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REVIEW

Molecular and physiological manipulations in rhizospheric bacteria

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Abstract
The plant growth promoting rhizobacteria (PGPR) has multifarious benefits to serve sustainable agriculture. Different consortia of microbes are currently being in use to increase yield of different commercial crops including wheat, canola, lettuce, tomato, banana, maize, chickpea and brinjal. Introduction of PGPR strains can help restore the soil health and prevent the ill effects of fertilizers. However, complete replacement of chemical fertilizers need engineered microbes with enhanced PGPR potential. Researchers are working seamlessly to engineer microbes with augmented indole acetic acid production, disease resistance, chitinases activity, nitrogen fixation and phosphorus solubilisation potential. Advances in gene editing tools and omics technologies have eased the process of gene manipulation in bacteria allowing non-PGPR strains to work as PGPR inoculants in rhizosphere. In this review, we have discussed microbe-based rhizospheric engineering approaches for the manipulation of different gene of interest for improving the PGPR potential of PGPR and non-PGPR strains. This review is fundamental in providing first-hand information on different success stories of genetic manipulations of PGPR strains done in recent years and provides understanding of the process in shaping future rhizosphere microbiome.

Keywords Gene editing tools · Gene manipulation · Microbiome · Plant growth promoting rhizobacteria (PGPR) · Rhizosphere

Background

Plant –root interface is considered as a unique niche for plethora of microorganism that act as key players in plant development and its resilience to various abiotic and biotic factors. Rhizosphere is estimated to host approximately 10^{10}–10^{12} CFU/g soil of bacteria from 10,000 different species (McNear 2013). Plants attract the bacteria by secreting various molecules which are collectively termed as rhizodeposits. Plants spend upto 17% of its total photo assimilates as rhizodeposits. Rhizodeposits includes root exudates, sloughed off plant cells, mucilage, diffusates**,** organic acids, proteins, amino acids, sugar and phenolic compounds to be used for microbial growth. In exchange, these bacteria regulate the growth of host plant by supplying soluble nutrient, enhancing stress tolerance and disease resistance (Zheng et al. 2018). The rhizospheric plant–microbe interactions have attracted much attention. The studies objectified towards comprehensive sustainable agriculture have reported that rhizospheric bacteria have a crucial role in crop health and yield. The application of rhizobacteria in agricultural field is associated with increasing crop yield, soil health and plant's tolerance (Simarmata et al. 2019). Rhizobacteria augment the plant development by various direct and indirect pathways. Direct pathways includes nutrient management by phosphate solubilisation, potassium solubilisation, biological nitrogen fixation, micronutrient supply, secretion of phytohormones like auxins, gibberallic acid, cytokinins and disease resistance by secretion of antibacterial, antifungal and anti-nematode factors (Kaur and Kaur 2018). On the other hand, indirect pathways include siderophore production, abiotic stress tolerance, ACC deaminase production, ammonia production. It has been reported that the climatic conditions, abiotic stress, plant host genetics, soil composition and anthropogenic activities significantly affects the availability and functioning of rhizobacteria in soil (Kaur and Kaur 2020; Igiehon and Babalola 2018).

The multifaceted advantages of plant growth promoting rhizobacteria (PGPR) can serve the agricultural benefits as

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a single entity towards replacing synthetic agrochemicals and chemicals fertilizers (Backer et al. 2018). Introduction of PGPR strains can help restore the soil health and prevent the ill effects of fertilizers. Living consortia of microbes are applied successfully to increase yield of wheat, canola, lettuce, tomato, banana, maize, chickpea and brinjal (Sneha et al. 2018). However, there is no single one fit biofertilizer that works for all crops and climatic conditions or that provides complete nutrient supply to plants. There is still a large gap between PGPR potential and its application as biofertilizer in fields for crop production (Vejan et al. 2016). These bacteria have shown high crop specificity and low success rate in varied climatic conditions. The inconsistency of rhizospheric bacteria to perform under different field conditions, limits its widespread usage for plant development. Differences in soil conditions and plant type effects the performance of rhizobacteria (Banerjee et al. 2006).The validation of application of rhizobacteria for field use is possible if their PGPR activity is enhanced through gene manipulations (Geetha and Joshi 2013).With the advancements in the molecular biology, synthetic biology and hand on genome editing techniques, there are vast opportunities to design a microbe that can provide all the three essential macronutrients (nitrogen, phosphorous, potassium) to the plants regardless of the crop. The knowledge of the mechanisms associated with the expression of plant disease resistance in PGPR has facilitated their better application by development of strains with enhanced activity or combination of different PGPR traits. This can also provide impetus to optimization of metabolic processes in PGPR to develop strains adapted to wide host range and environments. In this review, we summarize the recent advances in engineering plant growth promoting bacteria as living biofertilizer for sustainable agriculture (Tables 1 and 2).

Genetic manipulations in rhizobacteria to augment plant growth promoting potential

1. Nitrogen fixation

In sustainable agricultural system, yield of crop relies upon the soil's inherent fertility and microorganisms that regulate the transportability in addition to mineralization of essential nutrients for development of plants. Nutrient availability is appraised as the restricting factor of plant productivity (Choudhary et al. 2011). Nitrogen represents as one of the eminent nutrient principally required for the development of living beings specifically for the synthesis of amino acids. Bacteria belonging to genus *Azorhizobium, Sinorhizobium, Bradyrhizobium* and *Mesorhizobium* are reported as natural nitrogen fixers in leguminous plants while Frankia and Cyanobacteria are often found associated with non-leguminous plants (Kaur and Kaur 2018). PGPR strains like *Pseudomonas* sp. have shown the presence of *nif* genes required for nitrogen fixation. However, these microbe-plant associations are very specific and this symbiosis is observed in commercially important crops like rice, maize and wheat (James 2000). There is a need for the creation of potential recombinant strains exhibiting nitrogen fixating potential that can work for different crops. Earlier researchers have reported that transfer of functional *nif* (nitrogen-fixing) genes from *K. pneumoniae* to *P. putida* and *E.coli* have resulted in the mutant strains that were able to demonstrate nitrogenase activity by the express *nif* genes (Postgate and Kent 1987; Dixon and Postgate 1971). These studies confirmed that nitrogen fixation genes can be relocated from symbiotic to non-symbiotic bacteria through mobile genetic elements. Researchers are constantly working on the engineering of PGPR strains to boost plant growth under low nitrogen conditions.

In one such initiative, Setten et al. (2013) successfully transferred the nitrogenase encoding *nif*genes from *P. stutzeri* A1501 to PGPR strain *P. protegens* Pf-5. X940 cosmid-mediated gene transfer induced constitutive expression of nitrogenase enzyme activity by the mutant strain in a nitrogen free medium. Similar nitrogen fixation ability was shown by *P. putida*, *P. veronii* and *P. taetrolens* when transformed by X940 cosmid integrated with *nif* genes. The results of this study unambiguously confirm that this technology of transfer of *nif* genes can be used for genetic manipulation in different bacterial strains. *P. protegens* Pf-5, a well-known biological control agent was found to work effectively to enhance the growth of both dicots (Arabidopsis and alfalfa) and monocots (maize and fescue) via increasing the availability of ammonium ions and nitrates in soil in response to *nif* genes. Therefore, it can be speculated that genetically modified *P. protegens* can be functionally used in many commercial crops (Setten et al. 2013).

Wang et al. (2013a, b) tried to express the *nif* genes in the heterogenous prokaryotic model by integrating nine genes from *nif* gene cluster of *Paenibacillus* sp. WLY78 to *E.coli* JM109 under the control of its native σ^{70}-dependent promoter. The engineered *E. coli* JM109 synthesised active nitrogenase but its specific activity was only nearly 10% compared to the *Paenibacillus* sp. WLY78. The study raises an important ambiguity that the transfer of only *nif* genes to the heterogenous host may not yield the desired results and synthetic biology tools are necessary to increase the nitrogenase activity (Li et al. 2016). Promotor replacement is an artful synthetic biology approach to ease expression of nitrogen fixation which requires cluster of multiple genes organised into different operons. Transcription of these genes working in concert is managed by a complex cascade system of different promoter. These complex native regulatory elements and promoters can be replaced with simple expression systems. Wang et al. (2013a, b) cloned the entire *nif* gene

Table 1 Engineered PGPR for enhanced macronutrient availability

Trait	PGPR Used	Gene/Pathway engineered	Inference	References
Nitrogen fixation	*P. protegens* Pf-5 (NCBI ID- txid220664)	*nif* genes (nitrogen-fixing genes)	Recombinant strain displayed high nitrogenase activity Enhanced growth of alfalfa, Arabidopsis, maize and fescue observed	Setten et al. (2013)
Nitrogen fixation	*E.coli* (NCBI ID- txid562)	*nif* genes (nitrogen-fixing genes)	Expression of nitrogenase activity	Wang et al. (2013a, b)
Nitrogen fixation	*E. coli* (NCBI ID- txid562)	1. *suf* operon (Fe–S cluster assembly) 2. *fldA pfoAB* and *fer* (electron transporter genes) 3. *nifSU* gene (Fe–S cluster assembly) 4. *nifFJ* gene (electron transport specific for nitrogenase)	Expression of nitrogenase activity increased by 50.1%	Li et al. (2016)
Nitrogen fixation	*P. protegens* Pf-5 (NCBI ID- txid220664)	*nif* genes (nitrogen-fixing genes)	Remarkable nitrogenase activity was observed Fresh weight of cucumber and wheat increased High potential against *R. Solani* infection observed	Jing et al. (2018)
Phosphate solubilization	*E. Coli* (NCBI ID- txid562)	*gdh* gene (glucose 1-dehydrogenase) *pqqABCDE* gene (pyrroloquinoline quinone gene cluster)	Instigation of inorganic phosphate phenotype	Farhat et al. (2013)
Phosphate solubilization	*Herbaspirillum seropedicae* Z67 (NCIB ID-12540)	*pqqE* gene, *pqq* (pyrroloquinoline quinone gene)	Improved PGPR potential	Wagh et al. (2014)
Phosphate solubilization	*P. Fluorescens* (NCBI ID- txid294)	*gltA1*(citrate synthase) citC (citrate transporter genes)	Enhanced citric acid production	Adhikary et al. (2014)
Phosphate solubilization	*Ralstonia* sp. (NCBI ID- txid48736) *Pseudomonas putida* (NCBI ID- txid303) *Pseudomonas-simiae* (NCBI ID- txid321846)	phytases encoding genes	Expression of phytate solubilisation potential	Shulse et al. (2019)

cluster of 7 operons from *Klebsiella pneumoniae* to *E.coli* by replacing the native σ^{54} dependent promoters with T7 RNA polymerase–LacI expression system that yielded 100% nitrogenase activity. The newly constructed T7-dependent *nif* biological system cloned in *E.coli* when challenged with physiological and genetic conditions, bypassed the original complex regulatory circuits. However, only 42% nitrogenase activity could be seen by mimicking the same expression levels with variable-strength T7- dependent promoters. Therefore, the promoter choice can be vital in deciding the expression levels of a gene. Also, promoter replacement can be an important tool to switch the complex regulatory pathways into a more universal system and enhance the expression of *nif* gene in host (Dubeau et al. 2011).

Li et al. (2016) provided valuable insights towards enhancing the nitrogenase activity of the heterogenous host through genetic engineering. They cloned twenty eight selected genes from *Klebsiella oxytoca* and *Paenibacillus* sp. WLY78 and integrated them into two different vectors under the control of *Paenibacillus nif* promoter, followed by simultaneous transfer to the *E. coli* 78–7. These cloned genes included Fe–S cluster assembly (*suf* operon) & electron transport genes *fldA, pfoAB* and *fer* from *Paenibacillus* and Fe–S cluster assembly (*nifSU* gene) & electron transport specific for nitrogenase (*nifFJ* gene) from *K. oxytoca*. By this combined transfer of genes, the specific enzyme activity of nitrogenase from recombinant *E. coli* 78-7 was 50.1% of wild *Paenibacillus* (Fig. 1).

Another invasive strategy involves transfer of the entire nitrogenase island with promoter genes from the native to PGPR strain. This is done to rule out the sensitivities related to oxygen supply raised by the transfer of only *nif* genes.

Table 2 Engineered PGPR for enhanced IAA production and biocontrol potential

Trait	PGPR Used	Gene/Pathway engineered	Inference	Reference
IAA production	*Pseudomonas sp.* UW4 (NCBI ID- txid1207075)	*ami* (amidase), *nit* (nitrilase), *nthAB* (nitrile hydratase), *phe* (phenyl acetaldoxime dehydratase)	Enhanced IAA production by 2–3 times	Duca et al. (2018)
IAA production	*C.pinatubonensis* JMP134 (NCBI ID- txid264198)	*iaaM* (2-tryptophan monooxygenase), *iaaH* (indole-3- acetamide hydrolase), *luxI* (homoserine lactone synthase)	Enhancement growth and development in of *Arabidopsis* thaliana plants	Zúñiga et al. (2018)
Enhanced chitinases activity	*Burkholderia vietnamiensis* (NCBI ID- txid60552)	*Chi113* gene (Chitinase)	Enhanced activity against *R. solani, F. oxysporum, R. cerealis*	Zhang et al. (2012)
Chitinases activity	*Escherichia coli* BL21 (NCBI ID- txid511693)	*LbCHI31* (class Ib chitinase mRNA) and *LbCHI32* (class I chitinase mRNA) genes	Enhanced activity against *A. alternata*	Liu et al. (2013)
Chitinases activity	*Escherichia coli* (NCBI ID- txid562)	*ChiKJ406136* (chitinase-encoding gene)	Inhibit mycelium growth in *C. scoparium, C. parasitica, N. parvum Crous* and *F. oxysporum Schl*	Li et al. (2018)
Enhanced chitinases activity	*E.coli* BL21 (NCBI ID- txid511693)	*Pt*Chi19 gene (chitinase)	Inhibition of hyphae of *F. oxysporum, A. niger*, and *Armillaria mellea*	García-Fraga et al. (2015)
Enhanced disease resistance	*B. velezensis* (NCBI ID- txid492670)	*bdh* (2,3-butanediol dehydrogenase), *gdh* (glycerol dehydrogenase, *alsD* (acetolactate decarboxylase)	Increased production of acetoin	Chung et al. (2016); Peng et al. (2019)
Enhanced disease resistance	*P. protegens* Pf-5 (NCBI ID- txid220664)	*retS* gene (sensor kinase)	Diacetypholoroglucinol production increased by 20–30 folds	Jing et al. (2018)
Enhanced disease resistance	*P.protegens H78* (NCBI ID- CP013184.1)	*rsmE* (translational repressor gene) *lon* (ATP-dependent protease gene) *pltZ-pltIJKNOP* (ATP-binding cassette (ABC) transport operon)	Enhanced pyoluteorin production by 14.3 times	Shi et al. (2019)
Enhanced disease resistance	*P. chlororaphis* NRRL B-30761 (NCBI ID- txid587753)	*glpF* (glycerol diffusion facilitator) and *glpK* (glycerol kinase) genes	Enhanced production of Phenazine-1-carboxylic acid	Solaiman et al. (2016)
Enhanced disease resistance	*P. chlororaphis* GP72 (NCBI ID- txid1117110)	*glpF* (glycerol diffusion facilitator) and *glpK* (glycerol kinase) genes	Enhanced Phenazine-1-carboxylic acid production by 993.4 mg/L	Song et al. (2020)

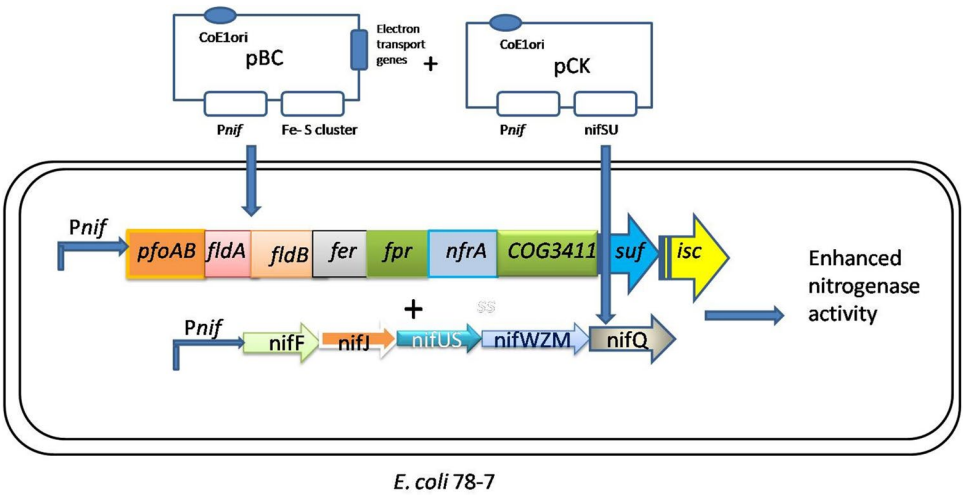

Fig. 1 Combined assembly of *Paenibacillus suf* operon and electron transporter genes with Fe–S cluster assembly (*nifSU* gene) of *K. oxytoca* in the recombinant E. coli 78-7. A total of 28 selected genes from *Paenibacillus* and *K. oxytoca* were placed under the control of *nif* promoter in two cloning and expression vectors (pBC & Pck) and then are transferred to E. coli 78-7. The combined assembly of the potential *K. oxytoca nifSU* genes with *Paenibacillus* electron transporter genes (*pfoABfldA*) leads to enhanced nitrogenase activity

Also, PGPR strain can be engineered for two or more characters simultaneously to enhance its PGPR potential. Such an initiative was carried out by Jing et al. (2018) in which they constructed a *P. protegens* Pf-5 strain to incorporate nitrogen fixation ability and enhance its innate antifungal traits. The nitrogenase island along with native promoter isolated from *P. stutzeri* DSM4166 was incorporated into a retS mutant strain. The engineered strain illustrated high nitrogenase activity as well as significant ammonium production as by acetylene reduction in L-medium. The mutant strains showed significant increase in fresh weight in dicots (cucumber) and monocot (wheat) and demonstrated high potential against *R. solani* infection (Jing et al. 2018).

2. Inorganic and organic phosphate solubilisation

After nitrogen, phosphorus is the second most substantial plant growth limiting nutrient. Even in the most fertile soils, the availability of phosphorous to plants is not more than 10 Mm (Aeron et al. 2019). Certain rhizospheric bacteria have the ability to convert inorganic phosphorus reserves to soluble form that can be assimilated by plants for their use. The phenotype for phosphate solubilisation is related to the synthesis of enzymes like phytase and phosphatase and secretion of low molecular weight organic acids. The presence of pyrroloquinoline quinone *PQQ* synthase gene discovered in *Erwinia herbicola* was correlated to mineral phosphate solubilisation (Goldstein and Liu 1987). They showed that the cloning of these genes to *E.coli* HB101 resulted into recombinant strain with comparable mineral solubilisation potential. Rodrı´guez et al. (2000) engineered *Pseudomonas* sp. PSS and *Burkholderia cepacia* IS-16 by transferring *PQQ* synthase gene from *Erwinia herbicola.* Enhanced mineral phosphate solubilisation (MPS) was observed in both the strains adding to their PGPR potential. Babu-Khan et al. (1995) and Liu et al. (1992) reported that expression of MPS trait is the result of indirect oxidation regulated by glucose dehydrogenase (GDH) which needs cofactor PQQ for formation of haloenzyme. *E. coli* K-12 synthesize apo-GDH but the cofactor (PQQ) is absent that results in lack of MPS phenotype. Babu-Khan et al. (1995) analysed that 369 bp single open reading frame that encodes the protein gabY, when cloned from *P.cepacia* (active solubilizer of inorganic phosphate) to *E. coli* K-12 resulted in the secretion of gluconic acid and induce MPS. It is rational here to suggest that *gabY* also play a key role in controlling expression of MPS and it can be considered as an option for genetic manipulation experiments in future. Farhat et al. (2013) cloned *gdh* gene and *pqqABCDE* gene encoding the apo- enzyme glucose dehydrogenase and its cofactor *pqq* from phosphate-solubilizing *Serratia marcescens* CTM 50650. Both the genes were subcloned and co-expressed in *E.coli*. The recombined strain was competent enough to solubilize wide range of inorganic phosphorous substrate including tricalcium phosphate, Gafsa rock phosphate and hydroxyapatite. These studies give a reason to support the fact that non-PGPR strains like *E.coli* offers potential for sustainable agricultural processes. However, field studies are needed, some of which are underway, to fully establish the potential of genetic manipulated strains.

Schulze et al. (2006) described that the process of nitrogen fixation by bacteria in field conditions is limited by the presence of insoluble phosphorous. Co-inoculation of PGPR strain with nitrogen-fixing ability and phosphorous mineralization has shown preferable nitrogen fixation and better plant growth in plants (Kumar and Chandra 2008). Instead of co-inoculation with two different PGPR strains, a single strain can also be engineered to express both these traits. For instance, *Herbaspirillum seropedicae* Z67, is a well characterized nitrogen-fixing endophytic bacteria known to niche roots, leaves and stem of agronomically important crops such as sugarcane, wheat, rice (Monteiro et al. 2008)

but it lacks MPS phenotype. Unlike phosphate solubilising bacteria, its genome includes encodes GDH apoprotein but lacks the genes that encodes for the biosynthesis of its cofactor PQQ. *pqqE* gene (pyrroloquinoline quinine) of *Erwinia herbicola*, *pqq* gene cluster of *Pseudomonas fluorescens* B16 and *pqq* gene cluster of *Acinetobacter calcoaceticus* (pSS2) was subcloned and over expresses in *H. seropedicae* Z67 to construct mutant strains designated as Hs pJNK1, Hs pOK53 and Hs pSS2, respectively. Engineered Hs pOK53 and Hs pSS2 reported significant rock phosphate solubilisation due to the activity of GDH enzyme (Fig. 2). The mutant strains exhibit enhanced growth rate in N- free media showing the PGPR attributes of the bacteria were improved by the genetic modifications (Wagh et al. 2014). All these studies have involved cloned the desired genes using plasmid which is very unstable. However, to avoid horizontal gene transfer and enhance stability of engineered traits, future studies should be focussed on the chromosomal insertion of *PQQ synthase* genes in bacteria. The genomic integration of artificial citrate operon was carried out by Adhikary et al. (2014) in six-PGPR *P. fluorescens* strains using MiniTn7 transposon gene delivery system using method of electroporation. The NADH insensitive *gltA1* (citrate synthase) and *citC* (citrate transporter) genes delivered to the genome of *P. fluorescens* conferred MSP due to enhanced gluconic acid and citric acid secretion. Although, this study provides interesting insights into the integration of phosphate solubilization genes but the efficiency of recombinant strains was not tested on the plants.

It is important to note that soil also contains organic reserves of phosphorous in chelated form which can be solubilised by bacteria that secrets enzymes like acid phosphatase and phytases (Rodríguez and Fraga 1999). With the objective of developing an effective phosphate biofertilizer, Fraga et al. (2001) studied the organic phosphate solubilisation genes (*Nap A*) encoding for acid phosphatase in *Morganella morganii*. *Nap A* gene was sub cloned from the host bacterium and transferred to *P. cepacia* IS-16 strain which is a known inorganic phosphate solubilizer. The transformed clones reported significant enhancement in extracellular acid phosphatase which helped in organic phosphate solubilisation. Phytate is another, biological phosphate reserve present in soil that can be solubilised through enzyme phytases. To explore the possibility of engineering of PGPR for phytate solubilisation for agronomical use, Shulse et al. (2019), used synthetic biology approach to integrate the phytases encoding genes directly into the chromosome of three bacterial hosts which were characterized inorganic phosphate solubilizers. The gene sequence of 82 phylogenetically diverse phytase genes encoding three different class of enzyme comprising BPP (propeller phytase), HAP (histidine acid phosphatase) and CP (Cysteine phosphatase) was refactored and then synthesized their optimal expression in Proteobacteria host. The refactored genes were engineered *Ralstonia* sp. strain UNC404CL21Col, *Pseudomonas putida* KT2440 and *P. simiae* WCS417r. High levels of phytate hydrolysis was seen in 41 mutant strains whereas 12 engineered strains conferred the high growth rates on *Arabidopsis thaliana* test plant when phytate is supplied as the sole phosphate source. This study paves the road to chromosomal based integration of novel genes through genetic engineering techniques to generate PGPR strains.

Potassium (K) is third macronutrient decisive in resistance of plants towards pest and abiotic conditions. There are close to 80 different enzymes involved in plant processes like starch synthesis, sugar metabolism, nitrate reduction and photosynthesis that require K as cofactor (Hussain et al. 2016; Gallegos-Cedillo et al. 2016; Almeida et al. 2015). K-solubilizing microbial community effectively release insoluble inorganic pools of total soil K through solubilisation (Saha et al. 2016; Meena et al. 2014). *Bacillus* spp., *Enterobacter* spp., *Burkholderia* spp., *Pseudomonas* spp. are reported to be involved in K- solubilisation (Dong et al. 2019; Etesami et al. 2017). The mechanism of K-solubilization by these bacteria is similar to phosphate solubilisation (Meena et al. 2014; Uroz et al. 2009; Parmar and Sindhu 2013) and involves the production of low molecular weight organic acids which help in K solubilisation from its

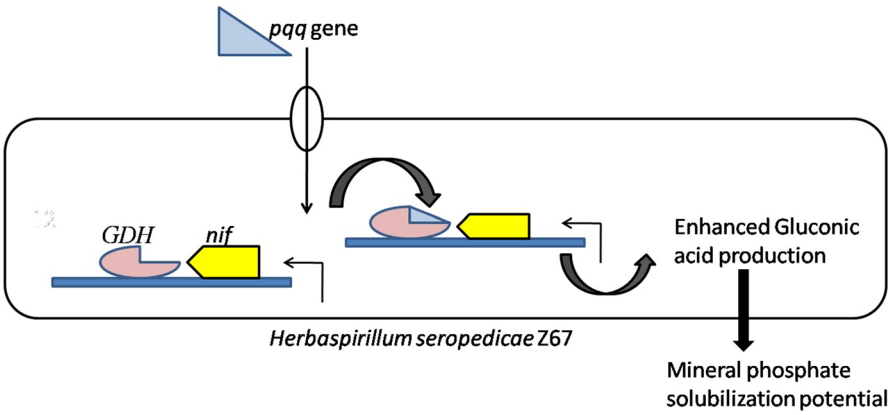

Fig. 2 Engineering of *Herbaspirillum seropedicae* to overexpress gluconic acid production. *pqq* gene cluster of *P. fluorescens* B16 was subcloned and overexpresses in *H. seropedicae* Z67 to construct mutant strains with significant rock phosphate solubilisation. Single or multiple PQQ genes can be cloned for constructing different mutant strains

parent minerals (Saiyad et al. 2015; Keshavarz Zarjani et al. 2013; Prajapati et al. 2013). Very few studies are available that highlights the mechanism of K solubilisation in bacteria with almost no studies on genetic basis of K solubilisation. Biofertilizer industry lacks interest in potassium solubilising bacteria (KSB) due to low efficiency and rapid loss of K solubilisation phenotype. The gene manipulation technology can be used to enhance the production of organic acids similar to phosphate solubilising bacteria. However, there is no literature available supporting this hypothesis.

3. Chitinases activity

Plant pathogens are the major cause of decrease in crop yield and quality (Savary et al. 2012).Fungi are the chief causative agent of numerous diseases in plants. Chemical fungicides constitute the major strategic therapy to treat fungal infections in plants. But as these chemical agents have high persistence in soil. They possess great environment risks by contaminating water, targeting beneficial soil microorganisms and declining beneficial non target pollinators. Engineering PGPR to enhance their activity against fungal phytopathogens has gained much interest in recent decades. It has been suggested that PGPR can inhibit phytopathogens by different mechanism, including production of defence-related compounds, antimicrobial substances, siderophore production, nutrition improvement and activation of plant defence mechanism (Kaur and Kaur 2018). However, the natural occurring rhizobacteria exhibit weak antifungal potential while most of them demonstrate high plant or pathogen specificity (Huang et al. 2006).

Various studies have reported to inhibit phytopathogenic fungi by inoculating plants with chitinase-producing microbes. Chitinase is an active enzyme that disrupts the β-1, 4-linkages of N-acetyl-D-glucosamine of chitin, which is a vital biomolecule present in fungal cell wall (Gomes et al. 2001). Disruption of the N-acetyl-D-glucosamine bonds causes the weakening of the cell wall and sequentially causing cell lysis. Engineering PGPR for production of chitinase, serves as one of the important step towards disease suppression in plants. In a study, an engineered PGPR, *B. vietnamiensis* P418 was constructed by introducing a chitinase-encoding gene *chi113* into its chromosome. The gene was isolated and cloned from *Bacillus subtilis* Ap113. The transformed cells had upgraded disease resistance potential against phytopathogenic fungi *R. solani* and *Verticillium dahliae* (Huang et al. 2006). Following this, *chi113* gene was integrated into the chromosome of *Burkholderia vietnamiensis* P418 using transposon delivery vector pUTkm1. The transformed strain showed no reduced fitness and no loss of inherent potassium and phosphate solubilising activity. P418-37 showed enhanced in-vitro growth inhibition of *R. solani, Fusarium oxysporum, Gaeumannomyces graminis, Rhizoctonia cerealis, Bipolaris sorokiniana* and *Verticillium dahliae* (Zhang et al. 2012). Transformed strain, P418-37 showed significant repression of cotton wheat sheath blight Fusarium wilt, and tomato grey mould.

There are ample opportunities for chitinase gene from plant origin to be cloned into bacteria with novel qualities of plant growth promotion. In a study, Jayaraj et al. (2004) rice chitinase cDNA (RC 7) chitinase gene encoding 35 kDa class I chitinase was cloned into plasmid vector, pDSK519, and integrated into *Azospirillum brasilense* SP51eFL1 by biparental mating. The protein expression was demonstrated through western blotting which confirmed the presence of 35 kDa cell lysate which hydrolyzed various chitin substrates and was immuno-reactive to the barley chitinase antibody. The fusion proteins from cell lysates of transconjugated strain exhibited strong antifungal activity, however, inherent nitrogen-fixing ability were slightly diminished.

Other studies show that non-PGPR strains can also be engineered for introduction of chitinase expressing genes against various fungal pathogens. Liu et al. (2013) isolated chitinase genes (*LbCHI31* and *LbCHI32*) from *Limonium bicolor*, and expressed it successfully in *E. coli* BL21 strain. The recombinant *E.coli* produced intracellular and extracellular chitinase with potential hydrolytic ability on cell walls of *A. alternate* (Fig. 1, Supplementary). In another study, *Pt*Chi19 gene encoding chitinase, isolated from *Pseudoalteromonas tunicata* CCUG 44952 T was integrated to the genome of *E.coli* BL21. The transformed strain harbouring *Pt*Chi19 exhibited prominent hyphal inhibition against fungal pathogens including *F. oxysporum, A. niger*, and *Armillaria mellea* (García-Fraga et al. 2015). Chitinase encoding gene *ChiKJ406136* was isolated from the complete genome of *Streptomyces sampsonii* KJ40, which was then cloned into *E. coli* to express recombinant chitinase protein. The mutant *E. coli* produced chitinase which inhibited the mycelium growth in *Cylindrocladium scoparium, Neofusicoccum parvum Crous, Cryphonectria parasitica* and *F.oxysporum Schl*. This study shows *E.coli* can be used as an expression vector for the production of commercial grade chitinase for agricultural use (Li et al. 2018).

4. Indole acetic acid production

Indole acetic acid (IAA) is the functional auxin that is predominantly associated with the development of apical meristem and growth of adventitious and lateral roots (Olatunji et al. 2017). Plant produces an endogenous pool of IAA depending upon the prevailing environmental conditions, tissue type and availability of tryptophan precursor, concentration of IAA produced being monitored by rate of synthesis, its sequestering in different plant regions, conjugation and degeneration. This endogenous pool of IAA is usually suboptimal or optimal to support plant growth.

Most of the rhizobacteria secretes IAA in the close vicinity to roots where plants can receive the hormone through roots. Acquisition of additional IAA produced by rhizobacteria enhances its concentration to optimal or supraoptimal levels, resulting in plant growth promotion. Miscellaneous strain of bacteria belonging to genus *Azospirillum, Enterobacter, Pseudomonas, Myroides, Alcaligenes, Xanthomonas* and *Acetobacter* have been reported to secret IAA and augment plant growth (Duca et al. 2018; Kaur and Kaur 2021).

The plant–microbe interaction is affected severely in stress conditions, suggesting that there is a need to engineer the natural IAA producing bacteria for over production of IAA. For this, *Pseudomonas* sp. UW4, an efficient PGPR strain acting efficiently in biotic as well as abiotic stress conditions was engineered for IAA-overproduction. Second copy of target IAA biosynthesis genes (ami, nit, nthA, nthB, phe) was introduced into the Pseudomonas sp. UW4 to construct transformant strain. *Pseudomonas* sp. UW4 transformants were reported to produce upto 2–3 times higher IAA (Duca et al. 2018). With the similar aim of constructing a superior bioinoculant that produce higher IAA, Malhotra and Srivastava (2006), worked with a natural isolate of *Azosprillium brasilense* SM. *Azosprillium* synthesize IAA via indole pyruvic acid (IPyA) pathway, which is subjected to extremely tight regulation. Constitutive indole acetamide (IAM) pathway genes, *iaaM* and *iaaH* from a heterologous host (*P. syringae*) were introduced to *A. brasilense* SM for the upregulation of IAA. With the IAM genes, bacteria were efficiently able to utilize indole acetamide as a substrate and produce 3 times higher levels of IAA than wild strain. Lateral branch development, root proliferation and dry weight of the sorghum plant were improved with the bioinoculation of engineered *A. brasilense* SM strain.

Deletion mutations can also be vital tool for the engineering of PGPR for higher production of IAA. Deletion mutations of structural gene *acdS* encoding 1-aminocyclopropane-1-carboxylate (ACC) deaminase resulted in mutant strain (*B. phytofirmans*YS2) that produced6 fold higher IAA than wild type, however, complete loss of ACC deaminase activity was also observed (Sun et al. 2009). Complementation of the ACC deaminase gene in the mutant YS2 (strain YS3) restores the ACC deaminase activity and high plant root elongation effect compared to the wild strain (Sun et al. 2009).

Non-rhizospheric bacteria offer better advantage over rhizospheric bacteria in terms of root colonization through metabolization of various plant root exudates (Ledger et al. 2012). Mechanism of quorum sensing (QS) offer them competitive advantage and better survival rates in particular niche. This encourages the quest to engineer these neutral bacteria to contribute towards the plant growth. *C. pinatubonensis* JMP134 is one such non-PGPR bacterium which is amenable to genetic manipulations. Zúñiga et al. (2018), constructed an QS dependent IAA biosynthesis device in strain *C. pinatubonensis* JMP134. A genetic circuit was created using Plux promoter and *luxI*, *luxR* genes from bacterium *V. fischeri* to *C. pinatubonensis*. A positive feedback QS circuit was capable of producing IAA and responding to N-(3-Oxohexanoyl)-L-homoserine lactone, 3-oxo-C6-HSL. *iaaM gene* (2-tryptophan monooxygenase) and *iaaH gene* (indole-3-acetamide hydrolase) from *P. savastanoi* 48 were assembled downstream the Plux-luxI sequence in the plasmid pSEVA-Plux-luxI. *In-vivo* and *in-vitro* test showed the self-induction of the device at higher concentration of bacterial cells leading to significant enhancement in lateral root expansion, primary root length, fresh weight and rosette area of *Arabidopsis thaliana* plants. This study emphasis the need that non-rhizospheric bacteria can be better utilized as a vehicle for agriculture applications.

5. Disease resistance

The pathogenic attack on plants produces an induced systematic resistance (ISR) initiated by the release of plant hormones. The PGPR residing in vicinity of effected plants release certain volatile compounds like 2,3-butanediol and 3-hydroxy-2-butanone (acetoin) that trigger ISR in plants and help control the disease progression. One of the simplest ways to combat the disease menace is to elicitate the defence pathway in plants as mediated by bacterial volatile organic compounds. Plants subjected to PGPR such as *Pseudomonas, Bacillus* and *Serratia* sp. have shown development of ISR and lower rates of pathogen attack (Kloepper et al. 2004). Biocontrol properties of PGPR may be improved by manipulating their genetic material to overexpress one or more traits (Glick and Bashan 1997). Recently, many researchers have worked towards enhancing the antibiotic secretion in PGPR, production of defence-related volatile compounds and overexpression of gene regulating secretion of broad spectrum antifungal compounds (Klemsdal and Tronsmo 1999).

Acetoin is a plant growth promoting volatile compound secreted by numerous PGPR strains including *B. subtilis, B. velezensis* and *B. amyloliquefaciens* IN937a. Acetoin production is critical for the elicitation of defence pathway in plants as the *B. subtilis* mutant strains (BSIP1174 and BSIP1173) fail to protect the plant against infection (Rudrappa et al. 2010). Acetoin production is also related to the binding of the PGPR strain to plant roots and enhanced plant growth promoting activity (Rudrappa et al. 2007). Higher acetoin levels are linked with the induction of defence enzymes such as polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL). Chung et al. (2016) have reported that *B. velezensis* is endowed with the innate ability to produce acetoin. However, the concentration of acetoin is generally lower than required functional

levels. Peng et al. (2019), constructed the knockout strains of *B. velezensis* by deleting *bdh*, *gdh* and *alsD* genes by the double crossover homologous recombination method. The recombinant strain showed similar growth pattern and a significant increase in acetoin production compared to wild strain. A mild but effective immune response was elicited in plants in a 'priming' state for triggering defence response against pathogen. Hydrogen peroxide accumulation and callose deposition was enhanced significantly helping plants to fight against infection. The concentrations of POD, PPO and PAL were found to be significantly higher in plant leaves inoculated with transformed bacterial strains (Peng et al 2019).

Gene manipulations techniques involving downregulation of certain genes controlling the production of antibiotics and related antimicrobial compounds are widely used as a futuristic approach towards sustainable methods of biotic stress tolerance. Various *Pseudomonas* sp. like *P. aeruginosa, P. fluorescens* FD6, *P. protegens* CHA0, *P. fuscovaginae, P. brassicacearum* LBUM300 are involved in secretion of diacetypholoroglucinol (DAPG), pyoluteorin (Plt), pyochelin, hydrocyanic acid, phenazine-1-carboxylic acid (PCA) and pyoverdine antimicrobial compound with antifungal properties (Huang et al. 2017). RetS, a regulatory protein is believed to negatively control the phenotype of antibiotic production in PGPR strains by acting as a contender of the GacS/GacA system. Inactivation of RetS a sensory kinase downregulate the production of related antibiotics in *P. fluorescens* FD6 (Zhang et al. 2015; Reimmann et al. 2005). Jing et al. (2018) knockout *retS* genes to improve the antibiotics biosynthesis in strain *P. protegens* Pf-5. The production of DAPG was increased by 20–30 folds with pronounced activity against *R. solani*.

Similarly, *P. protegens* H78 a PGPR well known to produce DAPG and Plt was engineered using multiple gene knockout strategies to enhance the production of Plt by 14.3 times compared to wild strain (Shi et al. 2019). Four potential negative regulators controlling Plt production including an inhibitory sequence lying within the operator of the *pltR* (transcriptional activator gene), translational repressor gene (*rsmE*) in the Gac/Rsm-RsmE pathway, negative regulatory gene *pltZ* transporter operon pltIJKNOP and ATP-dependent protease gene *lon* encoding enzyme that downregulates the positive regulators were deleted. Plt ABC-type pltIJKNOP transporter operon was overexpressed in the engineered strain. Although, there was a significant enhancement in Plt production, the study revealed a declined cell growth rate in the later growth cycle. Another important observation was the fact that simultaneous deletion of multiple genes from the same regulatory pathway did not have additive effects on Plt production. Therefore, engineering studies should focus on the bracing the metabolic flux into the Plt synthesis pathway by splitting its competitive pathways. This explains the urgency for the studies differentiating different regulatory pathways underlying Plt biosynthesis to enable the researchers to precisely engineer regulatory pathway for enhance Plt production without effecting cell growth.

Phenazine-1-carboxylic acid (PCA) is another important broad spectrum antifungal compound found effective against *Phytophthora capsici* and *Rhizoctonia solani AG1-IA, F.* oxysporum that affects pepper, rice, tomato respectively (Upadhyay and Srivastava 2011). It has reported that *P. chlororaphis* GP72 have the potential to produce commercial grade PCA from glycerol (Liu et al. 2016), but the rate of production is much low (Poblete-Castro et al. 2019). Solaiman et al. (2016) reported that metabolic utilization of glycerol in *P. chlororaphis* NRRL B-30761 can be enhanced by co-expression of genes *glpF* and *glpK* encoding glycerol uptake facilitator and glycerol kinase. Along the same line, Song et al. (2020) constructed *P. chlororaphis* GP72 mutant by co-expressing genes *glpF and glpK*. *GlpR* gene which negatively regulates the glycerol utilization and *mgsA* gene regulating the conversion of glycerol to pyruvaldehyde was knocked out using DNA manipulation techniques (Fig. 3). The PCA production after 36 h was enhanced to 993.4 mg/L in mutant strain compared to 729.4 mg/L in wild strain. This study provides future insights for better utilization of genetic manipulation techniques for engineering PGPR strains for the synthesis of value added antifungal compounds.

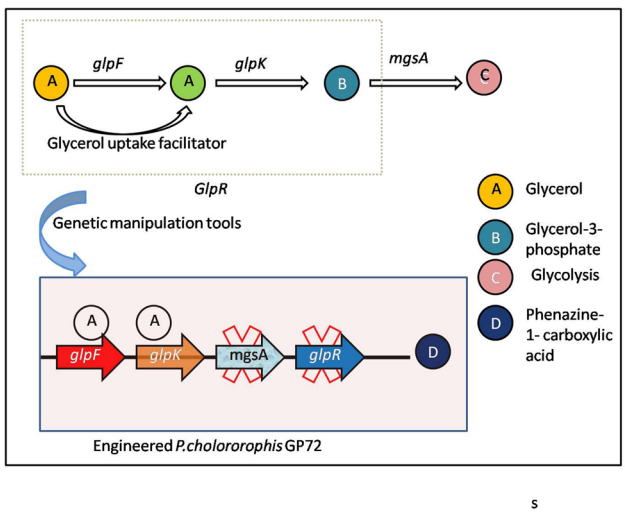

Fig. 3 Engineering of PGPR for enhanced production of Phenazine-1- carboxylic acid (PCA). *GlpR* gene which negatively regulates the glycerol utilization and *mgsA* gene regulating the conversion of glycerol to pyruvaldehyde was knocked out. *P. chlororaphis* GP72 mutant co-expressed *glpF and glpK* genes to overproduce PCA

Limitations in current findings and future prospective

Figure 4 shows different tools that are presently in use for carrying out gene manipulations in PGPR. Generous amount of work has been done to fabricate novel PGPR biological units with desired functions or to engineer existing biological systems to augment their endowed potentials. Towards this approach, synthetic biology tools such as indexing biological parts, genome sequencing, synthetic genome synthesis and genome editing have played a key role. In context of PGPR, ligation of desired genes/set of genes and desired biological parts including promoters, terminators have been employed to enhance the PGPR potential in rhizobacteria. Desired functional genes are integrated successfully into host's endogenous genome and overexpressed via transposon mediated modifications. Non-rhizospheric bacteria have also been engineered to show PGPR activity by the creation of synthetic genetic circuits. However, there are certain technical barriers in this approach that are being addressed by next generation sequencing methods. Biological parts that include promoters, terminators, operons are host specific and may not work in recombinant bacteria as expected. This calls out for need to build host-specific libraries of biological parts and the well-designed regulatory circuits. For the formation of new gene clusters biological parts should be simultaneously assembled (Lee et al. 2013).

Researchers have successfully knockout several downregulating genes through RNA interference (RNAi). Genome reduction approach by deletion or mutational inactivation of several genes is key tools in gene manipulation for enhancing PGPR activity in rhizobacteria. Off-target or unintended effects can often affects the RNAi-induced phenotypes (Martin and Caplen 2007). Fortunately, genome sequencing, transcriptome analysis, genome synthesis and RNAi technologies have helped designing of a predictable biological system. The current studies have focussed mainly on the isolation of desired gene and cloning it in host rhizobacteria or genome reduction approach to downregulate certain genes. Present studies are aimed at focussing single PGPR attribute of rhizobacteria and lack the characteristics of a so called 'single fit biofertilizer'. Most of these current studies have not yet advanced into field trails. Despite these limitations, there is still a need to pay attention on engineering of PGPR for agricultural use. Creation of robust biological systems requires precise gene manipulations techniques that make changes to specific gene loci. Development of engineered endonucleases with versatile DNA binding domains such as CRISPR/Cas9 nuclease (Clustered Regularly Interspaced Short Palindromic Repeats), Zinc Finger Nuclease (ZFN) and Transcription Activator-Like Effector Nuclease (TALEN) have provided a major breakthrough in gene targeting technology (Zhang et al. 2018). However, there is a paucity of studies depicting the success of ZFN, TALEN and CRISPR/Cas9 nuclease for engineering of rhizobacteria for agriculture application.

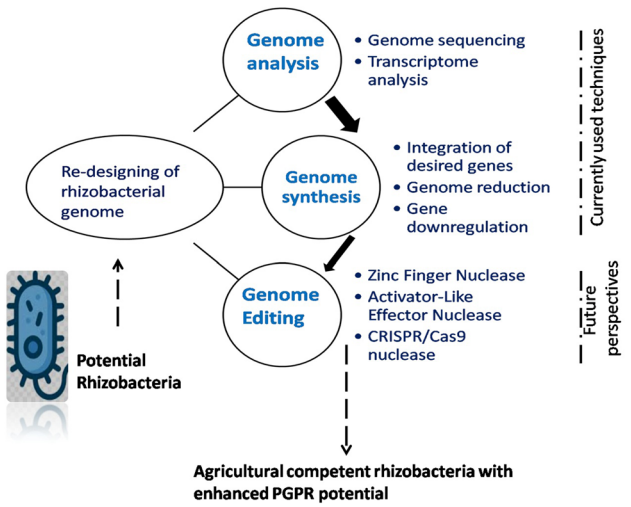

Fig. 4 Tools used for synthetic genome designing in PGPR. Genome sequencing, transcriptome analysis, genome synthesis and RNAi technologies are presently used for gene manipulation of PGPR. Construction and redesigning of PGPR in future requires the use of Zinc Finger Nuclease, Transcription Activator-Like Effector Nuclease and CRISPR/Cas9 nuclease

Conclusion

Although underutilized, microbial consortia are expected to play crucial role in soil fertilization to create nutritional synergy with ecosystem. Commercial success of PGPR has been restrained due to inconsistency to perform in varied soil types, environment conditions, plant types and ecological zones. With wide agro-climatic zones, diverse cropping systems and different soil types, it is mandatory to involve genetic manipulation techniques for improving the survival rates and enhancing the efficiency of PGPR strains. The advances in next generation sequencing techniques, high specificity of gene editing and bioinformatics tools have allowed the deep insights into translation and expression of different genes of interest (Von Bergen et al. 2013; Melcher et al. 2014). The next generation agriculture revolution is possible only if 'microbiome-driven cropping systems' becomes a reality. Kumar and Dubey (2020) have described that the use of synthetic biology systems to purposely revamp the plant–microbe association can also enhance the overall sustainability and efficiency of PGPR. Apart from discussed microbe-based approach, plant based and the *meta*-organism based approach can also act as key players in enhancing the role of PGPR for plant use. CRISPR-CAS9 approach can be use to reshape the plant genome and root

architecture to host PGPR and enhance their survival. In upcoming years, we can expect the use of metagenomics, other omic technologies, and genetic manipulation tools to be used in synergy for the selection of beneficial microbes. Although, voluminous amount of work has been done to engineer bacteria to enhance their beneficial characteristics; there is a need to redesign the strategies to evolve strains that can completely replace chemical fertilizers/pesticides.

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Declarations

Conflict of interest Authors declare that they have no financial or non-financial conflict of interest regarding publication of this article.

References

Adhikary H, Sanghavi PB, Macwan SR, Archana G, Kumar GN (2014) Artificial citrate operon confers mineral phosphate solubilization ability to diverse fluorescent *Pseudomonads*. PLoS ONE 9(9):107554

Aeron A, Khare E, Jha CK, Meena VS, Aziz SMA, Islam MT, Meena RK (2019) Revisiting the plant growth-promoting rhizobacteria: lessons from the past and objectives for the future. Arch Microbiol. https://doi.org/10.1007/s00203-019-01779-w

Almeida HJ, Pancelli MA, Prado RM, Cavalcante VS, Cruz FJR (2015) Effect of potassium on nutritional status and productivity of peanuts in succession with sugar cane. J Soil Sci Plant Nutr 15:1–10

Babu-Khan S, Yeo TC, Martin WL (1995) Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia*. Appl Environ Microbiol 61:972–978

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context mechanisms of action and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473

Banerjee MR, Yesmin L, Vessey JK (2006) Plant-growth-promoting rhizobacteria as biofertilizers and biopesticides. Handbook of microbial biofertilizers. Food Prod Press NY 28:137–181

Choudhary DK, Sharma KP, Gaur RK (2011) Biotechnological perspectives of microbes in agro-ecosystems. Biotech Lett 33(10):1905–1910

Chung J, Song GC, Ryu CM (2016) Sweet scents from good bacteria: Case studies on bacterial volatile compounds for plant growth and immunity. Plant Mol Biol 90(6):677–687

Dixon RA, Postgate JR (1971) Transfer of nitrogen-fixation genes by conjugation in *Klebsiella pneumonia*. Nature 234:47–48

Dong X, Lv L, Wang W, Liu Y, Yin C, Xu Q, Yan H, Fu J, Liu X (2019) Differences in distribution of potassium-solubilizing bacteria in forest and plantation soils in Myanmar. Int J Environ Res Public Health 16(5):700

Dubeau MP, Guay I, Brzezinski R (2011) Modification of genetic regulation of a heterologous chitosanase gene in *Streptomyces lividans* TK24 leads to chitosanase production in the absence of chitosan. Microb Cell Fact 10(1):1

Duca DR, Rose DR, Glick BR (2018) Indole acetic acid overproduction transformants of the rhizobacterium *Pseudomonas* sp. UW4. Antonie Van Leeuwenhoek 111(9):1645–1660

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

Farhat MB, Fourati A, Chouayekh H (2013) Coexpression of the pyrroloquinoline quinone and glucose dehydrogenase genes from *Serratia marcescens* CTM 50650 conferred high mineral phosphate-solubilizing ability to *Escherichia coli*. Appl Biochem Biotechnol 170(7):1738–1750

Fraga R, Rodríguez H, González T (2001) Transfer of the gene encoding the NapA acid phosphatase of *Morganella morganii* to a *Burkholderiacepacia* strain. Acta Biotechnol 21:359–369

Gallegos-Cedillo VM, Urrestarazum VM, Alvaro JE (2016) Influence of salinity on transport of Nitrates and Potassium by means of the xylem sap content between roots and shoots in young tomato plants. J Soil Sci Plant Nutr 16(4):991–998

García-Fraga B, Da Silva AF, López-Seijas J, Sieiro C (2015) A novel family 19 chitinase from the marine-derived *Pseudoalteromonastunicata* CCUG 44952T: heterologous expression characterization and antifungal activity. Biochem Eng J 93:84–93

Geetha SJ, Joshi SJ (2013) Engineering rhizobial bioinoculants: a strategy to improve iron nutrition. Sci World J 1:1–15

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15(2):353–378

Goldstein A, Liu ST (1987) Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Biotechnology 5:72–74

Gomes RC, Semedo LT, Soares RM, Linhares LF, Ulhoa CJ, Alviano CS, Coelho RR (2001) Purification of a thermostable endochitinase from *Streptomyces* RC1071 isolated from a cerrado soil and its antagonism against phytopathogenic fungi. J ApplMicrobiol 90:653–661

Huang YJ, Yang HT, Zhou HZ (2006) Chitinase gene from Bacillus subtilis: cloning sequencing and expression in *Burkholderia* B418. J Biol Control (Chi) 22:72–77

Huang X, Wang Z, Liu Y, Zhang X (2017) Complete genome sequence of *Pseudomonas protegens* H78 a plant growth-promoting rhizobacterium. Genome Announc 5(16):e00233-e1217. https://doi.org/10.1128/genomeA00233-17

Hussain Z, Khattak RA, Irshad M, Mahmood Q, An P (2016) Effect of saline irrigation water on the leachability of salts growth and chemical composition of wheat (*Triticum aestivum* L.) in saline-sodic soil supplemented with phosphorus and potassium. J Soil Sci Plant Nutr 16:604–620

Igiehon NO, Babalola OO (2018) Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture. Int J Environ Res Publ Health 574:897–911

James EK (2000) Nitrogen fixation endophytic and associative symbiosis field. Crops Res 65:197–209

Jayaraj J, Muthukrishnan S, Liang GH (2004) Transfer of a plant chitinase gene into a nitrogen-fixing *Azospirillum* and study of its expression. Can J Microbiol 50(7):509–513

Jing X, Cui Q, Li X, Yin J, Ravichandran V, Pan D, Fu J, Tu Q, Wang H, Bian X, Zhang Y (2018) Engineering *Pseudomonas protegens* Pf-5 to improve its antifungal activity and nitrogen fixation. Microb Biotechnol 13(1):118–133

Kaur R, Kaur S (2018) Biological alternates to synthetic fertilizers: efficiency and future scopes. Indian J Agric Res 52(6):587–595

Kaur R, Kaur S (2020) Variation in the phosphate solubilizing bacteria from virgin and the agricultural soils of Punjab. Curr Microbiol 77(9):2118–2127

Kaur R, Kaur S (2021) Plant growth-promoting potential of 'Myroides gitamensis' isolated from virgin soils of Punjab. Arch Microbiol 8(3):1–11

Keshavarz Zarjani J, Aliasgharzad N, Oustan S, Emadi M, Ahmadi A (2013) Isolation and characterization of potassium solubilizing bacteria in some Iranian soils. Arch Agron Soil Sci 59:1713–1723

Klemsdal SS, Tronsmo A (1999) Genetic manipulation for improvement of microbial biocontrol agents. Integrated pest and disease management in greenhouse crops. Springer, Dordrecht, pp 353–364

Kloepper JW, Ryu MN, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathol 94:1259–1266

Kumar R, Chandra R (2008) Influence of PGPR and PSB on *Rhizobium leguminosarum*bvviciae strain competition and symbiotic performance in lentil. World J Agric Sci 4:297–301

Kumar A, Dubey A (2020) Rhizosphere microbiome: engineering bacterial competitiveness for enhancing crop production. J Adv Res 24:337–352

Ledger ME, Harris RM, Armitage PD, Milner AM (2012) Climate change impacts on community resilience: evidence from a drought disturbance experiment. Adv Ecol Res 46:211–258

Lee BR, Cho S, Song Y, Kim SC, Cho BK (2013) Emerging tools for synthetic genome design. Mol Cells 35(5):359–370

Li XX, Liu Q, Liu XM, Shi HW, Chen SF (2016) Using synthetic biology to increase nitrogenase activity. Microb Cell Fact 15(1):1–11

Li S, Zhang B, Zhu H, Zhu T (2018) Cloning and expression of the chitinase encoded by ChiKJ406136 from *Streptomyces Sampsonii* (Millard & Burr) Waksman KJ40 and its antifungal effect. Forests 9(11):699

Liu ST, Lee LY, Tai CY, Hung CH, Chang YS, Wolfram JH, Rogers R, Goldstein AH (1992) Cloning of an *E. herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101: nucleotide sequence and probable involvement in biosynthesis of the coenzyme pyrroloquinoline quinine. J Bacteriol 174:5814–5819

Liu Z, Huang Y, Zhang R, Diao G, Fan H, Wang Z (2013) Chitinase genes LbCHI31 and LbCHI32 from *Limonium bicolor* were successfully expressed in *Escherichia coli* and exhibit recombinant chitinase activities. Sci World J 2013:1–9

Liu K, Hu H, Wang W, Zhang X (2016) Genetic engineering of *Pseudomonas chlororaphis* GP72 for the enhanced production of 2-hydroxyphenazine. Microb Cell Fact 15(1):131

Malhotra M, Srivastava S (2006) Targeted engineering of *Azospirillum brasilense* SM with indole acetamide pathway for indoleacetic acid over-expression. Can J Microbiol 52(11):1078–1084

Martin SE, Caplen NJ (2007) Applications of RNA interference in mammalian systems. Annu Rev Genom Hum Genet 8:81–108

McNear DH Jr (2013) The rhizosphere-roots soil and everything in between Nature. Educ Knowl 4(3):1

Meena VS, Maurya BR, Verma JP (2014) Does a rhizospheric microorganism enhance K+ availability in agricultural soils? Microbiol Res 169:337–347

Melcher U, Verma R, Schneider WL (2014) Metagenomic search strategies forinteractions among plants and multiple microbes. Front Plant Sci. https://doi.org/10.3389/fpls201400268

Monteiro RA, Schmidt MA, Baura VA, Balsanelli E, Wassem R, Yates MG, Randi MAF, PedrosaSouza FOEM (2008) Early colonization pattern of maize (*Zea mays* L Poales Poaceae) roots by Herbaspirillumseropedicae (*Burkholderiales oxalobacteraceae*). Genet Mol Biol 31:932–937

Olatunji D, Geelen D, Verstraeten I (2017) Control of endogenous auxin levels in plant root development. Int J Mol Sci 18(12):2587

Parmar P, Sindhu SS (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. J Microbiol Res 3:25–31

Peng G, Zhao X, Li Y, Wang R, Huang Y, Qi G (2019) Engineering *Bacillus velezensis* with high production of acetoin primes strong induced systemic resistance in *Arabidopsis thaliana*. Microbiol Res 227:126297

Poblete-Castro I, Wittmann C, Nikel PI (2019) Biochemistry genetics and biotechnology of glycerol utilization in *Pseudomonas* species. MicrobBiotechnol 13(1):32–53

Postgate JR, Kent HM (1987) Qualitative evidence for expression of *Klebsiella pneumoniae* nif in *Pseudomonas putida*. J Gen Microbiol 133:2563–2566

Prajapati K, Sharma MC, Modi HA (2013) Growth promoting effect of potassium solubilizing microorganisms on okra (*Abelmoscus esculantus*). Int J Agri Sci Res (IJASR) 1:181–188

Reimmann C, Valverde C, Kay E, Haas D (2005) Posttranscriptional repression of GacS/GacA-controlled genes by the RNA-binding protein RsmE acting together with RsmA in the biocontrol strain *Pseudomonas fluorescens* CHA0. J Bacteriol 187(1):276–285. https://doi.org/10.1128/JB1871276-2852005

Rodrıguez H, Gonzalez T, Selman G (2000) Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. J Biotechnol 84(2):155–161

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339. https://doi.org/10.1016/S0734-9750(99)00014-2

Rudrappa T, Quinn WJ, Stanley-Wall NR, Bais HP (2007) A degradation product of the salicylic acid pathway triggers oxidative stress resulting in downregulation of *Bacillus subtilis* biofilm formation on *Arabidopsis thaliana* roots. Planta 226:283–297

Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Paul WP, Bais HP (2010) The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 3(2):130–138

Saha M, Maurya BR, Meena VS, Bahadur I, Kumar A (2016) Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic Plains of India. Biocatal Agri Biotechnol 7:202–209

Saiyad SA, Jhala YK, Vyas RV (2015) Comparative efficiency of five potash and phosphate solubilizing bacteria and their key enzymes useful for enhancing and improvement of soil fertility. Int J Sci Res Publ 5:1–6

Savary S, Ficke A, Aubertot JN, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Food Sec 4:519–537

Schulze J, Temple G, Stephen JT, Beschow H, Vance PC (2006) Nitrogen fixation by white lupin under phosphorus deficiency. Ann Bot 98:731–740

Setten L, Soto G, Mozzicafreddo M, Fox AR, Lisi C, Cuccioloni M, Angeletti M, Pagano E, Díaz-Paleo A, Ayub ND (2013) Engineering *Pseudomonas protegens* Pf-5 for nitrogen fixation and its application to improve plant growth under nitrogen-deficient conditions. PLoS ONE 8(5):63666

Shi H, Huang X, Wang Z, Guan Y, Zhang X (2019) Improvement of pyoluteorin production in Pseudomonas protegens H78 through engineering its biosynthetic and regulatory pathways. Appl Microbiol Biotechnol 103(8):3465–3476

Shulse CN, Chovatia M, Agosto C, Wang G, Hamilton M, Deutsch S, Yoshikuni Y, Blow MJ (2019) Engineered root bacteria release plant-available phosphate from phytate. Appl Environ Microbiol 85(18):01210–01219

Simarmata T, Fitriatin BN, Setiawati MR, Herdiyantoro D, Suryatmana P, Hindersah R, Nurbaity A, Kamaluddin NN, Hanum F, Turmuktini T, Suhartatik E (2019) Development and formulation of beneficial rhizobacteria consortia to improve soil health and agricultural practice sustainability in Indonesia. Plant growth promoting rhizobacteria (PGPR): prospects for sustainable agriculture. Springer, Singapore, pp 63–74

Sneha S, Anitha B, Sahair RA, Raghu N, Gopenath TS, Chandrashekrappa GK, Basalingappa KM (2018) Biofertilizer for crop production and soil fertility. Acad J Agric Res 6(8):299–306

Solaiman DKY, Ashby RD, Crocker NV (2016) Genetic construction of recombinant *Pseudomonas chlororaphis* for improved glycerol utilization. Biocatal Agric Biotechnol 8:45–49

Song C, Yue SJ, Liu WH, Zheng YF, Zhang CH, Feng TT, Hu HB, Wang W, Zhang XH (2020) Engineering of glycerol utilization in *Pseudomonas chlororaphis* GP72 for enhancing phenazine-1-carboxylic acid production. World J Microbiol Biotechnol 36(3):1–8

Sun Y, Cheng Z, Glick BR (2009) The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderiaphytofirmans*PsJN. FEMS Microbiol Lett 296(1):131–136

Upadhyay A, Srivastava S (2011) Phenazine-1-carboxylic acid is a more important contributor to biocontrol *Fusarium oxysporum* than pyrrolnitrin in *Pseudomonas fluorescens* strain Psd. Microbiol Res 166(4):323–335

Uroz S, Calvaruso C, Turpault M-P, Frey-Klett P (2009) Mineral weathering by bacteria: ecology actors and mechanisms. Trends Microbiol 17:378–387

Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq Boyce A (2016) Role of plant growth promoting rhizobacteria in agricultural sustainability: a review. Molecules 21(5):573

Von Bergen M, Jehmlich N, Taubert M, Vogt C, Bastida F, Herbst FA (2013) Insights from quantitative metaproteomics and protein-stable isotopeprobing into microbial ecology. ISME J 7:1877–1885. https://doi.org/10.1038/ismej201378

Wagh J, Shah S, Bhandari P, Archana G, Kumar GN (2014) Heterologous expression of pyrroloquinoline quinone (pqq) gene cluster confers mineral phosphate solubilization ability to *Herbaspirillumseropedicae* Z67. Appl Microbiol Biotechnol 98(11):5117–5129

Wang L, Zhang L, Liu Z, Zhao D, Liu X, Zhang B, Xie J, Hong Y, Li P, Chen S, Dixon R (2013a) A minimal nitrogen fixation gene cluster from *Paenibacillus* sp. WLY78 enables expression of active nitrogenase in Escherichia coli. PLoS Genet 9(10):1003865

Wang X, Yang JG, Chen L, Wang JL, Cheng Q, Dixon R, Wang YP (2013b) Using synthetic biology to distinguish and overcome regulatory and functional barriers related to nitrogen fixation. PLoS ONE 8(7):e68677

Zhang X, Huang Y, Harvey PR, Ren Y, Zhang G, Zhou H, Yang H (2012) Enhancing plant disease suppression by *Burkholderia vietnamiensis* through chromosomal integration of *Bacillus subtilis* chitinase gene chi113. Biotech Lett 34(2):287–293

Zhang Q, Xiao Q, Xu J, Tong Y, Wen J, Chen X et al (2015) Effect of retS gene on antibiotics production in *Pseudomonas fluorescens* FD6. Microbiol Res 180:23–29. https://doi.org/10.1016/jmicres201507005

Zhang Y, Massel K, Godwin ID, Gao C (2018) Applications and potential of genome editing in crop improvement. Genome Biol 19(1):210

Zheng W, Zeng S, Bais H, LaManna JM, Hussey DS, Jacobson DL, Jin Y (2018) Plant growth-promoting rhizobacteria (PGPR) reduce evaporation and increase soil water retention. Water Resour Res 54(5):3673–3687

Zúñiga A, Fuente FDL, Federici F, Lionne C, Bônnet J, de Lorenzo V, González B (2018) An engineered device for indoleacetic acid production under quorum sensing signals enables *Cupriaviduspinatubonensis*JMP134 to stimulate plant growth. ACS Synth Biol 7(6):1519–1527