Int.J.Curr.Microbiol.App.Sci (2017) *6*(6): 33-41

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 6 (2017) pp. 33-41**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2017.606.003

Inside the Plants: Bacterial Endophytes and their Natural Products

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A B S T R A C T

Now a day's world population is facing several health problems caused by bacteria, virus, fungus, protozoan and other micro-organisms which could be due to drug resistant or parasitism and generates an alarming situation to world population for their survival. The requirement for novel and beneficial compounds like antibiotics, chemotherapeutic agents and agrochemicals to offer assistance in several aspects of the human life is ever increasing that are very effective and having negligible or very low toxicity to the environment. Since, production of these compounds is limited *in vivo* and negatively affect the biodiversity and hence, microorganisms may be utilized as a vital source for the production of these bioactive compounds in environmental friendly manner. Endophytic bacteria are the microbes among them, which devote its whole or part of life inside the plant without any negative symptoms to its host. They are reported to produce several bioactive compounds which could be utilized in medical, industries and moreover, in agriculture sector to fulfill the requirement of human's future need.

Keywords

Antibiotic, Anti-fungal, Bioactive compounds, Bacterial endophytes, Natural products.

Article Info

Accepted:
04 May 2017
Available Online:
10 June 2017

Introduction

Almost all plants of the world are a potential inhabitants of indigenous microbes principally recognized as endophytic microbes which can reside inside their tissue without giving any visible external symptoms which is responsible for nutrient assimilation and their processing, induction of defense system and synthesis of secondary metabolites (Pandey *et al.*, 2017). They may be actinomycetes, bacteria or fungi (Pandey *et al.*, 2016b). It is found that during the course of evolution certain microbes were able to enter the plant tissues, either with the help of synthesis of cell wall hydrolyzing enzymes like pectinease, cellulase or by developing some other mechanisms and reside inside the plant tissue and co-evolved. During co-evolution, they may be adapted towards the interior environment of the host plant involving the mechanisms of cross talk between the endophytes and the host plants (Pathak, 2011; Pandey *et al.,* 2016c). They colonize internal tissues of the plants either as obligate or in facultative manner without showing any immediate negative or external symptoms and reported to display the beneficial effects, put forward opportunities for discovering products and processes with potential applications in agriculture, medicine and biotechnology (Pandey *et al.,* 2012, 2014, 2015, 2016a, 2017). Bacterial endophytes stimulate plant growth, directly or indirectly thereby increasing their yield and several parameters utilized by living things for their life prospects (Gray and Smith, 2005; Pandey *et al.,* 2012, 2015, 2016). They offer an extensive range of benefits to the host plant against biotic and abiotic stresses (Hurek and Hurek, 2011). In return, the bacterial endophytes may be benefited by the various secondary metabolites and the growth regulators produced by the host plants (Schulz and Boyle, 2006). Endophytic bacteria were reported to produce novel bioactive compounds which were not previously found to be reported naturally in plants such as insecticides, antimicrobials, etc., (Ryan *et al.,* 2008).

Endophytic bacteria associated with medicinal plants hold a good opportunity to produce antibiotic to ameliorate negative effects of pathogenic fungi, bacteria and virus. There is a strong requirement for the invention of new metabolites and to explore alternative pathway by employing endophytic bacteria which are effective and cause less or no damage to the environment and replace the artificial chemicals and pesticides.

Natural products from bacterial endophytes

Now a day's world population is facing several health problems caused by bacteria, virus, fungus, protozoan and other micro-organisms which could be due to drug resistant or parasitism and generates an alarming situation to world population for their survival. Research based on the invention of medicinal and novel biactive compounds from endophytic bacteria is a promising task. There is a strong need for production of new drugs, especially antibiotics, anticancer agents, immuno-modulatory compounds, bioactive compound that is effective, but cause less or no damage to the milieu and replace the synthetic fertilizers and pesticides with more eco-friendly bio-fertilizers and bio-control agents. Bacterial endophytes may be used for the production of new pharmaceutical agents and agrochemical compounds. There is tremendous scope for the isolation of novel bioactive medicinal compounds from endophytic bacteria. Several classes of natural products such as antibacterial, antifungal antibiotics, antiviral, volatile insecticides, herbicidal, and plant growth promoting, plant protective agents have been reported to be produced by bacterial endophytes (Table 1). However, there are also some reports of production of harmful substances to predators of the host plants (Bacon *et al.,* 1977; Clay *et al.,* 1989; Suto *et al.,* 2002). Leucinostatin A production from *Acremonium* sp. isolated from *Taxus baccata* is found to be active against breast cancer (Strobel *et al.,* 1997). *Pseudomonas viridiflava* is a fluorescent bacteria located within the tissues associated with the leaves of many grass species produces ecomycins which represent a family of novel lipopeptides. The structure of these lipopeptides involves common amino acids such as alanine, serine, threonine, glycine and some unusual amino acids like homoserine and β-hydroxyaspartic acid, which are active against pathogenic fungi such as *Cryptococcus neoformans* and *C. albicans*. Pseudomycins, another group of antifungal compounds is also produced by plant-associated pseudomonads (Harrison *et al.*, 1991; Miller *et al.*, 1998; Strobel and Daisy, 2003).

An ample range of biologically active compounds has been isolated from bacterial endophyte but they still remain a relatively untapped source of novel natural products. Most researches focus on fungal-based antimicrobial bioactive products. A plentiful low-molecular- weight compounds which are active at low concentrations against a range of animal and plant pathogen have been isolated from bacterial endophytes. However, comprehensive screenings for antiviral compounds from bacterial endophytes have yet to be reported. Many endophytes are members of common soil bacterial genera, such as *Pseudomonas* and *Burkholderia* (Lodewyckx *et al.*, 2002) and are well known for their secondary metabolites that include antibiotics, anticancer compounds, insecticidal, antifungal, antiviral, and immunosuppressant agents. Bioplastics are biomaterials that are receiving increasing commercial interest. Genomic analysis indicates that many species of bacteria have the potential to produce bioplastics (Kalia *et al.*, 2003). Bioplastic poly-3-hydroxybutyrate (PHB) is polyester produced by *Bacillus megaterium* (Lemoigne, 1926). The most widely produced microbial bioplastics are poly-3-hydroxyalkanoate (PHA) and poly-3-hydroxybutyrate (PHB). *Herbaspirillum seropedicae*, reported to colonize a variety of plants and utilize a diverse range of carbon sources, accumulates significant levels of poly-3-hydroxybutyrate (PHB). Bacteria and higher plants having accumulation ability of PHAs may also help to produce novel heteropolymers for a range of applications (Aldor and Keasling, 2003; Catalan *et al.*, 2007).

Table.1 Bioactive compounds from bacterial endophytes

Bacterial endophyte	Source	Bioactive anti-fungal compound/ Function	Reference
Paenibacillus polymyxa	Wheat, Lodge pine, Green beans, *Arabidopsis thaliana* and Canola	Fusaricidin A–D (Antifungal)	Beck *et al.*, 2003; Li *et al.*, 2007
Serratia marcescens	*Rhyncholacis penicillata*	Oocydin A (Antifungal)	Strobel *et al.*, 2004
Bacillus sp.	Paddy	Antifungal activity	Wang *et al.*, 2009
Bacillus subtilis	Wheat	Antifungal protein E2	Liu *et al.*, 2010
Pseudomonas syringae	------	Pseudomycins	Harrison *et al.*, 1991
Bacillus pumilus MAIIIM4A	Cassava	Antifungal metabolites	De Melo *et al.*, 2009
Burkholderia brasiliensis M130	Rice root	EPS A and EPS B (Plant- microbe interaction)	Leigh and Coplin, 1992
Bacillus subtilis BS-2	Capsicum leaves	Antifungal protein (thermostable and UV-tolerant)	He *et al.*, 2003
B. cereus	Mustard	Chitinase (Antifungal)	Pleban *et al.*, 2003
Pseudomonas viridiflava	Grass species	Ecomycins	Miller *et al.*, 1998

		(Antifungal lipopeptides)	
Methylobacterium extorquens and *Pseudomanas synxantha*	Scots pine (*Pinus sylvestris* L.)	Adenine derivatives (Precursors in cytokinin biosynthesis)	Pirttila *et al.*, 2004
Pseudomonads	-	2,4-diacetylphloroglucinol (DAPG) (Antimicrobial)	Ramesh *et al.*, 2008
P. viridiflava strain EB274 and EB227	-	Ecomycins B and C (antifungal lipopeptides)	Harrison *et al.*, 1991
Streptomyces NRRL 30562	Snake vine [*Kennedia nigriscans*]	Munumbicins A, B, C and D (anti-microbial)	Castillo *et al.*, 2002
Streptomyces NRRL30566	Grevillea tree [*Grevillea pteridifolia]*	Kakadumycin A (antibiotics)	Castillo *et al.*, 2003
Streptomyces sp. strain GT2002/1503	*Bruguiera gymnorrhiza*	Xiamycin-A (anti-HIV activity)	Ding *et al.*, 2010
Streptosporangium oxazolinicum K07-0450	-	Spoxazomicins A-C (antitrypanosomal alkaloids)	Inahashi *et al.*, 2011
Bacillus licheniformis and *Bacillus pumilus*	Balloon flower (*Platycodon grandiflorum*)	Antifungal compound	Asraful *et al.*, 2010
Bacillus mojavensis	-	Leu^7 – surfactin (Anti-fungal)	Snook *et al.*, 2009
*Paenibacillus*sp. IIRAC-30	cassava (*Manihotes culenta*)	C15- lipopeptide (Anti-fungal)	Canova *et al.*, 2010
Bacillus amyloliquefaciens	*Scutellaria baicalensis* Georgi	fengycin homologues and surfactin homologues (anti-microbial)	Sun *et al.*, 2006
Streptomyces sp.	*Monstera* sp.	Coronamycin (antifungal and antimalarial)	Ezra *et al.*, 2004
Streptomyces NRRL 30562	Snakevine (*Kennedia nigriscans*)	munumbicins E-4 and E-5 (antifungal and antimalarial)	Castillo *et al.*, 2006
Shewanella sp. and *Pseudomonas* sp.	*Ageratum conyzoides*	2-amino-3-quinolinecarbonitrile and boric acid (Antibacterial)	Fitriani *et al.*, 2015

First report of indolesesquiterpenes (xiamycin B, indosespene, and sespenine together with the known xiamycin A) is from the culture broth of *Streptomyces* sp. HKI0595, a bacterial endophyte. It is used as a biocontrol agent and has been identified for strong antimicrobial activities against different pathogenic bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* which are resistant towards methicillin and vancomycin respectively (Ding *et al.,* 2011). Ammonia, butyrolactones, 2,4-diacetyl phloroglucinol, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, viscosinamide, xanthobaccin and zwittermycin A are the antibiotics produced by antagonistic bacteria (Whipps *et al.*, 2001). They produce hydrolytic enzymes that cause cell wall lysis, which can be used to control fungal pathogens (Backman and Sikora, 2008) and biosurfactants as antimicrobial compounds (Nielson *et al.*, 1999; Nielson *et al.*, 2000; Bais *et al.,* 2004). *Pseudomonas fluorescens* produces cyclic lipopeptides surfactants, such as viscosinamide (Nielson *et al.*, 1999), and tensin (Nielson *et al.*, 2000) with antifungal activity against *Rhizoctonia solani* and *Pythiu multimum* (Nielson *et al.*, 2000). Lanna-Filho *et al.,* (2013) partially characterized Protein fractions 42 and 75 from the *Bacillus amyloliquefaciens* and *Bacillus pumilus.* These protein fractions 42 and 75 were acting as elicitor in induced resistance against pathogen *Xanthomonas vesicatoria* in tomato plant and reduce the bacterial spot up to the extent of 63.5 and 56.6% respectively as compared with control plant along with an increase in the peroxidase (POX) and polyphenol oxidase (PPO) enzyme activities. These protein fractions were appearing as a single band of molecular mass of 28 and 43 kDa, respectively on SDS-PAGE silver staining. Endophytic bacterial isolates from healthy peanut plants were evaluated against the peanut bacterial wilt (BW) caused by *Ralstonia solanacearum*. Isolate BZ6-1 characterized as *Bacillus amyloliquefaciens* on the basis of morphology, biochemical and 16S rRNA analysis were identified as to the highest antimicrobial activity. The main antimicrobial compound surfactin and fengycin A homologs were examined by high performance liquid chromatography electrospray ionization tandem mass spectrometry (Wang and Liang, 2014). Endophytic bacteria *Lactobacillus* sp. isolated from the leaf tissues of *Adhathoda beddomei* were investigated for the presence of bioactive compound. Bioactive compound was extracted by solvent- solvent methods. Qualitative tests of the extracts showed the presence of carbohydrates, tannins, saponins, alkaloids, glycosides, proteins, amino acids and saponins while presence of phenolic compounds were reported to be 0.67 mg/ml (Swarnalatha *et al.,* 2015).

Acknowledgement

We are highly grateful to Department of Biotechnology, Government of India for providing research grant under the project “Institutional Biotechnology Hub” at College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh.

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How to cite this article:

Pramod Kumar Pandey, Siddhartha Singh, Mayanglambam Chandrakumar Singh, Amit Kumar Singh, Pratibha Pandey, Ajai Kumar Pandey, Mahesh Pathak, Mukul Kumar, Ramesh Chandra Shakywar and Raghubir Kumar Patidar. 2017. Inside the Plants: Bacterial Endophytes and their Natural Products. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 33-41.
doi: https://doi.org/10.20546/ijcmas.2017.606.003