J. Bangladesh Acad. Sci. 44(2); 69-84: December 2020 DOI: 10.3329/jbas.v44i2.51452

Journal of Bangladesh Academy of Sciences

Journal homepage: http://www.bas.org.bd/publications/jbas.html

Review Article

Anti-fungal secondary metabolites and hydrolytic enzymes from rhizospheric bacteria in crop protection: a review

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ARTICLE INFO

Article History
Received: 13 December 2020
Revised: 14 December 2020
Accepted: 15 December 2020

Keywords: Rhizospheric bacteria, Secondary metabolites, Crop protection.

ABSTRACT

Plant growth promoting rhizobacteria (PGPR) residing in soil rhizosphere provide enormous beneficial effects to a plant host producing diverse secondary metabolites and enzymes useful for plant growth and protection. Siderophores, antibiotics, volatile compounds and hydrolytic enzymes are the major molecules secreted by the PGPRs, which have substantial antifungal properties and can provide plant protection. These compounds are responsible for the lysis and hyperparasitism of antagonists against deleterious fungal pathogens. Siderophore-producing PGPRs function by depriving the pathogen of iron nutrition. Antibiotics have been reported to be involved in the suppression of different fungal pathogens by inducing fungistasis, inhibition of spore germination, lysis of fungal mycelia. The PGPRs also secrete a wide range of low molecular weight volatile organic compounds (VOCs) that inhibit mycelial growth, sporulation, germination of phytophathogenic fungi, etc. Hydrolytic enzymes, mostly chitinase, protease and cellulose, lyse the cell wall of fungi. Therefore, plant growth-promoting rhizobacteria can be considered as an effective, eco-friendly, and sustainable replacement to the chemical fungicides. There are many PGPRs that perform very well in controlled conditions but not in field conditions, and hence the commercializing of these products is not easy. Development of formulations with increased shelf life, a broad spectrum of action and consistent performance under field conditions can pave the way for commercializing the PGPRs at a faster rate.

Introduction

The dynamic zone of soil which closely surrounds plant roots is known as the rhizosphere. This zone is known as a microbe storehouse, and its characteristics are greatly influenced by plant root excretions (Shaikh and Sayyed, 2015; Jadhav and Sayyed, 2016). A large number of organisms such as bacteria, fungi, protozoa, and algae that directly or indirectly influence plant growth and productivity by modulating root activity and nutrition accessibility reside in this environment.

The most abundant organism in this zone are bacteria commonly known as PGPR, which

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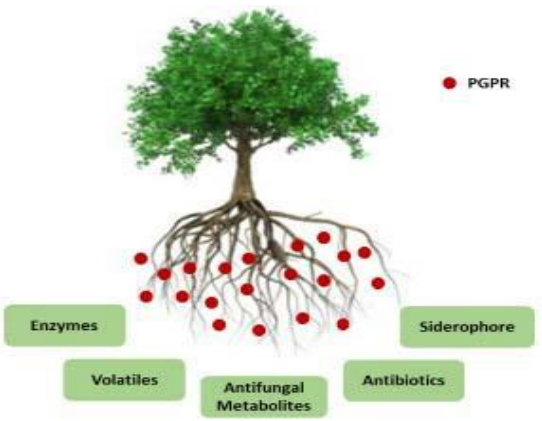
play unique roles in plant growth promotion by generating an overall favorable environment by modifying plant growth dynamics, metabolism and by amending disease resistance. Plants also release their exudates in order to create a suitable selective environment for the most beneficial bacteria by positive selection. Bacteria and also fungi residing in the rhizosphere make an array of useful bioactive compounds, very specialized in nature. These compounds are known as secondary metabolites (SMs). The absence of the secondary metabolites can have long term impairment on survival, prolificacy, or aesthetics on the organism producing the same. The secondary metabolites have long been of incomparable attention owing to their unique role in plant growth promotion together with biocontrol mechanisms. SMs serve as viable weaponry, useful against other bacteria and fungi that are pathogenic for plants. In identifying the probable benefits, researchers have pursued cost-effective and agriculturally valuable SMs that the rhizospheric microorganisms produce and have explored the rhizosphere for identifying the same. Their ability to provide protection against various plant pathogenic microorganisms by producing various bioactive antibacterial and antifungal components is becoming significant for sustainable crop production. PGPRs have been proposed to have a different name from their plant growth promoting counterpart, including other bacteria of similar functions of plant associated microbiome. They are beginning to be popularly known as 'Biocontrol PGPBs (Plant growth promoting bacteria)' (Bashan and Holguin, 1998).

Current strategies to control such phytopathogens are still inadequate. In terms of disease control, it is well-documented that beneficial plant-associated microbes can protect plants from disease infestations and prevent the deficiencies caused by restricted dependence on pesticides. Stimulating Induced Systemic Resistance (ISR) in plants may be one course of action of PGPR through which they suppress not only fungi or bacteria but also viruses (Van Loon et al., 1998; Hőfte and Bakker, 2007), but another slightly more familiar course of action is the direct suppression or antagonism PGPR showcases against those microorganisms. This may be achieved by mere competition for nutrients in some cases, but in most cases, PGPRs additionally produce antifungal compounds, antibiotics, bacteriocins, cell wall degrading enzymes (chitinase, glucanases, proteases), volatile compounds, siderophores, or other lethal compounds in lower concentrations to inhibit growth and metabolism of other microbes (Beneduzi et al., 2012).

Numerous studies have already established PGPR as an important antifungal agent active against various widespread plant pathogens such as *Fusarium*, *Rhizoctonia solani*, *Phytophthora cinnamomi* and many more (de Boer et al., 2003; Fatima et al., 2009; Méndez-Bravo et al., 2018). *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* are well known bacterial genera of plant growth promoting rhizobacteria that work through producing antifungal metabolites (Gray and Smith, 2005). Various bacteria that are predominantly studied and increasingly marketed as biological control agents include the genera *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*

and *Agrobacterium* (Diallo et at., 2011; Köhl et al., 2019). Therefore, screening of rhizospheric microbial communities is seen as a new approach for searching eco-friendly active natural products for their antifungal activity.



Antifungal secondary metabolites of PGPR

Because of an environmentally friendly nature and having biocontrol and plant growth promotion capabilities, it is believed that PGPRs has the potential to replace or augment many of the environmentally hazardous chemicals that are currently used in agricultural practices (Ali et al., 2017). The PGPB use different mechanisms of action to promote plant growth.

Since fungal plant pathogens cause severe losses in crop production annually, management of fungal diseases using biological control has been the focus of intense research (Shobha and Kumudini, 2012). PGPR offers significant growth inhibition and improved defense against fungal phytopathogens such as *Macrophomina phaseolina*, *Rhizoctonia* spp, *Fusarium* sp., *Aspergillus*, *Pythium* sp., etc. (Srinivasan, 2017) by producing siderophores, antibiotics, hydrogen cyanide, volatile organic compounds (VOCs) and other secondary antifungal metabolites (Figure 1) (Hayat et al., 2010).

Siderophores

Siderophores are low molecular weight iron binding molecules with molecular weights ranging from 400-1500 Da containing side chains and functional groups that can provide a high-affinity set of ligands (Beneduzi et al., 2012). These biomolecules are amongst the strongest soluble Fe^{3+} binding agents known and are secreted by microorganisms in response to iron starvation since it can scavenge iron from the environment and make the essential iron mineral available to the microbial cell (Kannahi and Senbagam, 2014).

Siderophore producing PGPR are present in close vicinity to plant roots in the rhizospheric region or surface of the plant where they not only provide iron nutrition to the plant, but also serve as the first defense against root invading parasites (Sayyed et al., 2013). Siderophore producing bacteria play an important role in antagonism against phytopathogens as they can scavenge the necessary iron from the environment, leaving potential plant pathogens deprived of the mineral (Campbell et al., 1986). And this iron deficiency causes growth inhibition, a decrease in nucleic acid synthesis, inhibition of sporulation and changes in cell morphology of the pathogens (Pandya and Saraf, 2014). Generally, the fungi are mostly affected by this limitation of iron availability as they are unable to absorb the iron–siderophore complex (Goswami et al., 2016). Thus siderophores, as effective weapons released by a PGPR, are able to conquer the battle for iron acquisition (Balhara et al., 2016).

Bacteria usually produce four types of siderophores: hydroxamate, catecholate, salicylate and carboxylate (Kannahi and Senbagam, 2014). The potent siderophore pyoverdins, produced by *Pseudomonas* species containing both hydroxamate and catecholate functional groups, are known for their high affinity to the ferric ion among most of the bacterial siderophores studied (Beneduzi et al., 2012). It can inhibit the growth of bacteria and fungi that present less potent siderophores in iron-depleted media *in vitro.* Siderophore-producing bacteria are mostly members of the genus *Pseudomonas*; among them, the most studied organisms are *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, which release pyochelin and pyoverdine type of siderophores (Goswami et al., 2016). Some other important siderophore producing bacteria include *Escherichia coli*, *Azotobacter*, *Bacillus* sp., *Rhizobium radiobacter*, *Burkholderia*sp., *Pantoeaallii* and *Mycobacterium* species (Ferreira et al., 2019).

Antifungal antibiotics

The biocontrol abilities of PGPR strains essentially depend on the production of antifungal antibiotics. The mechanisms of action of antibiotics include: inhibition of pathogen cell wall synthesis, alteration or deformation of the cellular membrane structures, slowing down the formation of initiation complexes on the small subunit of the ribosome, thus inhibiting, ribosomal RNA formation, inhibition of protein biosynthesis, etc. (Beneduzi et al., 2012). Besides the direct inhibitory action, antibiotics have a vital role in the induced systemic resistance (ISR) mechanism in plants (Kenawy et al., 2019).

GPRs belonging to *Bacillus* and *Pseudomonas* species play an active role in suppress-ing pathogenic microorganisms producing antibiotics. Such PGPRs secrete these extracellular metabolites that are inhibitory to plant pathogens even at low concentrations (Goswami et al., 2016).

The commonly produced antibiotics by PGPR that act on biocontrol mechanisms are phenazine, 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, surfactin, iturin, fengycin, viscosinamide, kanosamine, zwittermicin-A, polymyxin, circulin, pantocin, subtilin, and subtilosin (Kenawy et al., 2019). The antifungal antibiotic phenazine, produced by *Pseudomonads*, possesses redox activity and can suppress fungal pathogens of plants such as *Fusarium oxysporum* and *Gaeumannomyces graminis* (Mazurier et al., 2009; Warren et al., 2016). An effective and extensively studied antibiotic DAPG produced by *Pseudomonas fluorescens* has been reported for the destruction of the fungal cell membrane of *F. oxysporum* sp. *Niveum* (Hua et al., 2020). Pyrrolnitrin, also produced by *Pseudomonads* can inhibit *Rhizoctonia solani* during damping-off of cotton plants (Howell and Stipanovic, 1980).

Antibiotics, such as polymyxin, circulin and colistin, produced by a majority of *Bacillus* sp. are active against both Gram-positive and Gram-negative bacteria, as well as many pathogenic fungi. *Bacillus* sp. also produce the antibiotic zwittermicin A and kanosamine, which contribute to the biocontrol of several soil fungi or fungus-like microorganisms (Beneduzi et al., 2012).

The antibiotics synthesized by PGPR are classified into volatile and nonvolatile

complexes. The volatile antibiotics include alcohols, aldehydes, ketones, sulfides, and hydrogen cyanide, and the non-volatile antibiotics are polyketides, cyclic lipopeptide amino polyols, phenylpyrrole, and heterocyclic nitrogenous compound. This group of non-volatile antibiotics has broad-spectrum action against several phytopathogens (Kenawy et al., 2019).

Various endogenous signals are responsible for regulating antibiotic synthesis, such as two component sensor kinases (TCS), N-acyl homoserine lactones, and sigma factors and the gene clusters associated with the biosynthesis of antibiotics are highly conserved (Kenawy et al., 2019).

Hydrogen cyanide (HCN)

Hydrogen cyanide (HCN) is a volatile secondary metabolite that is synthesized by many rhizobacteria and has a powerful antagonistic effect on many organisms. Its cyanide ion inhibits metalloenzymes, principally the copper containing mitochondrial cytochrome c oxidases, thus inhibiting the electron transport and disrupting the energy supply to the cell, which leads to the death of the living organisms. HCN production is mostly associated to Gram-negative *Pseudomonas* sp., but many bacterial genera also have the ability to produce HCN, including species of *Alcaligenes*, *Aeromonas*, *Bacillus* and *Rhizobium* (Abd El-Rahman et al., 2019). *P. putida* produces HCN to stop the infection by *F. solani* in alfalfa (Sarhan and Shehata, 2014) and *P. fuorescens* has been reported to control plant's black root disease caused by the fungi *Thielaviopsis basicola* (Kenneth et al., 2019) and *M. phaseolina* (Reetha et al., 2014). Both siderophores and HCN are known to be intricately linked to antifungal property of a PGPR (Bakthavatchalu et al., 2012). HCN and CO_2 are formed from a glycine precursor in an oxidative reaction catalyzed by HCN synthase (Laville et al., 1998). In *P. fluorescens*strain CHA0, the set of membrane bound *hcnABC* genes (hydrogen cyanide synthase) were found to be associated with the biosynthesis of HCN (Sayyed, 2019).

Pseudomonas chlororaphis strain PA23, a biocontrol agent capable of suppressing the pathogenic fungus *Sclerotinia sclerotiorum*, has been found to secrete the antibiotics pyrrolnitrin and phenazine, together with degradative enzymes and siderophores (Nandi et al., 2017). This strain also produces hydrogen cyanide. Analysis of an *hcn* mutant in this strain revealed decreased *in vitro* antifungal activity. HCN is commonly used as a biocontrol agent in the agriculture system on the basis of significant toxicity against phytopathogens. But one study found the lack of correlation of the level of HCN produced by rhizobacteria with their bio-control activity *in vitro*, suggesting that HCN does not act directly in the process of biocontrol (Michelsen and Stougaard, 2012). The proposed new role for HCN production by rhizospheric bacteria is; increasing phosphate availability by getting involved in the chelation of metals ions, which leads to an indirect increase in nutrients availability for the rhizobacteria and their plant hosts (Abd El-Rahman et al., 2019).

PGPR Volatile compounds and their anti-fungal potentials

The composition of the nutrient medium is important for plant growth and disease resistance capacity, but the composition of the gaseous atmosphere also plays an important role in the proper development of plants by stimulating growth and by preventing infection. This gaseous atmosphere involves different types of volatile organic compounds produced by the plants and their surrounding microorganisms (Fincheira and Quiroz, 2018). The PGPR is an exuberant source of a wide range of low molecular weight VOCs (Ryu et al., 2005). Despite being quite understudied than their non-volatile counterparts, the volatile organic compounds with their low molecular weight, high vapor pressure and low water solubility are also becoming an attractive target of research, especially in the field of agricultural expeditiously as their utility is being identified. They have been reported to be effective in plant growth promotion, controlling pathogens, post-harvest disease resistance, as biofumigators, and supposedly also named as 'green chemicals' (Kanchiswamy et al., 2015).

PGPRs produce VOCs that have antifungal potential, protect plants from harmful phytopathogens, and thus act as biopesticides. These antifungal VOCs inhibit mycelial growth, sporulation, germination of phytopathogenic fungi, or induce systemic resistance in a plant itself (Quintana-Rodriguez et al., 2015). As biopesticides, these VOCs are much more sustainable than traditional chemical pesticides, so they protect a plant and promote its growth in an environment friendly manner. These VOCs comprise several classes of bioactive chemicals, including alcohols, aldehydes, acids, esters, ketones, thiols, terpenes, etc., which are bioactive compounds and can act as biofumigants to control postharvest diseases (Junker and Tholl, 2013). A partial list of VOCs and their possible mode of antifungal mechanism produced by PGPR are shown in Table 1. These VOCs, facilitated by their physicochemical properties, can easily diffuse and evaporate through the gas filled and water pores in the rhizosphere and soil, act as effective semi chemicals and are crucial to the inter- and intra-kingdom interactions. So, VOCs are seen as a solution to increase agronomic yield and match the pace of demand without obstructing either the economy or the environment.

PGPR antifungal hydrolytic enzymes

PGPRs are an enormous source of hydrolytic enzymes responsible for the degradation of plant and microbial biomass. The application of these hydrolytic enzymes producing rhizospheric microbes is an eco-friendly solution to combat plant pathogens.

Hydrolytic enzymes like chitinase, glucanase, protease and cellulase are able to degrade the fungal cell-wall and cause the cell lysis of fungal pathogens (Figure 2) (Shaikh and Sayyed, 2015; Jadhav and Sayyed, 2016; Shaikh et al., 2016). Many of the microbial strains like *S. marcescens*, *B. subtilis*, *B. cereus*, *B. subtilius*, *B. thuringiensis*, etc. have the potential to produce hydrolytic enzymes for the biocontrol of phytopathogens like *R. solani*, *F. oxysporum*, *S. rolfsii*, *P. ultimum* etc. (Felse and Panda, 2000; Someya et al., 2000; Mabood et al., 2014).

Table 1. List of some antifungal volatile producing PGPR.

PGPR	Fungi	Antifungal VOCs	Mode of action	Reference
Bacillus velezensis ZSY-1	*Alternaria solani* and *Botrytis cinerea*	Phenol-2,4-bis (1,1-dimethylethyl); Phenol (4-chloro-3-methyl); Pyrazine (2,5-dimethyl); benzothiazole	Inhibit the growth of fungi.	Gao et al., 2017
Arthrobacteragilis UMCV2	*Botrytis cinerea*	Dimethyl hexadecylamine (DMHDA)	Mycelial growth inhibition	Velàzquez Becerra et al., 2013
Bacillus amyloliquefaciens	*Fusarium oxysporum* f. sp. *niveum*	2-nonanone and 2-heptanone	мycelial growth inhibition	Wu et at., 2019
Bacillus spp. S18	*Fusarium solani*	Ethyl etradecanoate; ethyl dodecanoate; 2,10-bornanediol; pentadeca-5,10-diyn-1-ol; 2,2-dime-thylheptane; 1-hexanol; and phenacyl formate	Mycelial growth inhibition	Gutièrrez-Santa Ana et al., 2020
Pseudomonas fluorescens, *Pseudomonas corrugata*	*Sclerotinia slerotiorum*	2-ethyl 1-hexanol	Germination inhibition and mycelial growth inhibition	Fernando et al., 2015
Pseudomonas stutzeri E25 and Stenotrophomons maltophilia CR71	*Botrytis cinerea*	Dimethyl disulphide (DMDS)	Mycelial growth inhibition and induced systemic resistance	Rojas-Solis et al., 2015
Bacillus subtilis DZSY21	*Curvularia lunata*	2-Methylbutyric acid; 2-heptanone and isopentyl acetate	Mycelial growth inhibition	Xie et al., 2020
Bacillus megaterium BP17	*Phytophthora capsici, Pythium myriotylum, Atheliarolfsii, Gibberella moniliformi, Colletotrichum gloeosporioides, Rhizoctonia solani, Magnaporthe oryzae, Ralstonia solanacearum*	Pyrazine, 2-ethyl-3-methyl; Pyrazine, 2,5-dimethyl; Pyrazine, 2-ethyl and Pyrazine, 2-methyl.	Mycelial growth inhibition	Munjal et al., 2016

PGPR	Fungi	Antifungal VOCs	Mode of action	Refernece
Pseudomonas sp. ST–TJ4	Botryosphaeria berengeriana, Cytospora chrysosperma, Fusicoccus aesculi, Phomopsisri cinella, Phytophthora cinnamomi, Rhizoctonia solani, Sphaeropsis sapinea	Phenazine, 1-undecene; methyl siloxane; octamethylcyclotetra siloxane	Mycelial growth inhibitionand spore germination inhibition	Kong et al., 2020
Staphylococcus sciuri strain MarR44	*Colletotrichum nymphaeae*	Mesityl oxide; 2-methylpropyl ester; 4-Methyldecane; 4-Penten-2-one,4-methyl; toluene and o-Xylene	Mycelial growth inhibition and conidial germination inhibition	Alijani et al., 2020
Pseudomonas aureofaciens	*Pythium ultimum, Fusarium solani, Fusarium oxysporum,* and *Thielaviopsis basicola*	3-(1-Hexenyl)--5 methyl-5)-2H)-furanone	spore germination inhibition	Paulitz et at., 2000
Bacillus atrophaeus HAB-5	*Colletotrichum gloeosporioides*	Chloroacetic acid; tetradecyl esters; octadecane and hexadecanoic acid methyl ester	Mycelial growth inhibition	Rajaofera et al., 2019
Bacillus acidiceler	*Phytophthora cinnamomi*	2,3,5-trimethylpyrazine, 6,10-dimethyl-5,9-undecadien-2-one and 3-amino-1,3-oxazolidin-2-one	Mycelial growth inhibition	Méndez-Bravo et al., 2018

The cell wall of most of the fungi is made up of chitin, which is an unbranched, long-chain polymer of glucose derivatives composed of β-1,4-linked units of the amino sugar N-acetyl-D-glucosamine (NAG). Hydrolytic enzyme chitinases are capable of breaking down glycosidic bonds in chitin (Jadhav et al., 2017). A number of researchers have demonstrated the inhibition of fungal growth by chitinases of *Streptomycetes* (Skujins et al., 1965; Schlumbaum et al., 1986; Fridlender et al., 1993). The importance of chitinase activity was further demonstrated by the loss of biocontrol efficacy in *Serratia marcescens* chitinase mutants in which the *chiA*gene had been inactivated (Jones et al., 1986). Introduction of an chitinase gene from *S. marcescens* in *Pseudomonas* and the plant symbiont *Rhizobium meliloti* could successfully control the pathogens *F. oxysporum* var. *redolens* and *Gaeumannomyces graminis* var. *tritici* (Sundheim, 1992).

Different types of proteases produced by PGPR play significant role in cell wall lysis of phytopathogenic fungi. Several *Bacillus* species from the rhizosphere like *Bacilluscereus*, *Bacillus stearothermophilus*, *Bacillus mojavensis*, *Bacillus megaterium*, and *Bacillus subtilis* are known to produce protease (Sookkheo et al., 2000; Beg and Gupta, 2003).

Cellulases are complex enzymes consisting of a mixture of endo-1,4-β-glucanase and exo-1,4-β-glucanase. The enzyme catalyzes the hydrolysis of 1,4-β-D-glycosidic linkages in cellulose and plays a significant role in nature by recycling this polysaccharide. (Fridlender et al.1993) reported the hydrolytic inhibition of *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium ultimum by* β-1,3-glucanases of *Bacillus cepacia* (Fridlender et al., 1993). Singh et al., (1999) reported two strains of *Paenibacillus* and *Streptomyces* sp. which produce β-1,3-glucanases that inhibitthe growth of *F. oxysporum* (Singh et al., 1999). Vazquez-Garciduenas et al., (1998) reported the presence of seven β-1,3-glucanases produced by the strain, *T. harzianum*under diverse growth conditions (Vàzquez-Garcidueñas et al., 1998).

Genome mining for determining the biocontrol capacity of PGPBs

Until recently, traditional approaches were used to identify of biosynthetic pathways of the PGPRs by first extracting the bioactive compounds with desired properties and subsequently locating the responsible genes by biochemical techniques. However, scientists soon noticed that SMs are usually encoded by genes that group together in a genetic locus, which came to be known as a biosynthetic gene cluster (BGC) (Kjaerbolling et al., 2019). A BGC consists of genes required for the synthesis of the bioactive molecules and regulatory elements, such as transcription factors and promoters. Sometimes, it also encodes for transportation genes for exportation of the produced SMs and resistance genes that prevent self-destruction in the producers. The availability of a large number of whole-genome sequence data of rhizospheric organisms has led to the mining of the same. Advancements in omics technologies and bioinformatic tools have been conducive to a

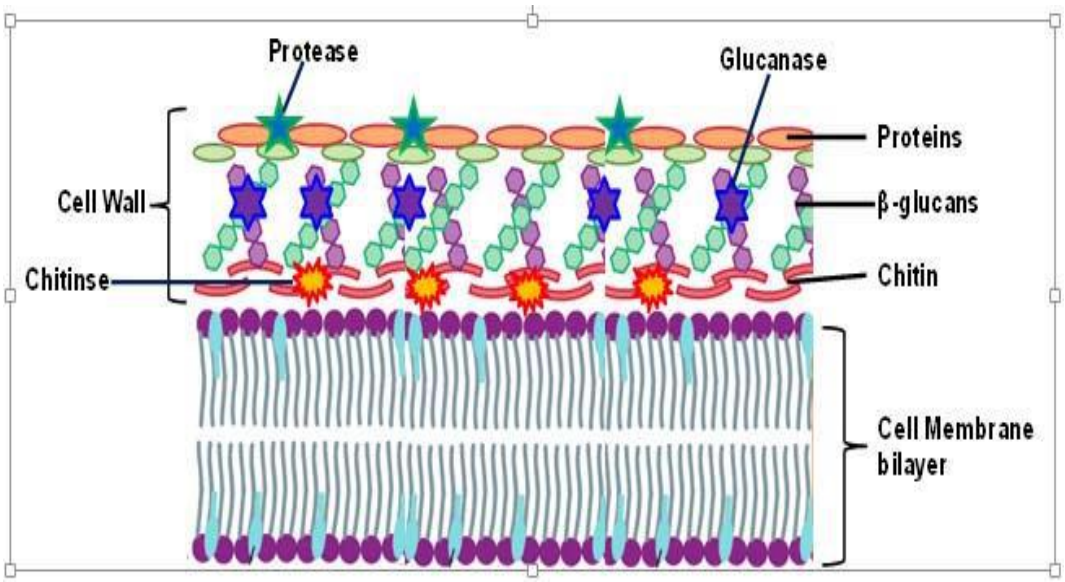


Fig. 2. Fungal cell wall and membrane composition. Hydrolytic enzymes target cell wall components.

paradigm shift from traditional culturing and screening methods to bioinformatic tools and genomics to uncover BGCs that were previously unidentified or transcriptionally silent (Chen et al., 2019). Functional annotation of the existing data has allowed the identification of genes related to biocontrol traits such as secondary metabolite and siderophore biosynthesis, volatiles and detoxification, hydrolytic enzymes having antifungal activity. A very recent study has combined genome analysis data with structural identification technologies using ultra-high-performance liquid chromatography coupled to quadruple time-of-flight tandem mass spectrometry (UHPLC-MS/MS) (Su et al., 2020). It was used to interpret the chemical origins of metabolites with significant biological activities. This study led to the discovery of a number of gene clusters encoding antimicrobial compounds in the genome of strain *Bacillus subtilis* NCD-2, including that of fengycin, a strong antifungal compound. Similarly, an *in-silico* approach has identified 25 putative SMs in the genome of *Verticillium dahliae* strain JR2 (Shi-Kunne et al., 2019). Two putative siderophores, ferricrocin and triacetylfurasinine C (TAFC), 1-8 Dihydroxynapthalene (DHN)-melanin and fujikurin compounds have been predicted to be members of the secondary metabolite arsenal of *V. dahlia*e.

Biocontrol formulations

To ensure the ready availability of biocontrol agents, it is necessary to have techniques for mass production of the same. It is necessary to have formulation protocols standardized so that the shelf life of the formulation is within the desired limits. It is only under such conditions the industries would be prepared to

get involved in the commercial production of plant growth promoting rhizobacteria (PGPR). As mentioned earlier, PGPR with wide scope for commercialization includes *Pseudomonas fluorescens, P. putida, P. aeruginosa, Bacillus subtilis* and other *Bacillus* spp. The potential PGPR isolates are formulated using different organic and inorganic carriers, either through solid or liquid fermentation technologies. The delivery route is through the treatment of the seeds, bio-priming, seedling dip, soil application, foliar spray, fruit spray, hive insert, sucker treatment and sett treatment. The dry weight of healthy pods and the total number of pods were found to increase significantly for peanuts when formulated *B. subtilis* B4 was used under greenhouse and open field conditions, compared to the controls, which showed no significant differences (Ahmad et al., 2019). Even the use of a commercial fungicide did not perform as better as the formulated PGPR.

Application of PGPR formulations with strain mixtures performs better than individual strains for the management of pests and diseases of crop plants, in addition to plant growth promotion. Supplementation of chitin in the formulation effectively increases the efficacy of the antagonists (Manjula and Podile, 2001). In North America, about 33 products have been registered to be used commercially either in the greenhouse or in the fields (Reddy, 2014). However, the safety of certain PGPR has to be ensured since their ability to infect human beings as opportunistic pathogens pose a great threat.

Concluding remarks

Nature is bestowed with enriched biodiversity of PGPR. The potential of rhizobacteria in crop protection by producing different defensive antifungal metabolites like antibiotics, hydrolytic enzymes, and other metabolites is expected to provide sustainable and eco-friendly plant disease control alternatives. In order to combat fungal diseases, the application of these rhizobacteria in the agricultural fields in the form of formulated products will give a greener and eco-friendly approach to sustainable agriculture. In agriculture fields, single or a consortium of these organisms has shown promising prospects in the biocontrol and plant growth promotion.

Despite significant progress made in different areas, the interaction between PGPRs and plant research is still in infancy. The interaction effect of antibiotics, hydrolytic enzymes, hydrogen cyanide, and active oxygen species involved in the induction of systemic resistance in the plant has to be explored extensively. Moreover, molecular engineering of these microbes that would enhance the efficiency of these organisms should also be focused deeply.

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