Isolation and Identification of Endophytic Bacteria from Rare Medicinal Plant Genera of Bilsapur City of Chhattisgarh

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Abstract: ***Endophyte reside in the living plant tissue are mostly unstudied and potential sources of new natural products for therapeutic preparations. Some endophytes produce the secondary metabolites as that of plant which make them promising sources of novel compounds. This study was conducted with the aim to isolate and identify endophytic bacteria from rare and endangered medicinal plants collected from Bilaspur district of Chhattisgarh .Isolation of endophytic bacteria was done on nutrient agar medium and characterized on the basis of morphological and biochemical characteristics. A total 18 bacterial endophytes were obtained from these different plants,and characterized with the help of morphological and biochemical tests and identified by BMDM.***

Keywords: Endophytes, nutrient agar medium, rare endangered medicinal plants, secondary metabolites

1. Introduction

Endophytic bacteria (such as *Gammaproteobacteria*, *Pantoea*, *Methylobacterium*, *Azospirillum*, *Herbaspirillum*, *Burkholderia*, *Rhizobium*, *Cellulomonas*, *Clavibacter*, *Curtobacterium*, *Pseudomonas Microbacterium*, *Burkholderia and Bacillus*) are significantly present inside the plants communities and not reported to exhibit any negative effect on them to date (Mano and Morisaki, 2008; Holliday, 1989; Schulz & Boyle, 2006; Lodewyckx *et al.*, 2002). Endophytic bacteria are colonize like phytopathogens inside host tissues, which makes them suitable as biocontrol agents (Berg *et al.*, 2005) especially with regards to plant pathogens (Sturz & Matheson, 1996; Krishnamurthy & Gnanamanickam, 1997), insects (Azevedo *et al.*, 2000) and nematodes (Hallmann *et al.*, 1997; Hallmann *et al.*, 1998). Fungal-, bacterial-, viral- infection and even harmful effect of nematodes can potentially be reduced by endophythic microbial communities (Kerry, 2000; Sturz *et al.*, 2000; Ping & Boland, 2004; Berg & Hallmann, 2006). Moreover, they also enhance plant growth (Bent & Chanway, 1998) by inducing phosphate solubilization activity (Verma *et al.*, 2001; Wakelin *et al.*, 2004), indole acetic acid production (Lee *et al.*, 2004), production of a siderophore (Costa & Loper, 1994), provide essential vitamins (Pirttila *et al.*, 2004) and alteration of nitrogen accumulation and metabolism (Compant *et al.*, 2005a; Compant *et al.*, 2005b). Apart from plant-endophytic mutual relationship studies for sustainable agriculture, the scientific communities are now look forward endophytic bacteria for novel secondary metabolites as drugs (Strobel *et al.*, 2004) and towards phytoremediation (Siciliano *et al.*, 2001; Barac *et al.*, 2004; Germaine *et al.*, 2004, 2006; Porteous-Moore *et al.*, 2006). As per the literature survey, present work has been done to explore the endophytic bacteria from healthy wild rare medicinal plant community of Bilaspur City in order to explore their secondary metabolites for wider range of applications towards benefit of mankind.

2. Materials and Methods

2.1 Sample collection

As per data base provided in the paper published by Tewari *et al.* (2014), healthy wild rare medicinal endangered plants viz., *Acorus calamus* L. (Buch), *Andrographis paniculata*. Burm.f. (Kalmegh), *Clerodendrum erratum* L. (Bharangi), *Convolvulus microphyllous* sieb. (Shank pushpin), *Tephrosia perpuria* L. (Sarphonk) were selected and collected from the Bilaspur City (on July 2017), location situated at 22.0796° N, 82.1391° E.

Table 1: Sample collected for the isolation of Endophytic Bacteria

S. No.	Sample		Geographical Location
	Botanical Name	Local Name	
1	*Acorus calamus* L.	Buch	22.1293° N, 82.1360° E
2	*Andrographis paniculata*. Burm. f.	Kalmegh	22.0867° N, 82.1988° E
3	*Clerodendrum erratum* L.	Bharangi	22.1293° N, 82.1360° E
4	*Convolvulus microphyllous* sieb.	Shank pushpin	22.0563° N, 82.1816° E
5	*Tephrosia perpuria* L.	Sarphonk	22.0867° N, 82.1988° E

Isolation of Endophytic Bacteria

Samples were washed with tap water, surface sterilized with 5% sodium hypochlorite followed by sterile-distilled water. 100 µl of the final wash (sterile-distilled water) was spread onto nutrient agar medium for control. For the test plate, 5.0 g surface sterilized explants (leaf, root and stem) were macerated in 10 ml of sterile distilled water and 100 µl of suspension was cultured onto nutrient agar media. Plates were incubated at 26-28^{o}C for 15 days, under consistent observation. Morphologically distinct colonies were marked and pure cultures of each marked colonies were prepared. Pure cultures were stored as Agar slant at 4^{0}C until used.

Identification of Endophytic Bacteria

Each bacterial pure cultures were subjected to morphological and biochemical characterization. Morphological characteristics observed were pigmentation, shape, elevation, edge, consistency and colony surface of bacterial colonies. Gram staining and Spore staining were performed. Biochemical characteristics includes Motility test, Catalase test, Coagulase test, Methyl red test, Voges-proskaeur test, Indole test, Starch hydrolysis, Citrate test, Oxidase test and Sugar fermentation. Bacterial strains were identified as per described by (BMDB) Bergey's Manual of Determinative Bacteriology (Bergey's Manual of Determinative Bacteriology,1964).

Statistical analysis

All the experimental setups were carried out in triplicates to minimize error. Means Data were taken. Data were calculated in MS-Excel.

3. Results and Discussion

Present research work has been conducted towards the isolation and identification of endophytic bacteria from rare medicinal plant genera of Bilsapur City of Chhattisgarh State of India. A total of five endangered rare medicinal plants (*Acorus calamus* L., *Andrographis paniculata*. Burm.f., *Clerodendrum erratum* L., *Convolvulus microphyllous* sieb. and *Tephrosia perpuria* L.) were selected to isolate endophytic bacteria. Eighteen endophytic bacteria (*Burkholderia* sp., *Pseudomonas* sp., *Bacillus* sp., *Clavibacter* sp., *Cellulomonas* sp., *Herbaspirillum* sp., *Rhizobium* sp., *Methylobacterium* sp., *Azospirillum* sp., *Curtobacterium* sp., *Microbacterium* sp., *Pantoea* sp., *Gammaproteobacteria* sp., *Acetobacter* sp., *Enterobacter* sp., *Burkholderia* sp., *Herbaspirillum* sp. and *Klebsiella* sp.) were isolated and identified as per their morphological and biochemical characteristics described by BMDB. It was noted that most of them are gram negative and these group of bacteria are known to be resistant against large groups of bio-control agents. To get better insight in microbial identification, their morphological and biochemical characteristics were cross verified by an online microbial identification database ABIS.

Maximum endophyte density 1.7×10^6 CFU/ml found in the leaf of *Tephrosia perpuria* L. while minimum 1.1×10^3 in the root of *Acorus calamus* L. Means as per data observed it could be said that leaf has maximum endophytic bacteria while root has lowest. Shyam *et al.* (2017) reviewed diverse group of endophytic bacteria and mentioned, in a maize root, average CFU of WP5gfp (*Rahnella* sp.) were 2.9×10^7/gram in root while 3.9×10^7/gram in leaf and stems.

Holliday (1989), Lodewyckx *et al.* (2002), Schulz & Boyle (2006), Mano and Morisaki (2008) Shyam *et al.* (2017) and Firdous *et al.* (2019) have been reported same kinds of endophytic bacteria from variety of plants. Every plant has certain consortium of endophytic bacteria. It was also noticed that almost same level diversity of endophytic bacteria present in plant communities inhabited in particular region however consortium of endophytes might be varied. Well documented literatures have been available and revealed that endophytic bacterial consortium that reside in rhizosphere soil, leaf, stem and root might be able to produce wide variety of novel secondary metabolites Rosenblueth *et al*., 2006; Compant *et al*., 2010; Reinhold-Hurek *et al*., 2011). Benefit associated with endophytic bacteria has been reported towards bioremediation (Newman and Reynolds, 2005), as a control agent that prevent the doorway of pathogen (Haas and Defago, 2005), siderophores (Hydroxamate- and catecholate- type) production (Sharma and Johri, 2003) and produce optimal level of phytohormone (Sgroy *et al*., 2009; Vanstraelen and Benkova, 2012). Such application of endophytic bacteria could further be explored and optimized.

Table 2: Details of the source plant for the isolation of Endophytic bacteria

S. No.	Plant	Plant Part	Endophytic Bacteria (CFU ml $^{-1}$)
1	*Acorus calamus* L.	Leaf	1.3×10^6
		Stem	1.4×10^5
		Root	1.1×10^3
2	*Andrographis paniculata*. Burm. f.	Leaf	1.2×10^5
		Stem	1.2×10^4
		Root	1.4×10^3
3	*Clerodendrum erratum* L.	Leaf	1.5×10^4
		Stem	1.1×10^5
		Root	1.3×10^3
4	*Convolvulus microphyllous* sieb.	Leaf	1.4×10^5
		Stem	1.1×10^6
		Root	1.3×10^3
5	*Tephrosia perpuria* L.	Leaf	1.7×10^6
		Stem	1.2×10^6
		Root	1.4×10^4

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