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Review Article

Antimicrobials compounds from extreme environment Rhizosphere organisms for plant growth

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A B S T R A C T

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Different rhizospheric bacteria from extreme environment shows different antimicrobial activity and this activity lead to the different antimicrobial compounds which help in plant growth. This paper shows the different antimicrobial compounds from extreme environments. This compound from rhizospheric bacteria (e.g. *Bacillus subtilis*) which helps in plant growth.

Introduction

Rhizosphere microorganisms are a heterogenous group of bacteria, including plant growth promoting bacteria, which can improve extent or quality of plant growth direct and indirect. Generally large array of bacteria, including species of Pseudomonas, *Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been reported to enhance plant growth (Ahmad et al., 2008; Barrie et al., 2008; Baudoin et al., 2010). PGP bacteria can exhibit a variety of characteristics responsible for influencing plant growth. The exact mechanisms by which PGP bacteria promote plant growth, are not fully understood, but are thought to include: (i) the ability to produce or change the concentration of plant growth regulators like IAA, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick 1995); (ii) N2 fixation (Boddey and Dobereiner, 1995); (iii) antagonism against pathophytogenic micro-organisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan et al., 1992) and cyanide (Flaishman et al., 1996) and (iv) solubilization of mineral phosphates and other nutrients (for 1990; De Freitas et al., 1997).

The rhizosphere microorganisms involve the biofertilizers can able to colonize internal tissues of plants, promoting the growth of plant through increase supply or availability of nutrients, such as nitrogen and phosphorus. (Richardson et al., 2009). Bacteria with capacity to release phosphate from insoluble P forms by organic acid production and enzymatic activity (I. e. phytase), also known as phosphobacteria, are commercialized to improve growth, yield and quality of crops as well as for biotechnological applications in other fields,

such as animal and human nutrition, aquaculture and environmental protection (Jorquera et al., 2008). In this paper concentrate on antimicrobial compound isolated from extreme environments, rhizosphere microorganism on plant disease. Among the rhizoshpere microorganisms involved in plant interaction with plant disease management from antimicrobial compounds of rhizospheric microorganisms, deserves special attention in plant growth promotion. In general, plant associated rhizobacteria migrate from extreme environmental soil to the rhizosphere of living plant and aggressively colonized the rhizosphere and roots of plants. These so-called rhizobacteria, as abundant symbiotic partners of plants, are considered plant growth promoting rhizobacteria (Kapulnik 1991).

Plant microbe interactions in extreme rhizosphere

Rhizosphere interactions are based on complex exchanges that evolve around plant roots. Plant microbe interaction in soil release water soluble compounds such as amino acids, sugars and organic acids that supply food for microorganisms and in return microorganisms provides nutrients for the plants. These all activity can make the rhizosphere the most dynamic environment in soil.

Microorganisms which do interact with specific plants. These interactions can be pathogenic, symbiotic, harmful, saprophytic or neutral. And interactions, which beneficial to plant include mycorrhizae, legume nodulation and production of antimicrobial compounds that inhibit the growth of pathogens. Rhizosphere microorganisms produce vitamins, antibiotics, plant hormones and communication molecules that all encourage plant growth. Microbial population in rhizosphere may benefit the plant in a variety of ways, including increased recycling and solubilization of mineral nutrients, synthesis of vitamins, amino acids, auxins, cytokinins and gibberellins which stimulate plant growth and antagonism with potential plant pathogens through competition and development of amensal relationships based on production of antibiotics. The action and interaction of some growth regulators like auxins regulate most of the physiological activities and growth in plants. Naturally occurring substances with an indole nucleus possessing growth-promoting activity is referred to as auxins. Chemically, it is Indole acetic acid.

The ability to synthesize fisherman is widely distributed among plant associated bacteria. 80% of the bacteria isolated from plant rhizosphere are to produce IAA. According to Halda-Alija, up to 74% of rhizobacteria identified and tested to produce IAA.Not only plants, but also microorganisms can synthesize auxins and cytokinins. The role of phytohormone biosynthesis of microorganisms is not fully elucidated. But it was indicated that there might exist a symbiotic association between plants and microorganisms. For plant growth apart from nitrogen, phosphorus is one of the most important nutrients. Phosphorus contributes to the biomass construction if micronutrient, the metabolic process of energy transfer, signal transduction, macromolecular bio-synthesis, photosynthesis, and respiration chain reaction (Shenoy and Kalagudi, 2005). In the rhizosphere bacteria continuously metabolize various organic compounds from root exudates. Therefore, their activities result in quantitative and qualitative alterations of the released root exudates. Bacteria in the rhizosphere can significantly influence the nutrient supply of plants by

competing for mineral nutrients and by mediating the turnover and mineralization of organic compounds. Therefore, bacteria in the rhizosphere can be a leading control of the turnover of nutrients in the soil (Robinson et al., 1989). Rhizosphere bacteria can influence plant growth also directly by releasing a variety of compounds, e.g., phytohormones or antimicrobial compounds (Perotto and Bonfante, 1997). Diversity of bacteria is affected by the plant age, the season and the soil conditions (Hrynkiewicz et al., 2010a). Rhizosphere bacteria can have a negative, neutral, or positive effects on plant fitness.

Detrimental microbes include both major plant pathogens and minor parasitic and non-parasitic deleterious rhizosphere bacteria (Barea et al., 2005). However, the effect of rhizosphere bacteria depends mostly on the genotype of the microorganisms and plants involved as well as on the environmental conditions (Brimecombe et al., 2007). *Pseudomonas* spp. and *Bacillus* spp. belong to the largest groups of rhizosphere bacteria (Brimecombe et al., 2007). PGPR are usually in contact with the root surface, and improve the growth of plants by several mechanisms, e.g., enhanced mineral nutrition, phytohormone production, disease suppression (Tarkka et al., 2008). Two groups of PGPR were described: one group is involved in the nutrient cycling and the plant growth stimulation (biofertilizers) (Vessey 2003) and the second group is involved in the biological control of plant pathogens (biopesticides) (Whipps 2001).

Bacteria may support the plant growth by several mechanisms, e.g.,: increasing the ability of nutrients in the rhizosphere (i), inducing root growth and thereby increase of the root surface area (ii), enhancing other beneficial symbioses of the host (iii) and by a combination of modes of action (Vessey 2003).

PGPR can increase the availability of nutrients, e.g., by enzymatic nutrient mobilization from organic matter and production of siderophores (Anderson et al., 1993; Whiting et al., 2001; Jing et al., 2007). Bacteria producing extracellular degrading enzymes are major decomposers of organic matter. They contribute essentially to the soil aggregation and nutrient availability (Johansen and Binnerup, 2002). In soils with low phosphate, bacteria can release phosphate ions from low-soluble mineral P crystals and from organic phosphate sources. These bacteria exude organic acids that dissolve the P crystals and exude enzymes that split organophosphate (Vessey 2003; Tarkka et al., 2008). Some rhizosphere bacteria also produce siderophores which can be absorbed as the bacterial Fe3+-siderophore complex by a number of plant species in the deficiency of iron (Vessey 2003). Microbial siderophores in the rhizosphere can significantly contribute to the biocontrol of soil-borne pathogens due to their competitive effects (Hiifte et al., 1994). PGPR can also promote the root growth. This can be caused by the ability of most rhizobacteria to produce phytohormones, e.g. indole-3-acetic acid (IAA), cytokinins, gibberellins, ethylene which promotes cell division and cell enlargement, extension of plant tissue and/or other morphological changes of roots (Salisbury 1994).

Effect of Rhizosphere Microorganisms on the Plant Growth

The rhizosphere is a hot spot of microbial interactions as exudates released by plant roots are a main food source for microorganisms and a driving force of their population density and activities. The rhizosphere organisms harbor many

organisms that have a neutral effect on the plant, but also attract organisms that exert deleterious or beneficial effects on the plant. Microorganisms that adversely affect plant growth and health are the pathogenic fungi, oomycetes, bacteria and nematodes.

Rhizosphere microorganism community have a neutral effect on plants, but are part of a complex food web that utilizes the large amount of carbon that is fixed by plants. These communities in rhizosphere members that exert deleterious or beneficial effects on the plant. Extreme microorganisms that adverse effects on the plant growth and health are the pathogenic fungi, oomycets, bacteria and nematodes, whereas microorganisms that are beneficial include nitrogen-fixing bacteria, endo- and ectomycorrhizal fungi, and plant growth promoting rhizobacteria.

Bacteria, fungi, nematodes, oomycetes these four are the main groups of plant pathogens (Agrios 2005), but true fungi, and oomycetes the major players in the soil. Only few rhizobacteria are considered to be soilborne, probably because non spore forming bacteria cannot survive well in soil for long periods. Bacteria also require a wound or natural opening to penetrate into the plant and cause infection. Examples are *Ralstonia solanacearum*, cause of bacterial wilt of tomato (Genin and Boucher, 2004), and Agrobacterium tumefaciens, the well studied causal agent of crown gall (Nester et al., 2005). Some filamentous bacteria (Streptomyces) can also infect plants and are better adapted to survive in the soil.

Soils can be disturbed by a wide range of factors concerning unfavorable agricultural management or industrial activities. Fertility of various soils observed in the last decades decreased at an alarming rate due to the loss of organic matter as a result of erosion, oxidation, compaction, biological impoverishment as well as a wide range of pollutants. Plant growth affects the physical (e.g., formation of new soil pores) and chemical (e.g., formation of soil organic matter) quality of soils this is why plant growth at disturbed sites can increase soil fertility substantially. However, in many cases unfavorable conditions in disturbed soils may cause a lack of any vegetation or a diminished vegetation development. Rhizosphere microorganisms are especially critical for plant colonization of unfavorable soils, since they can alleviate biotic and abiotic stress of plants.

Nutrient- and Water-Deficiency

The world population is expanding rapidly and will likely be 10 billion by the year 2050 (Cakmak 2002). The expected increases in world population will result in a serious pressure on the existing agricultural land via intensification of crop production. The projected increase in food production must be accomplished on the existing cultivated areas because the expansion of new land is limited due to environmental concerns, urbanization and increasing water scarcity. At the same time, soil productivity is decreasing globally due to enhanced soil degradation in the form of erosion, nutrient depletion, water scarcity, acidity, salinisation, depletion of organic matter and poor drainage (Cakmak 2002). Nearly 40% of the agricultural land of the world has been affected by soil degradation (e.g., 25% of European, 38% of Asian, 65% of African, 74% of Central American agricultural land) (Scherr 1999). Agricultural production must be increased in the existing land, and therefore crop production must be intensified per unit of agricultural land. Mineral nutrients are the major contributor to enhancing crop production, and in maintaining soil productivity and preventing

soil degradation. Generally, improving the nutritional status of plants by maintaining soil fertility is the critical step in the doubling of food production of the world. Impaired soil fertility by continuous cropping with low supply of mineral nutrients is considered a major risk for food production and ecosystem viability (Pinstrup-Andersen et al., 1999; Tillman 1999).

Reduced soil fertility and crop production results in an increased pressure to bring more land into crop production at the expense of forests and marginal lands. Such areas are generally poor in fertility and sensitivity to rapid degradation when cultivated (Cakmak 2002). A group of especially promising rhizosphere organisms of applications on nutrient-deficient disturbed soils are mycorrhizal fungi. They can beatifically colonize the root cortex and develop an extramatrical mycelium which helps the plant to acquire mineral nutrients and water from the soil. They play a key role in nutrient cycling in ecosystems and their external mycelium, in association with other soil organisms, from water-stable aggregates necessary for a good soil quality (Azcón-Aguilar and Barea, 1997). Moreover, it was demonstrated that arbuscular mycorrhizal fungi produce glomalin-a glycoprotein, which has been suggested to contribute to the hydrophobicity of soil particles and participate in the initiation of soil aggregates (Barea et al., 2002).

Extreme pH

The soil pH is correlated with various biological and other chemical soil properties. About 40% of cultivated soils globally have acidity problems leading to significant decreases in crop production despite the adequate supply of mineral nutrients such as N, P and K (Herrera-Estrella 1999; von Uexküll and Mutuert, 1995). In acid soils major constraints to plant growth are toxicities of hydrogen (H), aluminium (Al) and manganese (Mn) and deficiencies of P, calcium (CA) and magnesium (Mg). Among these constraints Al toxicity is the most important yield-limiting factor (Marschner 1991). Availability of P to plant roots is limited both in acidic and alkaline soils, mainly, due to formation of sparingly soluble phosphate compounds with Al and Fe in acidic and Ca in alkaline soils (Marschner 1995). Plant species have evolved adaptive mechanisms to improve their ability to cope with soils having low levels of available P by the formation of mycorrhizal association (Marschner 1995; Dodd 2000).

Organic pollutant

Organic pollutants can be degraded by plants through biochemical reactions taking place within the plants and in the rhizosphere. The remediation of soils containing diverse organic pollutants, including organic solvents, pesticides, explosives and petroleum is possible with the use of plants and their rhizosphere processes (phytodegradation) (Mirsal 2004). Phytodegradation of organic pollutants may be enhanced by bacterial activities. In this process, plants interact with soil microorganisms by providing nutrients in the rhizosphere which leads to an increased microbial activity and degradation of toxic pollutants (Mirsal 2004).

Antimicrobial compounds of extreme environmental bacteria

Most bacteria produce antimicrobial compounds such as broad spectrum classical antibiotics, metabolic products viz. Organic acids and lytic agents such as lysozyme. The gram positive bacterium *Bacillus subtilis*

produces a large number of antibiotics, which are classified as ribosomal or non-ribosomal. The non-ribosomal antibiotics may play a role in competition with other microorganisms during spore germination.
The high proportion of antimicrobial compounds producing strains may be associated with ecological role, playing a defensive action to strains into an established microbial community. The phospholipid antibiotic produced by *Bacillus subtilis* has the broadest spectrum activity against gram positive and gram negative bacteria. This study is taken with the objective of isolating of alkaliphilic *Bacillus subtilis* from the soil and to assess the antimicrobial effect of a phospholipid compound produced and activity was tested against test organisms (*E. Coli, Pseudomonas aeruginosa, Candida tropicalis, Staphylococcus aureus*).

Production of Phospholipid antimicrobial Compound

This method was followed for the production of phospholipid compound. *Bacillus subtilis* was grown in NG medium (containing 10 gm Nutrient broth; 10gm Glucose; 2 gm Sodium chloride; 5 mg of CuSO4.5H2O; 7.5 mg of FeSO4.7H2O; 3.6 gm MnSO4.5H2O; 15 mg of CaCl2.2H2O; and 9 mg of ZnSO4.7H2O; (per liter) adjusted to pH 10 with Sodium hydroxide supplemented with 50 µg for tryptophan per ml at 300C for 24 hours. 10% of this inoculum were reinoculated in the fresh NG medium and incubated under shaking at30°C for 72 hours. Then this production medium was centrifugation at 10000rpm for 10 minutes, Cells were collected. This cellular content was extracted three times using 10ml of 50% n- Butanol each time, then aqueous layer was collected & evaporated to concentrate at room temperature. Resulting crude extract was resuspended in 4ml of Methanol; this crude sample was again acetate further purification was carried out using the method of Bligh and Dyer.

Bioassay of Phospholipid antimicrobial

The crude extract of antimicrobial compound with ethyl acetate was used for bonsai. The 24 hours old cultures of test organisms, *Staphylococcus aureus, E. coli, Candida tropicalis, Pseudomonas aeruginosa* were seeded in sterile Muller-Hinton (MH) agar and seeded MH agar plates were prepared. Then wells were made on MH agar. The wells were filled with 100µl of crude extract of phospholipid compound. The plates were kept in a refrigerator for the diffusion of the compound. The plates were then incubated at 35±0.50C for 24 hours and then the diameter of the zone of inhibition was noted.
Extremophiles organisms include the *Bacillus* genus made up of gram-positive aerobic or facultative endospore-forming rod shaped bacteria. Extremophiles includes in *Bacillus* genus are metabolically chemoorganotrophs being dependent on organic compounds as a source of carbon and energy.

Among bio-preservatives, more than 500 antimicrobial compounds have been described so far. *Bacillus* genus has been reported to produce more than 45 antimicrobial molecules; some of these compounds are of clinical value, others are Assayed *in vitro* to control food microbes and the remaining one's control plant diseases. According to their biosynthetic pathway, these metabolites can be grouped into two different classes: the first class comprises ribosomally synthesized peptides, including bacteriocins whereas the second class comprises small microbial peptides synthesized enzymatically by non-ribosomal pathways.

Mechanism of Indole 3-acetic acid

Indole-3-acetic acid (IAA) L-tryptophan, an amino acid, serves as a physiological precursor for biosynthesis of auxins in higher plants and in microbes (Frankenberger &Arshad, 1995). Indole-acetic acid is known to stimulate both a rapid response (e.g. Increased cell elongation) and a long-term response (e.g. Cell division and differentiation) in plants (Cleland, 1990). More specifically, IAA is a fisherman that is known to be involved in root initiation, cell division and cell enlargement (Salisbury, 1994). A significant activity of PGPR is the production of auxin-type phytohormones that affect root morphology and thereby improve nutrient uptake from the soil (Barea et al., 2005). (Lucy et al., 2004) have shown that IAA-producing PGPR increase root growth and root length, resulting in a greater root surface area, which enables the plant to access more nutrients from the soil.

The capacity to synthesize IAA is widespread among soil- and plant associated bacteria. By and large, microorganisms isolated from the rhizosphere and rhizoplane of various crops are more active in producing auxins than those from root free soil because of the rich supplies of substrates exuded from roots compared with non-rhizosphere soil (Strzelczyk & Pokojska-Burdzeij, 1984). A 3-fold higher IAA content was found in the rhizosphere compared with nonrhizosphere environments (Narayanaswami & Veerraju, 1969). It has been estimated that 80% of bacteria isolated from the rhizosphere can produce IAA (Patten & Glick, 1996; Ahmad et al., 2006). Similarly, over 66% of wild Arabica coffee-associated rhizobacteria secreted IAA. A survey of the IAA biosynthesis pathways utilized by plant-associated bacteria reveal that pathogenic bacteria such as Pseudomonas syringae, Agrobacterium tumefaciens and Erwinia herbicola synthesize IAA predominantly via the indole-3-acetamide (IAM) pathway. Synthesis by this route is generally constitutive. PGPR such as Rhizobium, Bradyrhizobium and Azospirillum species synthesize IAA, mainly via the indole-3-pyruvic acid (IPyA) pathway, which may be subject to more stringent regulation by plant metabolites (Patten & Glick, 1996). Other rhizobacteria may produce cytokinins (Timmusk et al., 1999) and gibberellins (Khan et al., 2006).

Siderophore production

Living organisms require iron as a component of proteins involved in important life processes such as respiration, photosynthesis and nitrogen fixation. Iron is one of the major elements in the earth's crust, but soil organisms such as plants and microbes have difficulty in obtaining sufficient iron to support their growth because of formation under aerobic conditions of ferric oxides, which cannot be readily transported into cells. Under such iron starvation, bacteria, fungi and plants secrete small, specialized, efficient iron (III) chelator molecules commonly known as siderophores (Drechsel & Jung 1998). After the iron-siderophore complexes have formed, these now soluble complexes are internalized via active transport into the cells by specific membrane receptors (Glick et al., 1999). Following either cleavage or reduction to the ferrous state, the iron is released from the siderophore and used by a cell (Glick et al., 1999).

Many bacteria are capable of producing more than one type of siderophore or have more than one iron-uptake system to take up multiple siderophores (Neilands, 1981). A considerable number of wild Arabica coffee-

associated rhizobacteria (67%) produce siderophores. Wide arrays of beneficial plant-associated bacterial genera, e.g. Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum and Rhizobium secrete various types of siderophores (Glick et al., 1999; Loper & Henkels 1999). Siderophores function mainly in the solubilization, transport and storage of iron (Stephan et al., 1993). Some other important mechanisms by which siderophore producing bacteria contribute to the promotion of plant growth.

Hydrogen cyanide production

In general, cyanide is formed during the early stationary growth phase (Knowles & Bunch, 1986). Cyanide occurs in solution as free cyanide, which includes the cyanide anion (CN-) and the non-dissociated HCN. It does not take part in growth, energy storage or primary metabolism, but is generally considered to be a secondary metabolite that has an ecological role and confers a selective advantage on the producer strains (Vining, 1990). Cyanide is a phytotoxic agent capable of inhibiting enzymes involved in major metabolic processes and is considered one of the typical features of deleterious rhizobacterial isolates (Bakker & Schippers, 1987). Nevertheless, at present its applications in areas of biocontrol methods are increasing (Voisard et al., 1989; Devi et al., 2007). Cyanogenesis in bacteria accounts in part for the biocontrol capacity of the strains that suppress fungal diseases of some economically important plants (Voisard et al., 1989). For instance, for many pseudomonads, production of metabolites such as hydrogen cyanide (HCN) is the primary mechanism in the suppression of root fungal pathogens. Cyanogenic bacterial species havealso been found to be effective in killing the subterranean termite Odontotermes obesus, an important pest of major agricultural crops and forest plantation trees, under in vitro conditions (Devi et al., 2007), in addition to suppression of plant parasitic nematodes (Siddiqui et al., 2006). Hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However, producer microbes, mainly pseudomonads, are reported to be resistant (Bashan & de-Bashan, 2005).

Antibiotic activity

The extreme environments have been de fined as habitats that experience steady or fluctuating exposure to one or more environmental factors, such as salinity, osmolality, desiccation, UV radiation, barometric pressure, pH, temperature, nutrient limitation, and trophic dependence on surface environments (Boston et al., 2001 ; Seufferheld et al., 2008) . Despite being relatively protected from factors that, in surface environments, have negative impacts on microbial life (seasonal climate variations, extreme weather, desiccation, predation by other organisms and UV radiation) (Leveillé and Datta, 2010) caves are regarded as extreme habitats due to the absence of light and nutrient limitation. Extreme environments are deemed one of the most promising sources of useful compounds. Several studies have focused on screening secondary metabolites produced by microorganisms that inhabit such environments as potential sources of useful compounds: extremozymes (Singh et al., 2011), exopolysaccharides (Nicolaus et al., 2010), biosurfactants (Banat et al. 2010), antitumorals (Chang et al., 2011), radiation-protective drugs (Singh and Gabani 2011), antibiotics, immunosuppressants, and statins (Harvey 2000).

Table.1 Screening bacterial isolates for phospholipid compounds**:**

Bacterial isolates	**Antimicrobial activity of phospholipid compounds**			
	Staphylococcus aureus	*E.coli*	*Psedomonas aeruginosa*	*Candida tropicalis*
BS1	++	++	++	++
BS2	++	++	++	++
BS4	-	-	-	-
BS7	++	++	++	++
BS8	++	++	++	++

A term has recently been coined to designate the range of biologically active, low-molecular mass compounds that are produced by bacteria, yeast, plants, and other organisms—the parvome (Davies 2009) . Only a few of these molecules have already been isolated, identified, and used as therapeutic agents (Allen et al., 2010) . Antibiotics are a group of anti-infective therapeutic agents, derived from microbial sources that are used to treat bacterial infections. Antibiotics were first defined by Waksman (1945), as "chemical substances of microbial origin that possess antibiotic powers." More recent definitions do not include microbial origin, but are similar to Waksman's in that they are essentially anthropocentric, describing only a specific function (antimicrobial activity) of interest to mankind, not the chemical nature of the molecules that display it, nor their role in natural microbial populations.

However, the fact that a particular metabolite behaves as an antimicrobial in the laboratory does not mean it exclusively plays the role of a "chemical weapon" in nature. This antagonistic role has been proven only in certain instances (Davies 2009). Antibiotics have a number of other effects on bacterial physiology, such as affecting the ability to swarm, the capacity to form biofilms, or inducing bacterial lysogens to enter the lytic cycle. Research carried out at the University of British Columbia, Canada, has suggested that the so-called antibiotics act as intercellular signaling agents (Davies 2009).

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