Life Sciences, Medicine and Biomedicine LSMB

Research Article

Bacterial Endophytes: A Reservoir of Bioactive Anti-Microbial Compounds

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https://doi.org/10.28916/lsmb.2.1.2018.8
Received 3 January 2018, Revisions received 15 January 2018, Accepted 26 January 2018, Available online 28 January 2018

Abstract

Bacterial endophytes are found on all types of plants and is a potential source of bioactive compounds which can be utilized to fight against multi-resistant pathogens and could be further develop into new leads for antibiotic development. However, the research done on the bacterial endophytes is relatively new and has potential to grow as it is theorized that each plant has one or more bacterial endophytes inhabiting them. This review aims to review the studies that have been done previously and give new insights on the latest trends in this field of research.

Keywords: Bacterial endophytes, Bioactive compounds

1.0 Introduction

The discovery of *Escherichia coli* which is resistant to colistin, an antibiotic of last resort in China and further report of colistin-resistant strain in USA had ended the Golden Age of Antibiotics (McGann et al., 2016).This occurrence of antimicrobial strain is not an isolated case, there have been reports of anti-microbial strain of various microorganisms in Asia. Studies done by Asian Network for Surveillance of Resistance Pathogens (ANSORP) have reported that the antibiotic resistance of *Streptococcus pneunomiae* have increased over the years especially among Malaysian isolates (Song et al, 2004)

The recent report by Survey of Antibiotic Resistance (SOAR) in 2016 indicated that in Singapore,there was a significant decrease in susceptibilty of *Haemophilus influenzae* towards cefuroxime during the period of 2012 – 2014 compared to the 2009 -2011 period (Torumkuney et al., 2016). This echoes a report by ANSORP in 2012 that an increase in multi-drug resistance among the isolates from 11 Asian countries (Kim et al., 2012). This worrying development of multidrug resistance is also being seen in other pathogens such as *Staphylococcus aureus, Enterococcus sp, Enterobacteriaceae sp, Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Kang & Song, 2013).

In Malaysia, the National Surveillance of Antibiotic Resistance (NSAR) 2015 reported that the resistance of pathogenic bacteria such as *Staphylococcus aureus, Enterococcus sp, Acinetobacter sp, Haemophilus influenza, Pseudomonas aeruginosa, Enterococcus clocae* and *Escherichia coli* have increased but there were no multi-drug resistant strains detected. However, with the increased frequency of airtravel and the effect of globalization, this may change in the near future. Antimicrobial resistance do not only affect the mortality rate of the country,but also affects the Gross Domestic Product (GDP) of the country. This is because with increased mortality rate, there will be lesser manpower to generate the country's economy.

Antibiotic-resistant bacteria have become increasing widespread worldwide. Antibiotic resistant is defined as the reduction of effectiveness of an antibiotic during treatment of infectious diseases. Gram-negative bacteria are more resistant toward antibiotic than Gram-positive bacteria due to presence of outer lipopolysaccharide membrane in their cell wall which limits access of antibiotic into their targets in the bacterial cells (Aravamuthan et al., 2010).

Other mechanisms that contribute to antibiotic resistance in bacteria include modification of antibiotic target site. For example, bacteria modified their penicillin-binding proteins (PBP) which is the target site produce β-lactam resistance. Enzymatic inactivation of antibiotic also an important resistance mechanism exploited by bacteria. The best known example of enzymatic inactivation is production of β-lactamase which modify the β-lactam ring. In addition, an active efflux system is a major mechanism in antibiotic resistance which reduce the influx of antibiotic into the bacterial cells (Nikaido, 2014 Kenneth et al., 2002; Nikaido, 2001). Therefore, the search and discovery of a new antimicrobial compound is necessary to overcome the emergence of these antibiotic-resistance bacteria.

However, the discovery of new antimicrobial compounds either from natural products has declined over the years and have come to a standstill compared to the Golden Antibiotic Age (1940-1960). This phenomena is due to various reasons; (1) the difficulties in synthesizing the organic compounds, (2) the high cost and batch difference in large scale fermentation, (3) the advances in high-throughput combinatorial chemistry, (4) the high failure of drug candidates to pass the clinical trialand (5) the search for anti-cancer compound (6) the lack of fund on building a dedicated pipeline on drug discovery in academia (Davies, 2006).

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Although, the outlook for to derive a new anti-microbial compound from natural products as a source is grim, there are a renewed focus on the search of lead candidates especially from endophytic sources as illustrated in Figure 1.

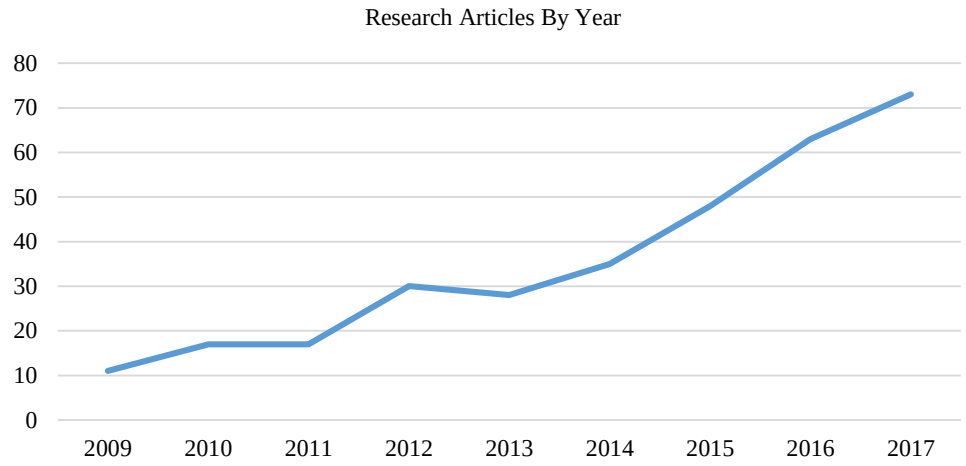

Figure 1: The focus of anti-microbial research on microorganisms from 2009 to 2017. Data adapted from the database of Sciencedirect.com

Natural product is defined as a chemical compound or compound produced by a living organism (Webster, 1913; Nature Chemical Biology, 2007). The producer of the natural product could be animal, plant or microorganism such as bacteria or fungi. Natural product could be divided into primary metabolites or secondary metabolites.

The primary metabolites are the chemical compounds that are essential for the organisms' growth, development and energy source. Primary metabolites are often carbohydrates, lipids, amino acids and nuleic acids. These type of compounds are used as precursors to basic metabolic pathways in the organisms. The structure and component of these chemical compounds are not much varied throughout the different kingdom. Secondary metabolites can be defined as low-molecular weight organic compounds that do not play a role in growth of an organism but produced as an adaption for specific function in nature. The production of secondary metabolites arises from primary intermediates through an action of enzymes (Vining., 1990). Secondary metabolites have many functions such as defence and protection against pathogens and used as traditional medicines for human (Sansinenea & Ortiz, 2011; Yazaki, Sasaki, & Tsurumaru, 2009). Secondary metabolites belong to various classes of chemical substances such as alkaloids, terpenoids, glycosides and peptide (Jenke-Kodama, Müller, & Dittmann, 2008).

Secondary metabolites can be produced either from plant or microorganism. Secondary metabolites also have been isolated from microbes. About 45% of known bioactive microbial metabolites are produced predominantly by Actinomycetes species. These secondary bioactive microbial metabolites exhibit antibacterial, antifungal, antiprotozoal, antitumor and antiviral properties (Bérdy, 2012).

2.0 Endophytes

Endophytes are microorganisms that live within the plants' tissues without causing any damage to the host (Lodewyckx et al., 2002). Entophytes could be classified as fungi, bacteria or algae (Schulz & Boyle, 2006).

Endophytes primarily assist in promoting the growth of plants that they inhabited as shown in Figure 2**.** They assist in changing the pH of the soil,water. Studies done on endophytes that were isolated from rice, potato,sugercane and tomato shown that the endophytes that inhabit the plants were able to promote the growth and also protect the plant from phytopathogens.

Facultative endophytes grow outside its host plant. Meanwhile, obligate endophytes are dependent on their host plant for their growth and survival (Hardoim et al., 2008). Endophytes are divided into 3 categories which are bacteria, fungi and algae.However, this review will only focus on bacterial endophytes.

2.1 Strategies in Isolation of Endophytes

Previous studies on endophytic research reported various reasoning in the selection of plants from which the endophytes were isolated. (Jinfeng, Mohamad Rafi, Chai Hoon, Kok Lian, & Yoke Kqueen, 2017; Jothy et al., 2011;Sim et al, 2009; Cheah,2001). The rationale choosing the plants for endophytic study is summarized in Figure 3.

Regardless of the reasons in choosing the plant samples, the most important step is to remove all bacteria,fungi or algae that inhabits the surface of the plant. The basic elements used in surface sterilization are ethanol, sodium hypochlorite and rinsing with water in the final step. In order to check that the surface sterilization is done properly, the parts of the plants that had been sterilized are pressed on the isolation agar to leave an imprint or a small quantity of the water used in the final rinsing is dropped onto the isolation agar and both were incubated and any growth of microorganisms were noted (Jinfeng et al., 2017).

3.0 Secondary Metabolites from Endophytes

Bacterial-endophyte closely interact with plant that has high potential in synthesising wide range of secondary metabolites. It is believed that endophytes are capable of producing identical bioactive compound or metabolites as their host plants. This situation will decrease the need to harvest slow growing plant and also protect the world's biodiversity (Strobel & Daisy, 2003). These secondary metabolites play a role in defence and competition and also act as signals for interaction and communication with the plant host (Brader, Compant, Mitter, Trognitz, & Sessitsch, 2014). Apart from that, the secondary metabolites also can act an agents needed for nutrient acquisition especially during iron uptake by siderophore (Neher, Johnston, Zidack, & Jacobsen, 2009).

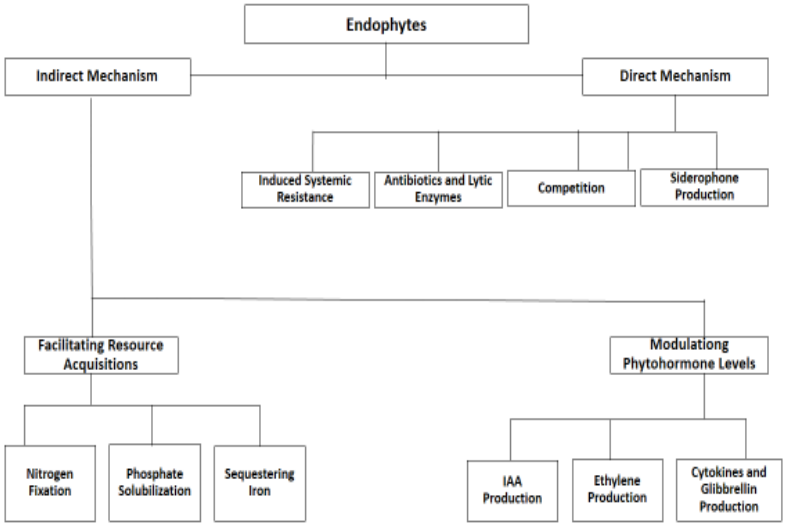

Figure 2: Various Mechanisms of How Endophytes Assist in Plant Growth

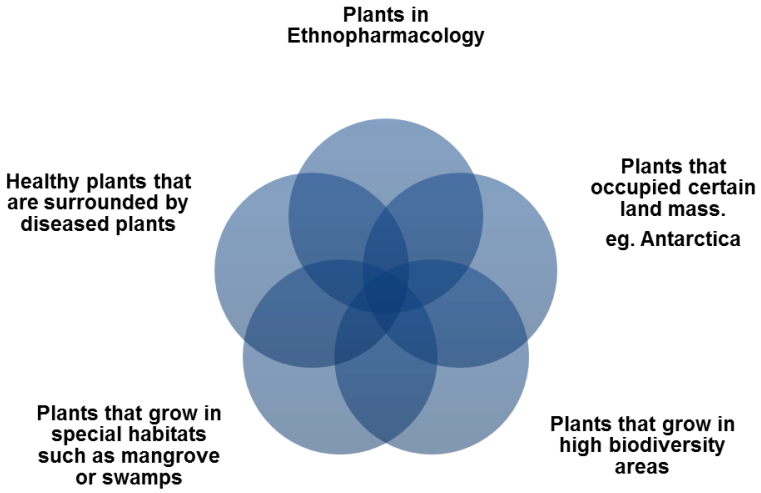

Figure 3: The Rationale Behind Choosing The Plants for Endophytic Research.
Intensity of the shaded area on the diagram indicate the higher possible of endophytes with greater bioactive metabolites

According to previous study by Miller et al., (1998), an antimicrobial compound called ecomycins was found to be produced by *Pseudomonas viridiflava*, an endophytic bacteria isolated from grass species. Castillo et al., (2003) have also stated that *Streptomyces sp*, a bacterial-endophyte from *Grevillea pteridifolia* produce bioactive secondary metabolites namely, kakadumycin A and echinomycin.

Studies have been done on antibacterial activity of bacterial-endophyte from medicinal plants has been summarized in Table 1. According to Castillo et al., (2003), endophytic *Streptomyces sp. NRRL 30562* isolated from snakevine produce antibiotic that showed antimicrobial activity against many pathogenic bacteria and fungi. Arunacchalam et al., (2012) stated that bacterial-endophyte isolated from *Andrographis paniculata* have showed activity against both Gram-positive and Gram-negative pathogenic bacteria.

Based on Bhoonobtong et al., (2012), bacterial-endophyte isolated from *Memecyclon edule*, T*inospora cordifolia, Phyllodium pulchellum* and *Dipterocarpus tuberculatus* had showed antibacterial activity against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Diethyl ether and chloroform extract of UD25 isolates had successfully inhibited *Staphylococcus aureus* with bacteriostatic activity. Meanwhile, ethyl acetate and diethyl ether extract may inhibited the growth *of Bacillus cereus* and *Escherichia coli* showing bactericidal activity. This bacterial-endophyte has been reported as *Bacillus amyloliquefaciens* through 16S rDNA gene analysis.

A bacterial-endophyte has been isolated from a medicinal plant *Plectranthus tenuiflours*.. Out of 28 isolates, 8 isolates (28.5%) were identified to have antimicrobial activity against at least one of the test pathogens in the study which are *Samonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,Streptococcus agalactiae LC, Proteus mirabilis LC* and *Candida albicans.* Bacterial-endophyte of *Bacillus sp* and *Pseudomonas sp* have showed significant antimicrobial activity against entire tested pathogens (El-Deeb, Fayez, & Gherbawy, 2013).

Pseudomonas aeruginosa, a bacterial-endophyte from *Brassica oleracea* was reported to display significant antibacterial activity against *Escherichia coli* (13mm), *Klebsiella pneumonia* (20mm), *Staphylococcus aureus* (25mm) and *Salmonella typhimurium* (12mm). *Pseudomonas aeruginosa* also showed significant antibacterial activity against *Klebsiella pneumonia* and *Staphylococcus aureus* compared to the inhibition zone of common antibiotics which were used as control which are Amoxicillin, Streptomycin and Ofloxacin (Sunkar et al., 2013).

Table 1 : Antimicrobial Compounds Produced by Various Endophytes and Its Host Plants

Endophytic Bacteria	Host	Bioactive compounds	References
Pseudomonas viridiflava	*Grass*	*Ecomycins B and C*	*Miller et al,1998*
Streptomyces sp NRRL 30562	*Kennedia nigriscans*	*Munubicins A – D*	*Castillo et al., 2002b*
		Munubicins E4-E5	*Castillo et al., 2006*
Streptomyces sp NRRL 30566	*Grevillea pteridifolia*	*Kakadumycins*	*Castillo et al., 2003*
Streptomyces sp MSU-2110	*Monstera sp*	*Coronamycins*	*Ezra et al., 2004*
Streptomyces sp CS	*Maytenus hookeri*	*24-demethyl-bafilomycin C1*	*Lu and Shen, 2003*
		24-demethyl-bafilomycin A2	*Lu and Shen, 2004*
		24-demethyl-bafilomycin A1	*Li et al., 2010*
		21-O-methyl-24-demethyl-bafilomycin A1	
		17,18-dehydro-19,21-di-O-methyl-24-demethyl-bafilomycin A1	
		24-demethyl-bafilomycin D	
Streptomyces sp. Ls9131	*Maytenus hookeri*	*Dimeric dinactin*	*Zhao et al. 2005*
		Dimeric nonactin	
Streptomyces sp. TP-A0556	*Aucuba Japonica*	*Demethylnovobiocins*	*Igarashi 2004*
Streptomyces griseus subsp	*Kandelia candel*	*7-(4-aminophenyl)-2,4-dimethyl-7-oxohept-5-enoic; 9-(4-aminophenyl)-7-hydroxy-2,4,6-trimethyl-9-oxo-non-2-enoic acid*	*Guan et al. 2005*
		12-(4-aminophenyl)-10-hydroxy-6-(1-hydroxyethyl)-7,9-dimethyl-12-oxo-dodeca-2,4-dienoic acid	
Streptomyces sp. MaB-QuH-8	*Maytenus aquifolia Mart.*	*Celastramycins A and B*	*Pullen et al. 2002*
Streptomyces sp. BCC72023	*Oryza sativa L.*	*Efomycin M*	*Supong et al., 2016)*
		Efomycin G	
		Oxohygrolidin	
		Abierixin	
		29-O-methylabierixin	
Streptomyces sp. BO-07	*Boesenbergia rotunda (L.) Mansf A.*	*3'-hydroxy-5-methoxy-3,4-methylenedioxybiphenyl*	*T.Taechowisan et al. 2017*
		3'-hydroxy-5,5'-dimethoxy-3,4-methylenedioxybiphenyl	
Streptomyces albidoflavus	*Bruguiera gymnorrhiza*	*Antimycin A18*	*Zhu, Wang, Zeng, Zhang, & Yan, 2010)*

4.0 Designs for Isolation of Bioactive Compounds

In literature, there are mainly 3 types of designs in isolation of bioactive compounds from the endophytic bacteria which is illustrated in Figure 4. The most common design in acquiring a bioactive compound is using the bioguided fractional assay which is denoted in P1 and P2 (Sun et al, 2006) . However, there are research which uses the design denoted in P3(Casella et al, 2013).

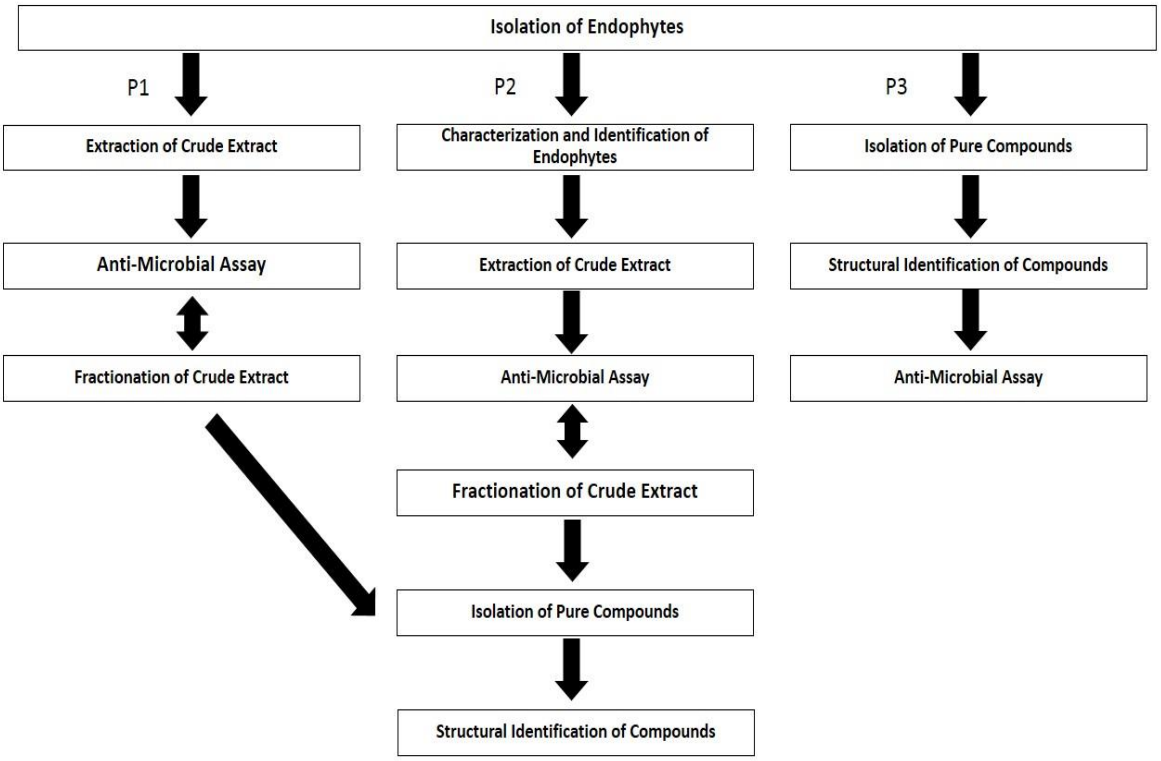

Figure 4: Designs in Isolation of Bioactive Compounds

5.0 Challenges in Isolating Bioactive Compounds From Endophytes

Isolation of bioactive compounds is a tedious process as these isolated endophytes may not produce any potential bioactive compounds due to (1) lack of suitable source to act as a precusor in production of the bioactive compounds since some of the starting material is produced by the host, (2) the growth conditions are not suitable for the endophytes, according to a study done by Paranagama, Wijeratne and Gunatilaka (2007), when the group change the source of water from tapwater to distilled water, only then the bacteria started to produce bioactive compounds and (3) lack of external stresses to induce the production of bioactive compounds and lastly,(4) some of the endophytes required epigenetic modifiers to "unsilence" certain production pathways (Venugopalan and Srivastava, 2015; Craig and Newman, 2013;Berdy, 2005; Strobel and Daisy, 2003)

6.0 Conclusion

Generally, studies on bacterial endophytes are limited compared to the fungal endophytes. Thus, the potential of isolating useful and novel bioactive compounds is far greater. Research on endophytes do not entirely depends on microbiologists, it needs a plethora of researchers from interdisciplinary fields such as chemistry, taxonomy, molecular biology, pharmacology and bioinfomatics. Scientist should explore and expand to various endophytes for novel drug discovery and also microbial diversity. The focus on existing compounds that were isolated could be expanded to other treatments like cancer, emerging multi-drug resistant pathogen or other diseases.

7.0 Declaration

The authors declare no conflicts of interest in this work.

8.0 Acknowledgements

The authors are grateful to Geran Putra Malaysia (GP-IPS/2017/9556300) for the research funding and the Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for the facilities.

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