Endophytic Bacteria- Unexplored Reservoir of Antimicrobials for Combating Microbial Pathogens

Disha Menpara and **Sumitra Chanda***

Phytochemical, Pharmacological and Microbiological Laboratory,
Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India
*Author for correspondence email: svchanda@gmail.com

Endophytic bacteria found ubiquitous in all plant species in the world, reside in the inner tissues of living plants without causing apparent symptoms of infection. An enormous, relatively untapped source of microbial diversity is represented by the endophytes. They have attracted increasing attention as they are efficient producers of antimicrobial agents and seem to have unique genetic and biological systems that may have applications outside the host plant in which they normally reside. Antibiotics are potent antimicrobial agents with high specificity. However the relentless emergence of antibiotic-resistant strains of pathogens, together with the retarded discovery of novel antibiotics has led to the urgent need to find alternative treatments. Thus screening for antimicrobial compounds from bacterial endophytes is a promising way to overcome the increasing threat of drug resistant strains of human and plant pathogen. An attempt has been made in this review to accumulate the data on endophytic bacteria isolated from plants that produce metabolites with antimicrobial activity against plant and human pathogenic bacteria.

Keywords Endophytes; Antimicrobials; Antibiotic resistance

1. Dire need for novel drugs and agrochemicals

There is an ever growing need for new and useful compounds to provide assistance and relief in all aspects of the human condition. Infectious and parasitic diseases are the next leading cause of death after cardiovascular diseases, causing 15.6% of all deaths in women and 16.7% in men [1]. Each year infectious diseases cause 14 million deaths worldwide, with mortality increasing even in the United States at an annual rate of 4.8 percent. Thus human race is facing the problem of tremendous increase in bacterial and fungal infections worldwide. The problem is worsened by antibiotic resistance, as well as the emergence of new pathogens with the potential for rapid global spread [2]. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases.

Antibiotics encompass a chemically heterogeneous group of organic, low-molecular weight compounds produced by microorganisms that are deleterious to the growth or metabolic activities of other microorganisms [3]. Antibiotics have very specific mode of action, affecting the vital processes like DNA, RNA, protein and cell wall synthesis. However, the recent upsurge of antibiotic resistant microbes has created havoc. Antibiotic resistance has turned out to be a global problem wherein more and more bacteria are developing resistance to antibiotics mainly conferred by randomly mutated genes [4, 5]. The problem of resistance has resulted in increased morbidity, mortality and costs of health care. This has prompted for more research for potent antimicrobial compounds to tackle drug resistant organisms like *Staphylococcus* sp., *Mycobacterium tuberculosis*, *and Streptococcus* sp. [6].

To meet the requirement of mammoth sized population, food production has to be increased. Phytopathogens are the major cause of plant diseases resulting in severe loss of yields [7]. As synthetic agricultural agents have safety and environmental problems, they have been currently targeted for removal from the market, which prompts us to find alternative ways to control farm pests and pathogens [8].

2. The new alternative

Despite a focused interest on synthetic products, bioactive natural products retain an immense impact on modern medicine. The foundation of modern medicine was antibiotics, but they are now becoming ineffective. Hence, initiatives are to be made to raise awareness to the problem of ineffectiveness of antibiotics. In the battle against the ever-increasing drug resistance of human and plant pathogens, we urgently need new alternatives. Exploration of novel niche of biodiversity leads to discovery of novel natural products [9]. Plants harbour an untapped source of bioactive metabolites called endophytes. Perusal of literature suggests that they are efficient producers of antimicrobial agents. Thus to tackle the rising meance of antimicrobial resistance, these plant associated microbiota i.e. endophytes hold a great promise.

3. Endophytes

3.1. Introduction

Nearly every living and non-living niche on earth is occupied by microorganisms. Plants are naturally associated with microorganism in various ways. Many microorganisms are present as epiphytes residing on plant surfaces whereas some are as endophytes which colonize internal plant tissues [10]. Endophytes (usually bacteria or fungus) reside in inner tissues of plants, without causing any apparent damage to the plant they inhabit [11-13]. Thus their colonization inside plants is symptomless.

The focus of this review is bacterial endophytes. They have been known for >120 years [14] and can be isolated from surface sterilised plant tissues. The methods used to isolate and characterize endophytic bacteria from different plant species has been very well reported [15-18]. Although the plant-endophyte interaction has not been fully understood, it has been reported that many isolates provide beneficial effects to their hosts like preventing disease development by synthesizing novel compounds and antifungal metabolites [19].

3.2. Ecology of endophytic bacteria

Endophytic bacteria are ubiquitously found in most plant species [17, 20]. They have been isolated from herbaceous plants as well as from woody trees. The microecology of endophytes indicates that they occupy intercellular spaces [21-23]. Endophytes might be finding their host by chemotaxis, electrotaxis, or accidental encounter. The main entry for endophytic bacteria appears to be through wounds [24]. Thus the entry occurs through root zone as well as through aerial zone [25]. It has also been reported that the entry can also occur through stomata and lenticels [26]. The definition of endophytes has the concept of a symptomless, non-disease producing infection that results in a series of interactions that range from no effects on the hosts (neutralism), beneficial to the hosts and bacteria (mutualism), or to that of benefiting only one member (commensalism). Thus endophyte–plant host interactions are different from pathogen–plant host interactions since neither associate really 'wins'; neither disease symptoms develop on the plant host nor is the bacteria eliminated by the plant host.

Bacterial endophytes benefit from inhabiting the plant's interior because it is a protected niche in which there is relatively little competition from other microorganisms for a constant and reliable source of nutrition. The internal colonisation protects endophytes from exposure to extreme environmental conditions, such as temperature, osmotic potentials and ultraviolet radiation. Thus the internal colonization by endophytes provides an added ecological advantage to them over epiphytes.

3.3. Diversity of endophytic bacteria

A wide variety of bacterial endophytes have been colonising plants. Several different bacterial endophyte species can be isolated from a single plant [27]. This diversity of endophytes ranges from Gram positive to Gram negative bacteria which include genera like *Achromobacter, Acinetobacter*, *Agrobacterium, Bacillus, Brevibacterium, Burkholderia, Chromobacterium, Curtobacterium, Enterobacter, Kocuria, Lysinibacillus, Methylobacterium, Microbacterium, Paneibacillus, Pantoea, Phyllobacterium, Pseudomonas, Rahnella, Rhodanobacter, Stenotrophomonas, Streptomyces, Xanthomonas* etc.[28-33]. However, in general, Streptomyces sp. and Bacillus sp. were the most predominant species [28, 29 and 32]. The diversity of endophytic bacteria can be studied by cultivation-dependent and cultivation-independent techniques (metagenomic analysis). Cultivation-based techniques help in the recovery and testing of isolates, whereas cultivation-independent techniques help to screen the variations in the total endophytic communities [34].

3.4. Antimicrobials from endophytic bacteria

From a drug discovery point of view, the novel bacterial strains are attractive, as they are likely to contain new genes in theory and are promising for novel products, thus the chance of finding novel pharmaceutical bioactive compounds from endophytic bacteria is substantial.

In the recent years, endophytic bacteria have garnered a good attention. It is may be because of their intimate and non detrimental association with plants that results in production of variety of antimicrobial compounds [35]. It has only recently been recognized that endophytic bacteria play an important role in resistance to disease and that signals exist to mediate cross talk between the endophyte and its host.The secondary metabolites of endophytic bacteria have applicability in medicine and also act as biocontrol agents [36, 37] . They can also be used as food preservatives in the control of food spoilage and food-borne diseases, a serious concern in the world food chain [38]. In the field of plant pathology, antibiotic-producing bacteria are used as a resource for new antifungal compounds and for biological control of pre and post harvest diseases [39]. Table 1 shows some reported bacterial endophytes with antimicrobial activity. Thus in any of the perspectives, they have not disappointed and they contribute to their host plants by producing plenty of substances that provide protection and ultimately survival value to the plant. Although endophytic fungi are most commonly worked out for production of antimicrobials, endophytic bacteria are also showing promising results with the progression of studies related with them [40]. Thus, there is great application value to develop antimicrobial drugs from endophytic bacteria. The discovery of an antimicrobial effect of a crude extract of the culture broth is the first step needed for the discovery of a new antibiotic. The second step is the identification and finally the third step is structure elucidation of potent metabolite for the development of antibiotic. Figure 1 shows the general protocol for isolation of endophytes and the separation of antimicrobial compounds from them.

Plant (selected on the basis of certain rationale)

↓

Surface sterilization of plant tissues prior to isolation of endophytic bacteria

↓

Endophytic bacteria

↓

Preliminary screening for antimicrobial activity

↓

Testing of antimicrobial activity of crude extracts

↓

Isolation of chemical constituents

↓

Structure elucidation of potent metabolite for the development of antibiotic

Fig. 1 Flow chart of antimicrobial compounds separation from endophytes.

The culture filtrate of the endophytic *Bacillus subtilis* strain EDR4 of wheat roots had an antifungal protein named E2 which exhibited strong antifungal activity against *Fusarium graminearum, Macrophoma kuwatsukai, Rhizoctonia cerealis, Fusarium oxysporum f.sp. vasinfectum, Botrytis cinerea and G. graminis var. tritici (Ggt).* The protein was purified by $(NH_4)_2SO_4$ precipitation, hydrophobic interaction chromatography, anion exchange chromatography and PAGE [41].

Endophytic isolates of *Bacillus licheniformis* and *Bacillus pumilus* from Balloon flower (*Platycodon grandiflorum*) roots obtained from a mountain area of Daepyung-Myun, Chinju, Korea showed maximum antifungal activity against *Phytophthora capsici, Fusarium oxysporum, Rhizoctonia solani,* and *Pythium ultimum* [42].

Culture extracts and filtrates of patented endophytic bacterium *Bacillus mojavensis* were found to have Leu^7-surfactin. This was shown with the help of HPLC-MS and CID analysis. This endophytic bacterium was patented for control of fungal diseases in maize and other plants [43].

In one of the recent studies, a functional gene-based molecular screening strategy was used to target non ribosomal peptide synthetase (NRPS) and type I polyketide synthase (PKS) genes in endophytes isolated from eight medicinal plants collected in Yunnan, China. Bioinformatic analysis of these biosynthetic pathways indicated that these endophytes were capable of producing bioactive compounds. The culture broth extracts of endophytes demonstrated antifungal and antibacterial activities. Thus from the perspective of natural product discovery, PKS and NRPS gene screening is a valuable method for screening isolates of biosynthetic potential [44].

The endophytic bacteria *Paenibacillus* sp. IIRAC-30 was isolated from cassava (*Manihot esculenta*). The ethyl acetate extract obtained from endophytic culture grown in Potato Dextrose medium, indicated presence of C15-lipopeptide belonging to surfactin series. The *Paenibacillus* sp. IIRAC-30 suppressed *Rhizoctonia solani* [45].

Bacillus amyloliquefaciens was isolated from Chinese medicinal plant *Scutellaria baicalensis* Georgi. Culture filtrate of the bacteria displayed antagonism against some phytopathogenic, food-borne pathogenic and spoilage bacteria and fungi. Subjecting the extract to HPLC-MS analysis showed fengycin homologues and surfactin homologues [46].

Ezra et al. 2004 reported antifungal and antimalarial activity shown by antibiotic Coronamycin which was produced by *Streptomyces* sp. isolated as an endophyte from an epiphytic vine, *Monstera* sp., found in the Manu region of the upper Amazon of Peru. Coronamycin had an MIC value of 2 µg/ ml against the pythiaceous fungus *Pythium ultimum* and showed anti *Plasmodium falciparum* activity with IC50 values of 9±7.3 ng/ml [47]. Antifungal and antimalarial activity were also shown by *Streptomyces* NRRL 30562 which was originally isolated as an endophyte from *Kennedia nigriscans*, snakevine, in the Northern Territory of Australia [48]. Mass spectrometric and HPLC analyses indicated presence of munumbicins E-4 and E-5.

Endophytes belonging to the members of the genus *Streptomyces* were isolated from plants collected from different sites of the tropical rainforests in Xishuangbanna, Yunnan province, China. 41 isolates showed significant antimicrobial activities. Among them, 65.9% were active against *Escherichia coli*, 24.4% against *Staphylococcus aureus*, 31.7% against *Staphylococcus epidermidis* and 12.2% against *Candida albicans* [49].

4. Facing challenges

Antimicrobials synthesized by endophyte holds great promise to solve the problem of bacterial resistance to some commonly used drugs [50] and overcome the burning demand of discovering highly effective and low toxic antibiotics to fight against resistant bacterial species. But there are currently bottlenecks limiting the development of endophytes for use in biotechnology and agriculture. First, certain endophytic antimicrobial compounds have unspecific toxicity, that is, they are toxic to human beings and other organisms along with pathogenic bacteria and fungi. Second, certain antimicrobial compounds have moderate antimicrobial activities and hence they cannot be used as a potential medicine. Third, there is very less knowledge regarding the biochemistry of synthesis and regulation of antimicrobial compounds in endophytes. Despite their activity against human, animal, and plant-pathogenic microorganisms, there is still limited evidence that antibiotics also have this function *in situ*.

Hence, to overcome the above mentioned hurdles, research in this direction is indispensable. It is very essential to find better bioactive antimicrobial substances without any side effects to humans and plants. For this, genetic engineering tools can come to rescue. The studies regarding the regulatory gene in synthesis path of antimicrobial compound would be very helpful to increase the yield of active substances synthesized by endophytes. Moreover, by modifying the structure of metabolites from endophytes, one can improve the efficacy and enhance their antimicrobial activity. Modifying the structure can also reduce the toxicity of antimicrobial compounds which ultimately decreases the side effects. Furthermore, antibiotics most probably do not act in isolation but are working in concert with other metabolites produced by the same cell and hence this aspect should also be considered in interpreting the biological significance of antibiotic production by endophytic bacteria.

5. Concluding remarks and perspectives

In the past few years, there is significant increase pertaining to the field of endophytes, in terms of isolation of novel bacteria as well as isolation of bioactive antimicrobial metabolites. Bacterial endophytes have attracted attention of many researchers' on their theoretical study as well as their potential applications. Now is the time to review past researches in the arena of endophytic bacterial born natural products discovery and to scrutinize future prospects to further explore the biodiversity of endophytic bacteria and the bioactive secondary metabolites produced by them for biotechnological applications.

As reviewed above, plants are providing the endophytic environment, which undoubtedly is storehouse of myriad of new bacteria providing novel structurally diverse compounds that can be used in pharmaceutical, agriculture and industry. Thus it is obvious that work in this field holds enormous promise. But still the work related to endophytes is still in the juvenile stage.

We can hunt for novel endophytes from plants that grow in extreme environments. Further research at the molecular level in this field is clearly required for a better understanding of the host–endophyte interaction. Improving the productivity of some potential candidates by using genetic engineering and microbial fermentation processes might lead to the discovery of the much needed antibiotic for the treatment of infections caused by multidrug-resistant bacteria. One has to isolate and purify antimicrobial substances produced by endophytic bacteria in laboratory so as to identify its structure and mode of action. If any of the metabolites are showing potent activity, then they are the potential candidates for future drug development [51]. There is high probability for isolation of novel antimicrobial metabolites because of boon of novel bioassay techniques and extensive chemical separation science. Thus continuous investigations of endophytic bacteria from diverse habitats and application of new technologies is the key for future success.

We can conclude that the endophytic bacteria are manufacturer of plethora of bioactive antimicrobial metabolites which inhabit the unique niche i.e. plants are undeniably a hidden treasure worth exploring. Therefore, a rich pool of bacterial species is yet to be discovered and investigated over the coming years.

Table 1 List of some reported endophytes for antimicrobial activity

Host plant	Potent endophytes	Activity shown	Tested organisms	Reference No.
Panax ginseng	*Paenibacillus polymyxa GS01, Bacillus sp. GS07, and Pseudomonas poae JA01*	Antifungal	Phytopathogenic fungi	[32]
T. grandiflora Polyalthia sp. Mapania sp.	*Streptomyces fulvoviolaceus, Streptomyces coelicolor, Streptomyces caelestis*	Antifungal	Phytopathogenic fungi	[52]
Scutellaria baicalensis Georgi	*Bacillus amyloliquefaciens*	Antibacterial, Antifungal	Phytopathogenic, food-borne pathogenic and spoilage bacteria and fungi	[46]
Panax notoginseng	*Bacillus amyloliquefaciens subsp. plantarum , Bacillus methylotrophicus*	Antifungal	Phytopathogenic fungi and nematode	[29]
Azadirachta indica A. Juss.	*Streptomyces sp., Nocardia sp.*	Antibacterial, Antifungal	Phytopathogenic fungi, Human pathogenic bacteria and fungus	[53]
Plectranthus tenuiflorus	*Bacillus sp. Pseudomonas sp.*	Antibacterial, Antifungal	Human pathogenic bacteria and fungus	[54]
Wheat	*Bacillus subtilis*	Antifungal	Phytopathogenic fungi	[55]
Anthurium	*B. amyloliquefaciens*	Antibacterial	Phytopatogenic bacteria	[56]
Platycodon grandiflorum	*Bacillus licheniformis, Bacillus pumilus, Bacillus sp.*	Antibacterial, Antifungal	Phytopathogenic fungi and anti-human food-borne pathogenic organisms	[42]
Artemisia annua	*Streptomyces*	Antibacterial, Antifungal	pathogenic bacteria, yeast and fungal phytopathogens	[57]
Centella asiatica	*Bacillus subtilis, Pseudomonas fluorescens*	Antifungal	Phytopathogenic fungi	[15]
Panicum virgatum L.	*Bacillus subtilis, C. flaccumfaciens, Ps. Fluorescens, P. ananatis*	Antifungal	Phytopathogenic fungi	[58]
Raphanus sativus L	*Enterobacter* sp., *B. subtilis*	Antibacterial, Antifungal	Phytopathogenic fungi, Human pathogenic bacteria	[59]
Memecylon edule , Tinospora cordifolia,	*Bacillus amyloliquefaciens*	Antibacterial, Antifungal	Human pathogenic bacteria and fungus	[60]

Phyllodium pulchellum and *Dipterocarpus tuberculatus*				
S. lavandulifolia, H. scabrum, R. pulcher	*Bacillus sp.*	Antibacterial, Antifungal	Human pathogenic bacteria and saprophytic fungi	[61]
Aloe chinensis	*Paenibacillus* species	Antibacterial, Antifungal	Pathogenic bacteria and fungi	[62]
Epimedium brevicornu Maxim.	*Phyllobacterium myrsinacearum*	Antibacterial, Antifungal	Phytopathogenic fungi and phytopathogenic bacterium	[63]
11 mangrove halophytic plants	*Bacillus Thuringiensis and Bacillus pumilus*	Antibacterial	Shrimp pathogens	[64]
Kandelia candel	*Streptomyces* sp.	Antibacterial	Several pathogenic bacteria	[65]
Codonopsis lanceolata	*Bacillus pumilus B. subtilis B. licheniformis*	Antifungal	Phytopathogenic fungi	[66]
Polygonum cuspidatum	*Streptomyces sp.*	Antifungal	Pathogenic fungi	[28]
Manihot esculenta	*Paenibacillus sp.*	Antifungal	Phytopathogenic fungus	[45]
Bruguiera gymnorrhiza Rhizophora stylosa Kandelia candel	*Bacillus amyloliquefaciens*	Antibacterial, Antifungal	Phytopathogenic fungi and phytopathogenic bacteria	[67]
Monstera sp.	*Streptomyces sp.*	Antifungal, Antimalarial	Pythiaceous fungi and the human fungal pathogen, malarial parasite	[47]
Piper nigrum L	*P. aeruginosa, P. putida and B. megaterium*	Antifungal	Phytopathogenic fungus	[68]
Huperzia serrata	*Burkholderia sp.*	Antifungal	Phytopathogenic fungi	[69]
300 plants from upper Amazonian Rainforests	*Streptomyces sp. Micromonospora sp. Amycolatopsis sp.*	Antibacterial, Antifungal	Range of potential fungal and bacterial pathogens	[70]
Lycopersicon esculentum	*Streptomyces sp., Microbispora sp., Micromonospora sp. and Nocardia sp.*	Antibacterial, Antifungal	Phytopathogenic fungi and phytopathogenic bacteria	[71]

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