Traversing through the intricate interplay of plant microbiome front runners

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Abstract Bacteria produce a plethora of metabolites and substances, both intra- and extra- cellular, that govern the diversity as well as keystone functionalities in its close vicinity. A special group of bacteria, plant growth promoting rhizobacteria (PGPR), for example produce siderophores, exopolysaccharides (EPS), 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) and Indole acetic acid (IAA) that can contribute to its PGP traits. The activity of such PGPR as well as their metabolites allow them to interact with the rhizosphere in a way that is mutually beneficial to the bacterium and the plant. Siderophores and EPS are two metabolites that serve to enhance the quality of soil, maintain a healthy diversity in the microbial niche while also preventing the onslaught of phytopathogens that may deteriorate the plant and, in turn, the soil health. This makes the study of siderophores and EPS vital to any process that includes or aims to deal with the PGPR or any microbe with PGP qualities. It, thus, becomes imperative to investigate the determinants as well as the regulatory molecules responsible for governing the biosynthetic pathways for these two metabolites which, in tandem, invariably help in plant growth promotion. The phenomenon of quorum sensing plays an important role in plant defense regulation to defend the plant against various kinds of stresses like salinity, nutrient deficiency, drought and pathogen invasion. To apprehend the interactions between the determinants and their regulatory molecules attuning them at gene level can be a strategy to understand their metabolic pathways leading to broad spectrum resistance against various phytopathogens. This review focuses on various aspects of siderophores and EPS, including their regulatory determinants with respect to the PGP qualities of a candidate microbe.

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Introduction

Crop improvement and increased yields have been a primary focus to meet the demands of a growing human population. One way this can be achieved is by the use of beneficial bacteria known as Plant growth promoting Rhizobacteria (PGPR), which have a niche in the rhizosphere of plants. This class of bacteria not only helps in crop improvement but also produces certain metabolites

that confer resistance to plants against abiotic and biotic stresses. These metabolites include Siderophores, Exopolysaccharides, Lipopolysaccharides and Flagellin. The lipopolysaccharide present in outer membranes of gram-negative bacteria play an important role in preventing hypersensitive responses generated due to virulent or non- host bacterial adhesion in plants (Dow et al., 2000). Flagellin is a globular protein that forms the flagella and bacterial flagellin receptors act as elicitors to provoke an immune response in plants (Patel S. et al., 2017). Exopolysaccharides are high molecular weight compounds that have been abundantly studied for their diverse properties whereas siderophores are low molecular weight iron chelating compounds which are reported to play a role in disease resistance and bioremediation (Shukla et al., 2019; Dave et al., 2020). These determinants are regulated by processes such as Quorum Sensing (QS) that helps in root colonization and also response to cell density by gene regulation. Targeting and analyzing the QS inducers would be useful in disease and crop management. The biosynthetic pathways of siderophores and exopolysaccharides in various organisms may also be engineered in such a way that they induce defense mechanisms in plants as well as protect plants from abiotic stress and nutrient deficiency. On the other hand, the contribution of the biosynthetic pathways and their direct correlation with defense mechanisms and other abiotic stresses is not known. The role of the regulatory pathways like quorum sensing and biosynthetic pathways affecting these determinants can be explored to establish whether they play a role in enhancing defense responses of the plants against abiotic and biotic stress. Fine tuning QS pathways can also be a decisive approach to discern the regulatory potential of these determinants. This review mainly focuses on regulatory and molecular aspects of siderophores and exopolysaccharides as the key players of the plant microbiome against biotic and abiotic stresses.

Exopolysaccharides

Exopolysaccharides are high molecular weight compounds that provide structural integrity to the microbes and form the major component of biofilms. Biofilms are a prerequisite to root colonization which is an important attribute of PGPR and is essential for competing against pathogen invasion. There are numerous applications of exopolysaccharides apart from plant defense, for

example Xanthan gum produced by *Xanthomonas campestris* is used in food industries. EPS like Gellan and Dextran enhances rheological properties of food and pharma products (Cruz et al., 2000; Freitas et al., 2006; Abdalla et al., 2021) Many emulsifiers, stabilizers and gelling agents are prepared using EPS (Welman et al.,2003). In medical fields the two main properties of EPS insolubility and water holding capacity, have been exploited as they allow slow release of drugs. Due to hydrophilic properties and high charge, EPS is used in wastewater treatments and solvent dehydrations (Huang et al.,2022). Exopolysaccharides are also known to show antioxidant activity, for example EPS from the fungus *Cordyceps gracilis* exhibits radical scavenging, iron chelating and reducing power activities (Donot et al., 2012). EPS is currently being used in unique biomedical applications due to biocompatibility, mechanical strength, and biodegradability. Some examples include Gellan hydrogels which is used in bone engineering along with hydroxyapatite; Alginate chitosan-based hydrogels are used in stem cell encapsulation (Sharma et al., 2015). EPS Hydrogels are also used in drug delivery as they promote controlled release of drugs (Nurhayati et al., 2019). EPS is also used in bioremediation due to the presence of a substantial quantity of anion functional groups and helps to remove or recover metals from the rhizosphere through biosorption by effectively sequestering positively charged heavy metal ions (Nadzir et al., 2021). In the rhizosphere, EPS production by certain plant growth promoting microbes induces biofilm formation in response to the exposure of toxic metals which enhances tolerance of microbial cells by forming a protective cover as well as transforming toxic metal ions into non-toxic forms after adsorption (Ayangbenro AS et al., 2017). In a recent study on the isolation of bioluminescent and exopolysaccharide bacteria *Vibrio alginolyticus* PBR1 and *Vibrio rotiferianus* and their characterization found that these bacteria possess the ability to resist heavy metals like Pb and Sr with the help of exopolysaccharides Parmar et al., 2020). During drought stress EPS produced by the microbes are helpful in reducing detrimental effects due to their high-water holding capacity. EPS also helps plants tolerate salinity stress caused by decreased sodium uptake and osmotic stress. EPS also protects the bacteria against fluctuating temperatures; in the case of thermophiles, EPS acts as a protective shield against high temperatures (Dave et al., 2020) Fig 1 .
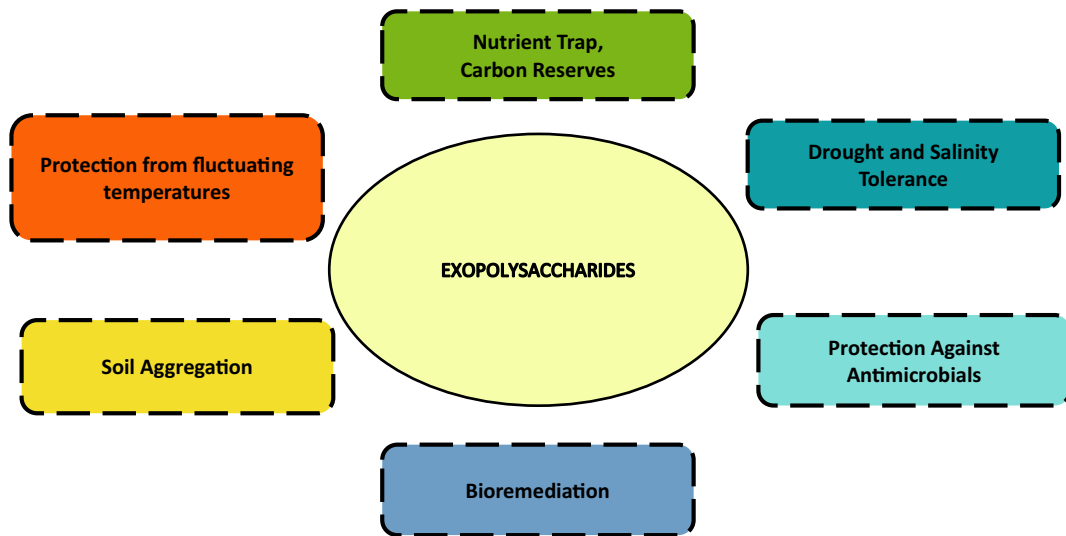
Fig. 1 The versatility of Exopolysaccharides in various fields

Characteristics of exopolysaccharides

Exopolysaccharides can be characterized by using various bioanalytical techniques thin layer chromatography (TLC) that is mainly used for acid hydrolyzed EPS wherein the spots are visualized using alpha-naphthol and sulphuric acid. The composition of monosaccharides is identified using Gas chromatography. The purity of EPS is determined using High performance liquid chromatography. The functional groups in EPS can be detected using Fourier Transform Infrared spectroscopy (FTIR) and molecular confirmation is done by Nuclear Magnetic Resonance (NMR) spectroscopy which is indicated by different chemical shifts (Benit et al., 2018, Shukla et al., 2020).

Carbohydrates

Sugar residues form the main constituent of EPS matrix. They are categorized into two types Homopolysaccharide consists of the same monomeric units of residues and heteropolysaccharide contains different units of monosaccharides. (Sutherland., 2007). Many exopolysaccharides contain inorganic and organic components that determine their properties. The exopolysaccharides produced by various microorganisms are diverse Xanthan Gum, Alginate, curdlan and cellulose *Pseudomonas aeruginosa,* most commonly studied microorganism for EPS, produces three main types: Polysaccharide synthesis locus (psl) which codes for eps Psl made up of galactose critical for formation of biofilms along with Alginate and Pellicle Polysaccharide (Pel). (Ryder et al., 2007; Oleńska et al.,, 2021).

Proteins

Enzymes also form a major part of the biofilm matrix and help in the degradation of polymers and in detachment of bacteria from the matrix whereas some enzymes also act as virulence factors during infections (Costa et al.,2018). The enzymes can be retained in the matrix as they form a complex with polysaccharides and this prevents them from being degraded by proteolysis and enhances their thermodynamic stability (Conrad et al., 2003). Lectins are proteins that help in stabilization and formation of matrix. For example, the glucan binding proteins help in biofilm formations of the dental pathogens *Streptococcus mutans* by aggregation, inhibition, cell wall synthesis and plaque cohesion (Lynch et al., 2007; Dave et al., 2020; Shukla et al. 2021).

Extracellular DNA (e-DNA)

DNA molecules are found in all cells and majorly form the extracellular medium. One such DNA is the e-DNA that forms an integral part of the biofilms and may vary between closely related species (Shukla et al., 2019; Aldecoa et al., 2017). The microbial origin of e-DNA in terms of evolution was first found in marine as well as terrestrial niches. The release of extracellular DNA occurs by secretion and autolysis (Albecoa et al., 2017).

The eDNA mechanisms are regulated by quorum sensing. e-DNA also imparts natural competence to the microbial cells which enables their transformation. The chelation of metal ions in case of *P. aeruginosa* is also carried out by e-DNA inducing antimicrobial resistance and increased virulence (Albecoa et al., 2017). The e-DNA is an essential component of EPS as it protects the microbes from physical, chemical, environmental stress and antimicrobial therapies which makes it a prime target to control biofilm formations by bacterial communities (Devaraj et al., 2019; Izano et al., 2008, Yang et al., 2007). e-DNA finds its applications in genetic exchange, adhesion and signaling (Shukla et al., 2019). eDNA is a potential source of nutrition as it consists of Carbon, Nitorgen and Phosphorus. Apart from eDNA which forms the major component of biofilms there are other molecules like RNAs. cGMPs which also play a role in biofilm formations (Castiblanco et al., 2016).

Lipids

Lipids contribute to hydrophobicity of EPS. For example, the bacterium *Sinorhizobium meliloti* produces exopolysaccharides which inhibits evaporation of water from the soil as they conjugate with the soil structure to retain water (Deng et al., 2015). Due to this property, they may be useful for microbially enhanced oil recovery and for bioremediation of oil spills (Putra et al., 2019). Biosurfactants such as rhamnolipids that have been found in the EPS matrix of *P. aeruginosa* display surface activity and help in microcolony formation, facilitating migration and preventing colonization of channels by other bacteria (Chavez et al., 2021).

Water

The hydrated matrix of EPS makes the microbial cells withstand deviations in water potential. The EPS matrix acts as a molecular sieve, sequestering cations, anions, non-polar compounds and particles from the water phase. During desiccation the EPS provides protection to the microbes and the biofilm formed by them. The EPS production begins in response to desiccation process which induces resistance to water stress by retaining large amounts of water (Tamaru et al., 2005; Shukla et al., 2019).

Biosynthesis of exopolysaccharides

The biosynthesis of microbial exopolysaccharides occurs through four major pathways in prokaryotes, which differ in their reaction mechanisms and the enzymes used in synthesis of each step. Initiation and elongation of polymers by enzymatic transformations are carried out by precursor molecules which lead to production of activated sugars. Biosynthesis pathways of polysaccharides lead to regulation of production of EPS and can be targeted to achieve disease resistance and enhanced biofilm formations for root colonization by PGPR in the rhizosphere. The **Wzx/Wzy dependent pathway** involves the translocation of C55 (undecaprenol diphosphate) across the membrane with help of the flippase enzyme Wzx protein. On the other hand, the Wzy protein carries out the process of polymerization which takes place in the periplasmic section of the cell. The extent of the polymerization process is governed by the Wzz enzyme. This pathway is responsible for synthesizing various polysaccharides: Lipopolysaccharide (LPS), EPS, Capsular polysaccharide (CPS) and ECA (Enterobacterial common antigen) (Whitefield et al., 2006; Schmid et al., 2015). The LPS molecule acts as the elicitor of immune response whereas the EPS molecule helps combat biotic and abiotic stresses altogether by helping in biofilm formations and increased water retention capacity respectively. The second pathway which plays an important role in synthesis of polysaccharides is **ABC dependent pathway** in which the ATP binding ABC transporters export glycans across the membrane. The glycans present on the cell surface play an important role in immune defenses, formation of biofilms and maintenance of the cell envelope and structure. The ABC and Wzx/Wzy pathways differ in their initiation and completion steps but ultimately both lead to formation of lipid linked polysaccharides outside the membrane. The third major pathway is the **Synthase dependent pathway** in which translocation and polymerization is carried out by a synthase protein (Schmid et al., 2016). The synthases act as glycosyltransferases and are an integral part in formation of cellulose, hyluronan, alginate and other components of the matrix. Another pathway involved in biosynthesis of EPS is the **Sucrase Pathway** which uses the enzyme sucrase to export the polysaccharide outside the membrane. The common molecule synthesized by this pathway is dextran (Schmid et al., 2018). Fig 2 depicts brief overview of the pathways.

Fig. 2 Different pathways for biosynthesis of Exopolysaccharides

Wzx/wzy dependent pathway
- Periplasmic space
- Arrangement by glycosyltransferase
- Wzx protein (Flippase)

Sucrase Pathway
- Polymerization outside the cell
- Transfer of glucose from sucrose on polysaccharide chain

ABC Transporter Pathway
- Cytoplasmic Side
- Formation of Homopolymers
- Translocation by efflux pump

Synthase Dependent Pathway
- Formation of both homo and hetero polymers
- Translocation and assembly by synthase

Siderophore

Siderophores are iron chelating low molecular weight compounds that help in combating environmental stress due to inadequate iron (Costa et al., 2018). Because of their high chelating ability, they cause mineral dissolution and help in uptake of other minerals like Manganese, Zinc and Molybdenum. Some strains of *Pseudomonas* have been reported to produce a siderophore known as pyoverdine which not only helps in scavenging iron but also in regulating the expression of different virulence factors such as exotoxin A and PrpL proteases (Visca et al., 2007). As high amounts of ROS can be detrimental to the cells, these siderophores are said to help in storage of iron thereby controlling ROS production and inducing oxidative stress tolerance. In medical fields, siderophore is used to reduce iron overload in patients whereas in pharmaceutical industries Desferrioxamine is one such siderophore produced by the bacterium *Streptomyces pilosus* used to treat patients with iron overload (Codd et al., 2017). The siderophores also exhibit antimicrobial activity such as the siderophore secreted by *Azospirillum lipoferum* that shows antimicrobial activity against bacterial and fungal strains (Shah et al., 1992). Sideromycins also have antibiotic activity and can be a useful approach to curb antibiotic resistance (Braun et al., 2009). Siderophores

are extensively used in agriculture due to their biocontrol activity against phytopathogens wherein they may prevent the pathogen from scavenging the bioavailable iron; for example pyoverdines secreted by PGPR *P. fluoroscens* help in biocontrol of phytopathogens (Trapet et al., 2016). A new field has emerged known as Siderotyping which is used to distinguish strains according to the type of siderophores they produce (Meyer et al., 2002). Siderophores also play a role in bioremediation as they solubilize metals and increase their mobility. Siderophores have high affinity towards other metals apart from iron and they may solubilize and mobilize a variety of metals like Fe, Cd, Cu, Ni, Pb, Ni, Pb and Zn (Schalk et al., 2011). The chelating actions of siderophore determines its binding affinity towards various metals (Golonka et al., 2019). Another process which helps achieve reduced metal toxicity is called Bioaugmentation which involves addition of microbes producing bio-surfactants and siderophores (Roskova etal., 2022).

Diverse functions of Siderophores which contribute to its multifaceted nature

Siderophores exhibit different conformations and share a conserved structure that has a functional group which ligates with molecules like transferrin and lactoferrin

(Marathe et al., 2015). Hydroxamate siderophores are structurally more complex and hydrophilic in nature. The property of denticity plays an essential role in iron affinity. Hexadentate siderophores have greater affinity towards Fe (III) than tetradentate siderophores. In tetradentate siderophores, two or three molecules of ligand bind to satisfy the six coordinating sites of ferric ions making them more stable than the bidentate ones (Chen et al., 2019). Some siderophores are similar in structure but differ by a single functional group; for example, Salmochelin produced by *Salmonella enterica,* is similar to enterobactin but differs in that it is glucosylated at the C5 position of at least one catechol ring (Fischbach et al., 2006). In stereochemistry of siderophores many hexadentate hydroxamates are stereo-chemically restricted to the cis isomers. Unlike most known siderophores, the ferrioxamines are not optically active (Hider et al., 2007).

Iron selectivity

The negatively charged oxygen atoms have the highest affinity to iron (III). The tighter interaction results due to the presence of a high charge on the oxygen atom. Siderophores form an octahedral conformation with charged oxygen atoms that give rise to a stable spin complex. The octahedral conformation may be distorted due to nitrogen or sulphur atoms in the absence of Fe. This distortion causes decreased affinity for iron (III) like that observed in Enterobactin (Schalk et al., 2012).

Structural range of Siderophores

There is a mononuclear coordination of hexadentate and bidentate ligands with iron (III). The most commonly found structure among siderophores is hexadentate. The catechol and hydroxymate groups confer protection against various lytic enzymes. The cyclic petides and N-C terminal blocked peptides ensure increased life span of these groups by providing resistance against peptidase enzymes. (Liu et al., 2002).
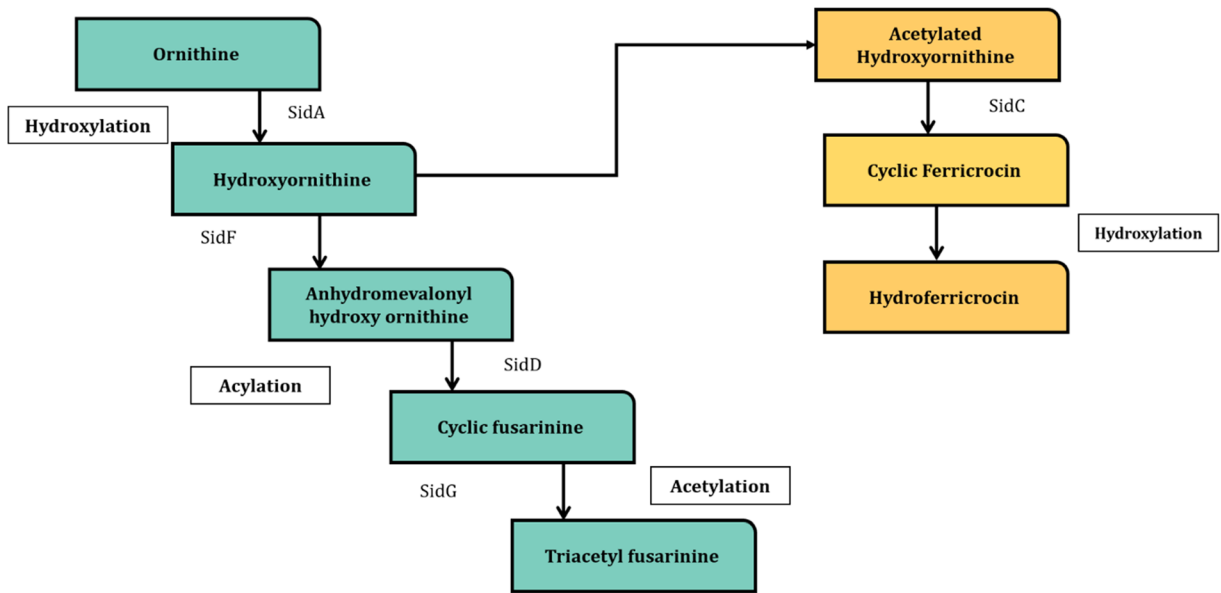
Biosynthetic pathway of Siderophores

The biosynthetic pathway of siderophores differs in both fungi and bacteria due to the different precursor molecules. Initiation of biosynthesis in fungi starts from citrate, amino acids, dihydroxybenzoate and N5-acyl-

N5-hydroxyornithine. The assembly of siderophores is done by non-ribosomal cytoplasmic synthase which forms a covalent linkage between amino acid and forms clusters as the peptide chain elongation proceeds from one domain to another domain (Roux et al., 2009). The siderophore operon of *Aspergillus fumigatus* has been widely studied and includes important genes like *sidA* which codes for an enzyme L-ornithine N5-monooxygenase that catalyzes the first step in siderophore biosynthesis as shown in Fig. 3 The ornithine is synthesized by large enzymes called non ribosomal peptide synthetases (NRPSs) (Kragl et al., 2007). The *sidD* gene, a non-ribosomal peptide synthetase (Blatxer et al., 2011)*, sidG* an acyl transferase and highly conserved genes *sidF, sidC* are required for biosynthesis of both the ferricrocin complex (FC) and the hydroxyferricrocin complex in siderophore synthesis (HFC) (Blatzer et al., 2011). There are two main pathways of biosynthesis: NRPS independent and dependent. In the NRPS dependent three main steps are involved: adenylation, thiolation and condensation. The molecules in this pathway are highly selective and can be seen mostly in bacteria whereas in the NRPS independent pathway a single synthetase enzyme carries out the single step process of synthesis. On the other hand, in bacteria such as *E. coli, Yersinia pestis* and *P. aeruginosa* the chorismate precursors initiate the biosynthesis of siderophores like enterobactin and pyochelin as highlighted in Fig 3. . Here the *entB* and *entS* are the enterobactin biosynthesis gene (Paul Dubey et al., 2015). The operon *entCDEBAH* present in these bacteria induce the synthesis of siderophores and genes like *fepA, fepB, fepC, fepD, fepE, fepG, fes* and *entS* which play a role in utilization and uptake of enterobactin (Peralta et al., 2016). (Fig. 4).

**Regulation of EPS and Siderophore**

Exopolysaccharides form the major component of biofilms which help in adherence and root colonization of PGPR against pathogenic microbes. Biofilm formation is controlled and regulated by another important phenomenon called QS, is a type of cell-cell communication which solely depends on bacterial community and density. The microbes in response to QS produce signals known as autoinducers which help in increasing the cell-population density. These signals induce alterations in gene expression (Wang et al., 2020). There are two

Fig. 3 Siderophore Biosynthesis Pathway in the fungus *Aspergillus fumigatus*

widely studied QS, the **LuxI/R** consists of the Luciferase operon (*lux1CDABE*), which is required for luminescence, is regulated by two main proteins – LuxI and LuxR. The *luxI* codes for autoinducer synthase which leads to accumulation of Acyl homoserine lactones (AHL) and AI N3- oxododecanyl HSL (3OC6HSL). The transcriptional activator LuxR is bound to 3O6HSLand this LuxR-AHL complex recognizes the operon *luxICDABE* and thus switches on the operon. This generates a positive feedback loop which increases the levels of AHL thereby activating the QS mode of the bacterium. The LuxI synthesizes AHL whereas the LuxR interacts with AHLs specific to the gram-negative microbe, but in gram-positive bacteria the QS signals are generated by oligopeptides and histidine kinases are used as receptors (Wang et al., 2020). The other system includes the **LuxS/AI-2** and is regulated by two enzymes, Pfs and LuxS, which convert SAH (S-adenosyl
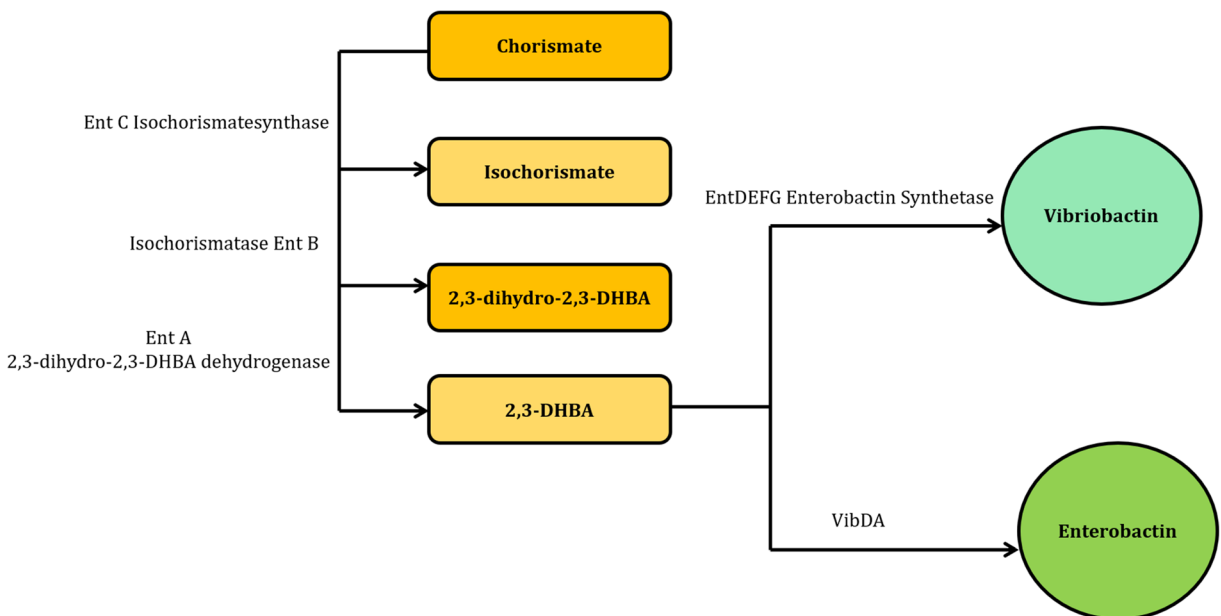
Fig. 4 Biosynthesis pathway of siderophores in *Eschrechia coli*, *Pseudomonas aeruginosa* and *Yersinia pestis*

homoserine) to adenine, homocysteine and the signal molecule 4,5-dihydroxy-2,3-pentanedione (DPD). The DPD is then exported and cyclized to AI-2, enabling both the synthesis of AI-2 and the detoxification of the toxic by-product SAH (Schauder et al., 2001). The presence of LuxS among bacteria possesses many attributes and components that can be rewired into engineered systems (Wang et al., 2020). These diverse signals and characteristics can be translated, fine-tuned, and explored to study different interactions and applications in plant growth promotion and disease resistance. On the other hand, **Iron** is essential as well as toxic to the bacteria. The pathogens, including *P. aeruginosa* indulge in intense competition for iron with the host (Ratledge et al.,2000; Braun et al., 2009; Griffiths et al., 1999). The pathogen has multiple systems to sequester iron and can regulate cellular iron acquisition and storage through a combination of positive and negative regulatory factors (Ratledge et al., 2000; Poole et al., 2003; Waters et al., 2005). The two best-studied *P. aeruginosa* iron systems are the pyoverdine system and the pyochelin system. Pyoverdine and pyochelin bind extra-cellular iron (Fe^{+3}) which is then transported into the cell together with these siderophores. Pyoverdine synthesis and secretion are regulated by means of the extra cytoplasmic function (ECF) factor PvdS. Expression of PvdS is regulated by iron and the ferric uptake regulator (Fur). The increase in concentration of QS molecules represses the siderophore synthesis which decreases at higher cell densities (Dertz et al., 2006) but in *P. aeruginosa* the siderophore production increases at high cell density which ensures that they are used by members of their own species (Stintzi et al., 1998; Darch SE et al., 2012). Sometimes the repression of siderophores at a higher cell density would allow the bacteria to divert resources towards growth promotion (Nadell et al., 2008). Hence the siderophores that are not stimulated by QS are primarily involved in iron scavenging and homeostasis whereas the siderophores stimulated by QS play a role in signals or virulence factors and defense mechanisms (Lamont et al., 2002). In *Vibrio vulnificus* the iron and quorum sensing together regulate the vulnibactin synthesis (Wen et al., 2012). It has been reported that the presence of iron and biofilm formation is tightly linked as iron deficiency gives rise to twitching motility (Singh PK et al., 2002). The pyoverdine system in *Pseudomonas* species is necessary for biofilm as in the absence of pyoverdine the delivery of iron to the innermost layers of the biofilm gets impaired despite production of lower affinity pyochelin. As the defect in pyochelin synthesis does have any effect on biofilm formation demonstrating that pyoverdine is required for efficient biofilm formation and functions (Visca et al., 2007). The other PGP traits along with siderophore and exopolysaccharide production are listed in Table 1.

Molecular mechanisms used by PGPR under biotic and abiotic stress

PGPR have positive influence on plants which ultimately leads to increased growth and productivity. The

Table 1 Different Plant Growth Promoting Rhizobacteria Showcasing various PGP traits

Serial No.	Name of PGPR	PGP Characters	Reference
1	*Bacillus pumulis, FAB10*	EPS, IAA, NH3, siderophore, PS, and HCN	Ansari et al.,2019
2	*Enterobacter* sp. *P23*	EPS, IAA, NH3, siderophore, PS, ACC deaminase, SA, and HCN	Sarkar et al., 2018
3	*Halomonas* sp. *Exo1*	EPS, IAA, NH3, N2 fixation, siderophore, PS, and HCN	Mukherjee et al., 2019
4	*Ochrobactrum pseudogregnonense and Bacillus safensis*	IAA, siderophore, PS, and ACC deaminase	Chakraborty et al., 2012
5	*Pseudomonas putida and Bacillus paramycoides*	IAA, NH3, siderophore, PS, and ACC deaminase	Pandey et al.,2020
6	*Alcaligenes* sp. *AF7*	EPS, IAA, siderophore, PS, Zn solubilization, and GA	Fatima et al., 2020
7	*Rhizobium* spp.	Siderophores, hydrogen cyanide, ammonia, EPS, IAA	Ahemad and Khan, 2012
8	*Bradyrhizobium* spp.	IAA, siderophores, EPS, hydrogen cyanide, ammonia	Meena et al., 2017

PGPR undergo molecular changes giving rise to certain physiological and metabolic changes that help the bacteria outcompete the pathogens attacking the plants and combat stress. For example, in *Arabidopsis thaliana* the PGPR *Paenibacillus polymyxa* B2 enhanced tolerance to drought stress by transcription of *ERD15* gene which codes for dehydration induced ERD15 proteins which are responsive to drought stress. In some cases, PGPR also tend to induce phytohormonal changes. The IAA produced by the bacteria stimulates root growth which helps in enhanced nutrients and water. It had been shown that when bean plants were inoculated with *Azospirillum brasilense* there was increased root surface and length due to production of IAA by the bacterium (Burdman et al., 2000; Cohen et al., 2009). During salinity stress salt increases which can alter the water content in plants. The PGPR introduced to plants help them tolerate salinity by altering gene regulation. For example, the rice plant when inoculated with *Bacillus amyloliquefaciens* upregulated four genes and downregulated two genes *GIG* and *SNF1* which contribute to increased tolerance to salinity stress (Tiwari et al., 2017). The PGPR in the rhizosphere are recognized by plants from the microbial compounds produced by them such as volatile metabolites, siderophores, oligosaccharides, LPS, flgagellin and EPS. These compounds trigger certain defense responses during pathogen invasion. There is an elicitation of immunes responses in plants leading to activation different signaling pathways giving rise to Induced Systemic Resistance (ISR). In ISR, the activation of signaling pathways of Jasmonic Acid/Ethylene in presence of PGPR enhances plant immunity and systemically primes the plant against pathogens as jasmonic acid and ethylene are stress hormones which are accumulated in response to stress and are key regulators of ISR. There are other secondary metabolites produced by PGPR that act as elicitors of ISR, for example phenazines produced by *Pseudomonas* species. Along with ISR, there is another type of defense mechanism known as Systemic Acquired Resistance (SAR) which is induced by pathogens leading to accumulation of salicylic acid and simultaneous activation of PR genes (pathogenesis related genes) that code for PR proteins which provide immunity to other parts of the plants. The SA levels endogenously increase in presence of the pathogen and rapid signals are produced which confers resistance to plant in a very short time whereas the immunity induced by PGPR in plants that is ISR, helps generate stronger and faster defense responses during pathogen attack (Yu et al., 2022; Patel and Saraf, 2017).

Carbohydrate receptors in plants

Carbohydrates participate in immune recognition and defense. Exopolysaccharides are the high molecular weight determinants secreted by bacteria which have carbohydrate as their major constituent. Exopolysaccharide has the ability to induce immune responses in plants in the presence of a pathogen. The exopolysaccharides also play a role in Reactive oxygen species (ROS) generated by plants in response to abiotic stresses. The exopolysaccharides have the potential to be antioxidant agents due to their ROS scavenging ability. During plant microbe interactions they act as energy sources as well as help in regulation of defense genes. The perception of exopolysaccharides by plants depends on their structure and functions (Trouvelot et al., 2015). Apart from microbial polysaccharides, plants have lectins which act as carbohydrate binding domains. The exopolysaccharides can thus bind to such receptors and elicit defense responses (Naithani et al., 2021). On the other hand, a receptor EPR3 in plants helps in non-self-recognition and response to microbial associated molecular patterns. The function of exopolysaccharides in PGPR depends on their molecular composition (Kawaharada et al., 2015; Wong et al., 2020). These receptors can be crucial targets for inducing defense in plants enabling them to avoid diseases.

Future prospects

Siderophore and EPS are multivariate functionally and structurally and have a positive influence on plants. These two metabolites along with others make the PGPR surmount other bacteria residing in the rhizosphere. Due to numerous beneficial traits of EPS and siderophores, they are being continuously studied in vitro and at the gene level. The EPS studies lack appropriate structure–function relationships for biological functions rendering them not suitable for commercialization. Complete information is needed on EPS structure as different bacteria produce uniquely structured EPS. High production costs and processing make it difficult to exploit EPS as opposed to artificial analogues (Shukla et al., 2021; Hussain et al., 2017). Along with EPS, exploiting siderophores is also challenging, from the structural and functional

relationships to the microbial environment which includes any structural or molecular changes in response to the microbial metabolites, and in-depth analysis of its role at low iron availability during iron starvation needs to be explored in detail (Ahmed et al., 2014). The regulatory molecules involved in abiotic stress require further study with respect to PGPR to envisage their use in mitigating stress. Quorum sensing is a phenomenon which is exhibited by many bacteria which depends on cell density. QS regulates the production of EPS and Siderophore as they play a role in biofilm formations necessary for microorganisms to survive in competitive environments. These determinants are amenable to scrutiny at every step and fine tuning of QS signals, metabolic engineering of EPS and siderophores will help uncover their role in regulation of plant defense responses. Techniques like CRISPR can also be used for gene editing and engineering of metabolites (Arpit et a et al., 2021). To strengthen the use of siderophores and EPS in multitude of applications and as potential tools of sustainable agriculture much of their regulatory roles with respect to molecular and physiological changes as well as defense signaling pathways needs to be studied to unveil more of their new functions. Better understanding of their chemical structures and molecular mechanisms as well as characterization will help decipher other new functions in order to develop new approaches that will be beneficial to living beings and environment.

Authors contribution   AK and DP contributed in conception and design of manuscript. AK compiled the literature and wrote the manuscript. AS and DP reviewed the manuscript and AS and AK revised it.

Declarations

Conflict of interest   The author declares no conflict of interest.

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