibiotics that deals with interfering phytopathogen mechanism, and the production of hydrolytic enzymes which degrade the cell wall of phytopathogens [42]. The mechanism of lytic enzymes is mainly based upon the composition of the fungal cell wall and due to its unique composition, it is an excellent target for antifungal metabolites development fungal cell wall mainly composed of chitin, glucans, mannans, and glycoproteins [43]. The cell wall has a fibrillar structure attached to proteins and lipids and a variety of polysaccharides and this material may lead to the extracellular degradation and non-permeable substrates and modification of the cell wall structure 80% of a fungal cell wall made up of polysaccharides and this structure made up of chitin, chitosan, Beta-glucans and various trisaccharide's and also composed of gel-like matrix called proteins and glycoprotein. PGPR play a crucial role in biocontrol agents to control plant diseases and improvement of crops certain biotic agents like harmful insects, parasitic weed and phytopathogens cause serious damage to control the quality and quantity of crops different strategies are employed [44-46]. To improve biocontrol strategies by manipulating the soil environment and the study of the mechanism of biocontrol agents is studied by the interaction between BCA and pathogens [47].

Chitinase

Growth inhibition of pathogens can be suppressed by the secretion of lytic enzymes. This is achieved by degrading the cell wall with different lytic enzymes such as glucanase, protease, chitinase, and lipases which are mainly produced by certain biocontrol strains of PGPR involved in the lysis of the cell wall of a fungal and fungal cell wall made up of chitin [48] and mechanism if degradation varies from species to species the main mechanism by decomposing or deforming the fungal cell wall components. These lytic enzymes can be also recognized as hydrolytic enzymes that directly contribute to the parasitization of phytopathogens and reduce the plants from biotic stresses. The role of chitinolytic enzymes is 4-Beta-ILT-acetylglucosaminidases splits chain into GlcNAc monomers in an exo-type fashion and endochitinases cleave randomly at internal sites over the entire length of microfibril. The other is exochitinase catalyzes the progressive release of diacetylchitobiose in a stepwise fashion such that no monosaccharides or oligosaccharides are formed [49]. And beta-glucans can act by two mechanisms by sequentially cleaving glucose residues from the non-reducing end. Endo-glucanase cleaves beta-linkages at random sites along the polysaccharides chain releasing oligosaccharides [50]. The PGPR produces a diverse number of enzymes like ACC-deaminase, cellulases, chitinase, lipases, proteases, and β-1,3-glucanase which are involved in the lysis of fungal cell walls [51]. Hence β-1,3-glucanase and chitinase-producing bacteria are effective to suppress their growth. The expression of lytic enzymes by PGPR can enhance the suppression of phytopathogens. For instance, chitinase produced by *S. plymuthica* strain C48 inhibits germ-tube elongation and spore germination in *Botrytis cinerea* [52]. Chitinase secreted by *Paenibacillus* sp., *Streptomyces* sp., and *Serratia marcescens* was found to constrain the growth of *Botrytis cinerea*, *Sclerotium rolfsii* and *Fusarium oxysporum* sp. *cucumerinum*. Lysobacter produces the enzyme glucanase which inhibits *Bipolaris* and *Pythium* sp. [53]. *Micromonospora chance* and *Actinoplanes philippinensis* inhibit *Pythium aphanidermatum* in cucumber through the secretion of β-1, 3-glucanase [54].

Cell wall lysis

This leads to the breaking down of glycosidic bond between chitin and thus act as biocontrol agents by degrading phytopathogens' cell wall. It mainly shows its effect on fungal growth on the cell walls, hyphal tips, and germ tubes [55] which leads to hyphal curling or bursting of hyphal tip and degraded cell wall by chitinase production.

Mycoparasitism

Mycoparasitism is act directly on the fungal thallus leading to its lysis [56]. These mycoparasites divided into two groups called necrotrophic which kill host cells before or after the invasion and release nutrients these are more destructive than the biotrophic one this is due to the production of antibiotics, toxins, and hydrolytic enzymes [57]. In Biotrophic parasitism, it follows a living rather that dead host structure [58] and they had the most restrictive host range.

Protease

Proteases play a significant role in the cell wall lysis of phytopathogenic fungi since chitin and/or fibrils of β-glucan are embedded into the protein matrix. Thus, proteolytic activity is a prerequisite to lyse whole fungal cells [59]. Proteases are widespread in nature; microbes are the preferred source of these enzymes due to their fast growth and easy cultivation and the ease in genetic manipulation to get the enzyme with desired properties for specific applications [60-61]. *Bacillus* sp. produces extra-cellular proteases; several *Bacillus* species like *Bacillus cereus, Bacillus stearothermophilus*, *Bacillus mojavensis*, *Bacillus megaterium*, and *Bacillus subtilis* are known to produce protease [62-65]. Bacterial proteases are generally extracellular, easily produced in greater amounts, and active under various environmental conditions. Proteases purified from *Bacillus* have significant activity, stability, broad substrate specificity, a short period of fermentation, simple downstream purification, and a low-cost production process [66-67]. Extracellular proteases of *Trichoderma* sp. also play a significant role in the lysis of cell walls of phytopathogenic fungi. Some of the proteases produced by *Trichoderma* sp. are involved in inactivating extracellular enzymes of phytopathogenic fungi [68]. The protease enzymes break down major proteins into peptide chains and/or their constituent amino acids of phytopathogens and thereby destroy their capacity to act on plant cells.

Mode of action of protease

Proteins are degraded by hydrolysis which involves the cutting of one or more peptide bonds by the addition of water to liberate peptides or amino acids. Enzymes that hydrolyze the proteins are called proteases. Each protease recognizes the chemical structures of certain specific amino acids and then catalyzes the breaking of the peptide bond.

Cellulase

Cellulases catalyze the hydrolysis of 1,4-beta-D-glycosidic linkages in cellulose and play a significant role. They are having various intra and intermolecular hydrogen bonds which leads to the formation of rigid, insoluble, crystalline microfibrils. It is amorphous and crystalline in nature. Because of their crystalline nature, they are resistant to enzymatic hydrolysis. Cellus are beloa ng to group of enzymes catalyzes the hydrolysis of cellulose produced by bacteria, fungi and protozoan. The ability of PGPR to produce cellulases mainly associated with the degradation of fungal cell walls and helps in suppression of phytopathogen [69]. Different enzymes play important role in the degradation of cell wall by producing cellulose\endoglucanase, exoglucanses and beta-glucosidases convert cellulose into Beta-glucose [70]. Cellulases is mixture of endo-1,4-betaglucanses which cleaves internal bond and exo-1-4beta-glucanases cleaves two or four units from end of cellulose strands and cellobiase.

Glucanase

Beta-1,3-Glucanases are widely present in bacteria, fungi and higher plants [71]. These enzymes play important role on degradation of cell wall in fungi, yeast and higher plants and are classified as exo- or endo-beta-1,3-glucanases. Various strains such as *Sclerotium rolfsii* and *Pythium ultimum* can inhibits the beta-1,3-glucanases that inhibited the growth of *F. oxysporum* reported the seven Beta-1,3-glucanases produced by *T. harzianum* strain under diverse conditions. Beta-1,3-glucans are major components in cell wall of yeast and fungus. The cell wall polysaccharides glucan is considered of beta-1,3-linked backbone having branches via beta-1,6-linkages. It also causes degradation of cell wall and penetration into host mycelium [72]. They are carried out by two mechanism exo-1,3 glucanase act by cleaving sequentially glucose residue to from nonreducing end and endo-glucanase cleaves linkages at random sites along the polysaccharides chain releasing smaller oligosaccharides [73].

Significance of lytic enzyme production

The agrochemicals and genetic approaches used as tools to control plant diseases, but they are not always effective. Moreover, several agrochemicals are nonbiodegradable and exert a harmful effect on the environment. The excessive usage of pesticides for plant disease management has increased pathogen-resistant strains [74]. In this regard, PGPR have been seen as an attractive strategy and a sustainable means of controlling soil-borne pathogens and diseases. The application of PGPR and PGPF in sustainable agriculture has been increased in several regions. The PGPR with biocontrol efficacy often provides long-term protection against soil-borne phytopathogens because of their rhizosphere competency, i.e., capacity to rapidly colonize the rhizosphere.

Production of antibiotic

An antibiotic is specifically defined as a heterogeneous community of low-molecular organic complexes that harm the production or metabolism of various microorganisms [75]. In the management of plant diseases, one of the important mechanisms is the production of antibiotics for the suppression of plant pathogenic microbes by PGPR such as *Bacillus* species and *Pseudomonas* species. They also help in the destruction of pathogens and thus generate inhibitory effects and antagonistic metabolites in their defense mechanism against harmful strains of microbes. *Pseudomonas* strain mainly produces Phenazines, Pyrrolnitrin, Phycocyanin, Azomycin, Cepafungins and *Bacillus* species synthesis Kanoamin, Bacillomyxin. They also induced systemic resistance mechanisms which is one important way of suppressing the pathogenic effects. Certain strains produce a wide range of antibiotics and have multiple effects on the pathogenic strain. In vitro and in situ, the development of the target pathogen was the most crucial aspect of plant growth that promotes rhizome pathological bacteria and promotes resistance to other pathogens [76]. Antibiotics are specifically classified as volatile and non-volatile, as aldehydes, alcohols, sulfides, ketones, and hydrogen cyanide come under the category of volatile antibiotics and non-volatile antibiotics include heterocyclic nitrogenous compounds [77]. Antibiotics show beneficial properties like antimicrobial, antiviral, and antioxidant [78].

Table 1 Classification of an antibiotics as volatile and non-volatile antibiotic products

Non-volatile	Volatile
Polyketides (DAPG)	Hydrogen cyanide
Pyoluteorin (Plt)	Aldehydes, Ketones, alcohols, Sulphides

Non-volatile antibiotics

Polyketides

These are known as antimicrobial, antifungal and antioxidant agents. It acts as a plant growth-enhancing rhizobacterial agent as well as a biocontrol agent. 2,4-Diacetylphloroglucinol is a phenolic polyketide compound that is obtained from *fluorescent Pseudomonas. Gaeumannomyces graminis* var. *tritici* is responsible for wheat diseases which can be suppressed by 2,4-DAPG antibiotic that act as suppressing agents which prevents certain diseases caused due to soil-borne pathogens [79]. The phytopathogen can be prevented by some

Pseudomonas strain which shows nematocidal activities [80]. In plants, the DAPG elicits ISR microorganisms and serves as a unique elicitor in plant disease management.

Pyoluteorin (Plt)

It is a natural antibiotic that is biologically synthesized from the hybrid nonribosomal peptide synthetase and Polyketide synthase pathway [81]. It is isolated from various PGPR strains mainly *Pseudomonas aeruginosa* strains act as toxic against certain forms of pathogenic bacteria, and fungi. It inhibits most of the pathogenic strains of oomycete like *Pythium ultimum*. Another example that can cause diseases to sugarcane is *Glomerella tucumanensis* responsible for red rot in roots which leads to difficulties in the absorbtion of water from the soil and leads to growth-related problems but pyoluteorin produced by *P. putida* has been found to be effective against this disease [82-83].

Heterocyclic nitrogenous compounds

They are low molecular weight compounds known as phenazines. These compounds are produced by a diverse group of species which includes *Pseudomonas, Burkholderia, Streptomyces* and *Brevibacterium.* Some produces blend form of phenazines derivatives known as phenazine-1-carboxylic acid (PCA)found in *Pseudomonas* strain. Several strains of PGPR have antibiotic and antitumor features and are active in their ability to suppress pathogenic plant fungi and nematodes [84-85]. PCA mainly contributes in the suppression of pathogenic disease caused by *G. graminis* pathovar in wheat which leads to damage in wheat crops and similar manner another strain of *Pseudomonas* important on controlling cocoyam root rot caused by *P. myriotylum* [86]. It also produces pyocyanin and phenazine-1-carboxylic acid which having antagonistic activity against *Aspergillus niger, F. oxysporum* and other sort of different pathogens [87]. These antibiotics specifically meant to suppress pathogenic effect.

Volatile antibiotics

Hydrogen cyanide (HCN)

It is volatile in nature means it get evaporates in gaseous form and it is mainly produced by Gram negative bacteria which includes *Chromobacterium violaceum, P. aeruginosa, P. flurorescens* produce cyanide as secondary metabolites [88]. It also shows nematicidal activities against certain pathogenic strain. One of infection known as alfalfa infection caused by *F. solani* can be use as biocontrol agents [89]. The amount of HCN produced by rhizobacteria in vitro may or may not act as biocontrol agents but it can improve geochemical process in the substrate includes chelation of substrates which leads to availability of nutrients .it also act by synthesizing pigments which are effective against the fungal pathogens. *Bacillus amyloliquefaciens subsp. plantarum* XH-9 are example of microorganisms involved in volatile antibiotic production.

Aldehydes, alcohols, ketones, and sulfides

These chemicals are act as biocontrol agents against the fungal formation, ascospores. The survival of sclerotia were entirely impeded by these substances when come directly in contact with sclerotia structure leads to reduction in inoculum capacity and prevents occurrence of diseases [90].

Induced systemic resistance

To combat pathogenic bacteria, fungi, and viruses, PGPR activates some form of protection mechanism. This will improve and adapt the plant much better [91]. The gene and gene products have not been well established for this form of biological control phenomenon. Unlike systemic acquired resistance (SAR), a protection state is triggered in the entire plant following primary pathogen infections [92]. To act against plant pathogens, a mechanism called induced systemic resistance (ISR) uses plant hormones like jasmonic acid (JA), salicylic acid (SA), ethylene and other organic acids for the stimulation and signaling in host plant for the defence purpose [93]. This mechanism is mediated through JA, ethylene, and SA biosynthesis pathways [94]. The interaction of these hormones is either antagonistic or synergistic to change the mechanism of defences [95]. A large number of secondary metabolites that have antibiotic activity are phenolic, favonoids, alkaloids, cyanide glycosides, etc. [96]. Antimicrobial active ingredients, such as phenols, can inhibit microbial development, and different phenolic metabolic cells that are less harmful to plant cells accumulate in the cells than aglycones. After infection, aglycone is released by hydrolysis which is toxic to both plant cells and microbes [97]. The defence response in the plant system can cause cell wall thickening, lignification, callus deposition, a build-up of phytoalexins and synthesis of many lytic enzymes [98]. To cope up with environmental stress, PGPR reaction toward ISR can be achieved through adjustment of physical and biochemical reaction to environmental stress and also by increasing physical and mechanical vigour of the cell wall. There are certain bacterial species which are found to be involved in the process of biocontrol including *Pseudomonas sp., Bacillus pumilus*, and *Enterobacteriaceae* [99]. ISR has wider scope when applied PGPR strain is used as a seed coat against *Pseudomonas syringae* causing angular leaf spot, *Colletotrichum lagenarium* causing anthracnose in cucumber and *Erwinia tracheiphila* leading to bacterial wilt. To combat pathogenic bacteria, fungi, and viruses, PGPR activates some form of protection mechanism. This will improve and adapt the plant much better [100]. The gene and gene products have not been well established for this form of biological control phenomenon. Unlike systemic acquired resistance (SAR), a protection state is triggered in the entire plant following primary pathogen infections [101].

Production of ACC deaminase

ACC deaminase activity is relatively common in the plant microbiome [102], emphasizing the importance of this activity to the interaction and communication between plants and PGPB. Microenvironments, such as the rhizosphere, are the preferred location for the isolation and characterization of rhizobacteria with beneficial activities. One strategy to determine the possible activity of ACC deaminase in PGPB is to perform a screening for the *acdS* gene. Population searches, *in silico* or *in vivo*, for the *acdS* gene in genomes or soil microorganisms and endophytes, have shown that this activity has a broad bacterial distribution [103-105]. In a study in which the phylogeny of the *acdS* gene was analysed, this enzyme was observed in bacterial groups as diverse as *Actinobacteria, Deinococcus/Thermus, α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria* and *Firmicutes* [106]. Interestingly, the authors report that some *acdS* genes were also found in plant and human pathogens; as well as the presence of Lrp-like regulatory proteins, including AcdR, which regulates the expression of *acdS* genes in proteobacteria [106]. Other groups of plant beneficial organisms, such as the fungus *Trichoderma asperellum*, which also contains ACC deaminase, have shown phytopathogenic biocontrol and plant growth promotion activity [107]. The previous example is one of the relatively few works where the activity of ACC deaminase in a non-bacterial microorganism has been documented. As a consequence of this observation, it would be

advisable to search for such activity in beneficial fungi, such as *Trichoderma*, since it has been widely used as a biocontrol organism and promoter of plant growth [108]. Moreover, it has been reported [109] that the *Arthrobacter protophormiae*, an ACC deaminase-containing bacterium, interacts with other beneficial microorganism, enhancing rhizobial nodulation and mycorrhizal colonization, thereby inducing salt tress tolerance to *Pisum sativum* plants; this is also the case for several other rhizobia. The first report of the presence of ACC deaminase in rhizobia was published in 2003 [110]. Previously, the enzyme had only been reported in free-living bacteria, yeast and fungi. This report opened new research into these bacteria, which are important for fixing atmospheric nitrogen during their symbiotic interaction with legume plants. More recently, the presence ACC deaminase has been reported not only in *Rhizobium*, but in several Rhizobiaceae (*Rhizobium, Sinorhizobium*, and *Agrobacterium*), *Phyllobacteriaceae* (*Phyllobacterium* and *Mesorhizobium*) and *Azospirillum* [106]. Many genera of PGPB bacteria also exhibit deaminase ACC activities, including *Aneurinibacillus, Arthrobacter, Achromobacter, Bac illus*.The specific role of a gene may be analysed by generating mutant strains, as well as isolating and expressing the target gene in heterologous hosts. In the case of the acdS gene of *Pseudomonas* sp. UW4 in work by [111], this gene was isolated and expressed in hosts such as *Escherichia coli DH5α, Pseudomonas putida* ATCC 17399 and *P. fluorescens* ATCC 17400 all of which do not naturally contain ACC deaminase, thereby enabling the transformed strains to grow in minimal medium containing only ACC as a nitrogen source; these strains were unable to grow in the absence of the expression of the exogenous acdS gene. Importantly, when these strains were transformed with an acdS gene, they were subsequently able to stimulate the elongation of the roots of canola seedlings. Following a similar strategy, [112] expressed the acdS gene of strain UW4 in two strains of *Mesorhizobium ciceri*, one that was salt sensitive and one that was salt tolerant. *M. ciceri* is a nitrogen-fixing symbiont of chickpea plants. The results of this work indicated that the transformed *Mesorhizobium* strains significantly improved their symbiotic activity compared to the wild-types, and that the expression of the acdS gene facilitated the interaction between the plant and the bacterial symbiote under saline conditions. By generating ACC deaminase deficient mutants of two endophytic fluorescent *pseudomonads*. These authors reported that tomato plants that were pre-treated with wild-type ACC deaminase-containing endophytic *pseudomonad* strains exhibited higher fresh and dry biomass, higher chlorophyll contents, and in general, were healthier when growing under high salinity stress compared to plants that had been pre-treated with the ACC deaminase deficient mutants of these two PGPB strains or negative controls in which plants were not treated with any bacteria.

Nanobiotechnological advances

Long-term protection against soil-borne phytopathogens because of their rhizosphere competency, i.e., capacity to rapidly colonize the rhizosphere. The PGPR enhances growth and protects plants against phytopathogens. The PGPR can act as a biofertilizer, biopesticide, phytostimulators and rhizoremediators. The PGPR multiply in soil, leaving no residual problem. A single PGPR can protect against multiple plant pathogens. The PGPR possess multifarious mechanisms including antibiosis, CWD enzymes and siderophore production and also induce SAR/ISR in plants. They are nontoxic to plants and humans. They are eco-friendly and easy to manufacture. BCAs are cheaper as compared to the agrochemicals. The PGPR can be handled easily and applied in the field. The use of PGPR is sustainable in long-term. The agrochemicals and genetic approaches used as tools to control plant diseases, but they are not always effective. Moreover, several agrochemicals are nonbiodegradable and exert a harmful effect on the environment. The excessive usage of pesticides for plant disease management has increased pathogen-resistant strains [13]. In this regard, PGPR have been seen as an attractive strategy and a sustainable means of controlling soil-borne pathogens and diseases. The application of PGPR and PGPF in sustainable agriculture has been increased in several regions. The PGPR with biocontrol efficacy often provides importance in field.

Nano biosensors can be used effectively in agriculture for sensing soil pH, moisture, wide variety of pathogens, plant hormones, plant metabolites, pesticide, herbicide, fertilizers, and metal ions. Appropriate and controlled use of nano biosensor can support sustainable agriculture for improving crop productivity. Nano biosensor is a modified version of a biosensor which may be defined as a compact analytical device/ unit incorporating a biological or biologically derived sensitized element linked to a physico-chemical transducer. With the progression in sciences, nano biosensors with superbly dedicated miniature sensors with highly miniaturization were designed and developed in 21st century based on the ideas of nanotechnology.

Recently, researchers have used an integrated approach by combining nano sciences, electronics, computers and biology to create biosensors with extraordinary sensing capabilities that show unprecedented spatial and temporal resolution and reliability. Nano sensors with immobilized bioreceptor probes that are selective for target analyte molecules are called nano biosensors. A nano biosensor is usually built on the nanoscale to obtain process and analyze the data at the level of atomic scale Nano biosensors open up new opportunities for basic research and provide tools for real bio-analytical applications, which was impossible in the past. They can be integrated into other technologies such as lab-on-a-chip to facilitate molecular analysis. Their applications include detection of analytes like urea, glucose, pesticides etc., monitoring of metabolites and detection of various microorganisms / pathogens.

Future aspects

Synthesis of PGPR based nanoparticles by using various methods which includes green synthesis method or sol gel method using certain form of precursors. Due to nanoscale, it can penetrate into the plant easily and leads to improve productivity. Also study the effectiveness of synthesized nanoparticles against the pathogenic bacteria by studying its mode of action against the pathogens by In-silico methods. To synthesis the encapsulated form of nanoparticles and to study its effectiveness in plant growth and promotion. Preparation of nano biosensors using agricultural wastes can be used as novel material as it minimizes the wastes and reuse it in innovative way which benefits the agricultural sector because use of nano biosensors is boon to agricultural sector which detect minute levels of fertilizers, herbicides, pesticides, pathogens, moisture and P^H.

CONCLUSION

In concern with the current scenario toward chemical pesticides and fertilizers, and their huge consumption, there is a focus on utilization of microbial inoculants and organic inputs

for its application in agricultural field. Hence, the potential of rhizobacteria in crop protection by producing different defensive antifungal metabolites like antibiotics, hydrolytic enzymes, and other metabolites is hoped to provide sustainable and eco-friendly plant disease control. Application of these rhizobacteria in agricultural field in the form of formulated product will give the greener and eco-friendly approach for the sustainable agriculture to combat the fungal diseases. Applying efficient rhizobacterial strain secreting various hydrolytic enzymes will help reduce the liberal use and doses of agrochemicals, which is the most important prospect in rhizobacterial/PGPR research. Commercial production of these organisms will have sustained release of antifungal metabolites in the environment, and these metabolites do not develop the resistance to target organism as in chemical pesticides. Application of single or consortium of these organisms has shown the promising prospect in the field of biocontrol and plant growth promotion. Study of hydrolytic enzymes of rhizobacteria will help in manipulating the bacterial community with biological control and plant growth promotion ability in rhizosphere zone of different sites. So, these rhizobacteria will be the key determinant in plant health and productivity with sustainability.

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