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Screening of Endophytic Bacteria from Anticancer Medicinal Plants

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Abstract: ***Endophytes, microorganisms that reside in the living plant tissue are mostly unstudied and potential sources of novel natural products for exploitation in therapeutic preparations. Some endophytes produce the same 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 anticancer medicinal plants. Five different plants were selected to enhance diversity as Catharanthus roseus, Oscimum sanctum, Alovera, Withania somnifera and Murraya konengii. Isolation of endophytic bacteria was done on Tryptic soy agar media and characterized on the basis of morphological and biochemical characteristics. A total 73 bacterial endophytes were obtained from these different plants, among them 10 were get characterized by culture dependant method.***

Keywords: Endophytes, Tryptic soy agar, anticancer medicinal plants, secondary metabolites

1. Introduction

Traditional medicines have long history of serving peoples all over the world. India is an herbal hub which gives novel and rich source for natural drug research and development (Dixit and Ali, 2010). Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bioprospecting of new plant-derived drugs. Cancer is a major public health burden in both developed and developing countries with higher mortality rate. Plant derived agents are being used for the treatment of cancer. There is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumorigenesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy. Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products. Plants recommended for cancer therapy involves, *Allivum sativum, Alovera, Azadirachta indica, Catharanthus roseus, Curcuma longa, Oscimum sanctum, Withania somnifera,* , etc. (Sakarkar and Deshmukh, 2011, Nema *et al*., 2012).

Plant associated bacteria that live inside plant tissues without causing any harm to plants are defined as endophytic bacteria. These plant associated bacteria colonize the rhizosphere, phyllosphere (epiphytes) and the inside of plant tissues (endophytes). Endophytic bacteria in association with the rhizospheric bacteria exert several beneficial effects on host plants, such as stimulation of plant growth, nitrogen fixation and resistance to plant pathogens (Mahajan *et al.*, 2014). The use of therapeutic plant species in medicine is old but now a day it is confirm that all types of plant sp. harbor endophytic bacteria product of which have several potential applications in pharmaceutical industry (Mahajan *et al*., 2014).

2. Materials and Method

2.1 Collection of anticancer medicinal plants

In the present study, total 5 five different plants viz. *Withania somnifera* (Ashwagandha), *Ocimum sanctum* (Tulsi), *Alovera* (Korfad), *Catharanthus roseus* (Sadaphuli) and *Murraya Koenigii* (kadipatta) recommended for cancer therapy were selected for isolation of endophytes. These plants were collected from locations situated in five different districts in Marathwada region for proper study of biodiversity of endophytic bacteria. Districts selected for study were Ambajogai, Aurangabad, Latur, Nanded, and Parbhani. Different plant samples viz. root, inner bark, leaves, flowers etc. were collected from each mature healthy plant and were immediately processed.

2.2 Isolation and identification of endophytic bacteria

a) Surface sterilization of plant material: The healthy plant parts of selected ethnomedicinal plants such as stem cuttings, leaves, fruits, and roots were used for isolation of endophytes. The plant samples were initially subjected for surface sterilization as per the methodology given by Ahmed *et al*., (2012) along with some modifications. Plant material was first cleaned by washing several times under running tap water and then cut into small segments aseptically. Surface sterilization was performed by sequentially rinsing the plant parts with 70% ethanol for 1 minute, then with 0.01% mercuric chloride for 5 minute which followed by 0.5% Sodium chloride for 4-5 minutes and finally it treated with sterile distilled water for 4-5 times.

b) Isolation of the endophytes: The surface sterilized plant parts viz. leaves and fruits were further ground with 6ml 0.9% NaCl solution using sterile pestle and mortar and kept aseptically for 15-20 min. for the release of endophytic bacteria from host tissue. The tissue extract was diluted with 0.9% NaCl solution and plated on tryptic soy agar medium plates. The plant parts viz. inner bark, roots were cut into pieces with sterile knife to excise inner tissues. The excised inner tissues were further inoculated on tryptic soy agar medium plates. All the plates were incubated at 30^0 C for 3-5

days. After incubation, various colonies were selected showing different morphological and growth characters. Each endophytic isolate was checked for purity and transferred to freshly prepared nutrient agar plate. Appropriate controls of plate were prepared to check out contamination without plant tissue inoculation.

Sterility check: To confirm that the plant surfaces were effectively decontaminated 1ml aliquots of the sterile distilled water that was used in the final rinse of surface sterilization procedures were plated onto nutrient agar medium and incubated at 28^{o}C for 48 hrs. Bacterial growth was observed after 48 hrs. Also, surface sterilized segments were rolled on nutrient agar plates, incubated at 28oC for 48 hrs. and checked for possible microbial growth (Hallmann *et al.*, 1997).

c) Maintenance of endophytes: The purified endophytic isolates were transferred separately to nutrient agar slants for further use.

3. Results and Discussion

A) Selection of plants for isolation of endophytic Bacteria
A rationale was used to select the plant for isolating novel endophytic microorganisms. In this present study, plants that have an ethno botanical history for use as anticancer agents were preferentially considered for isolating bacterial endophytes. Plants collected in this study were annual/ perennial herbs and shrubs which available everywhere or easily cultivated in local areas. As these were candidates for study, since the medicinal uses to which the plant may have been selected relates more to its population of endophytes than to its biochemistry itself. Significant variations in the populations of indigenous endophytes have been reported. These variations are attributed to plant source, plant age, tissue type, time of sampling and environment. Generally bacterial populations are larger in roots and decrease in the stems and leaves (Strobel and Daisy 2003).

Plants selected in this study were, *Withania somnifera* (Ashwagandha), *Ocimum sanctum* (Tulsi), *Alovera* (Korfad), *Catharanthus roseus* (Sadaphuli) and *Murraya koenigii* (kadipatta) were available everywhere and also with variable medicinal properties along with potential of producing secondary metabolites / phytochemicals having application in cancer therapy. All the plants selected in this study have wide therapeutic applications (Rungsung *et al.*, 2013). These plants are native to the Marathwada and commonly cultivated in every region. Observation table given below will show the list of collected plants selected for study containing its botanical and common name with its references.

1. Observation table: List of collected anticancer medicinal plants

Sr. No.	Common name of plant	Botanical name	Ecological status	References
1	Gheekumari	*Alovera*	Perennial plant	Nema *et al.*, 2012
2	Sadafuli	*Catharanthus roseus*	Perennial, annual shrub	Sain and Sharma, 2013
3	Kadipatta	*Murraya koenigii*	Perennial	Muthumani *et al.*, 2009
4	Tulsi	*Ocimum sanctum*	Annual	Pradeep *et al.*,2010
5	Ashwagandha	*Withania somnifera*	Perennial herb	Oza *et al.*,2010

B) **Isolation of the endophytes**
Adopting a standard protocol of Ahmed *et al.*, (2012) bacterial endophytes were isolated with some required modifications**.** A total of 73 isolates were isolated from five different plants collected from different districts of Marathwada region for obtaining biological diversity in endophytes. Medicinal properties of plants in most cases may have been depending upon population of endophytes than that of plant biochemistry. Endophytes are ubiquitous with rich biodiversity, which have been found in every plant species examined. Each individual plant is host for one or more endophytes (Strobel and Daisy, 2003).

Table 2: Number of endophytic bacteria isolated from anticancer medicinal plants

Sr.No.	Location	Plant name	Plant part used				
			Root	**stem**	**leaves**	**fruit**	**total**
1	Hingoli	*Alovera*	01	02	-	-	03
		Catharanthus roseus	02	02	01	-	05
		Murraya koenigii	01	01	-	-	02
		Ocimum sanctum	-	-	03	-	03
		Withania somnifera	-	01	01	-	02
		Total endophytes	**04**	**06**	**05**	**00**	**15**
2	Nanded	*Alovera*	01	01	-	-	02
		Catharanthus roseus	02	01	01	-	04
		Murraya koenigii	-	02	01	-	03
		Ocimum sanctum	-	-	01	-	01
		Withania somnifera	-	01	-	01	02
		Total endophytes	**03**	**05**	**03**	**01**	**12**
3	Parbhani	*Alovera*	02	02	-	-	04
		Catharanthus roseus	01	-	03	02	06
		Murraya koenigii	-	02	01	01	04
		Ocimum sanctum	03	01	02	-	06
		Withania somnifera	01	-	02	01	04
		Total endophytes	**07**	**05**	**08**	**04**	**24**

4	Aurangabad	*Alovera*	01	02	-	-	03
		Catharanthus roseus	03	02	03	-	03
		Murraya koenigii	01	-	02	-	03
		Ocimum sanctum	-	-	01	-	01
		Withania somnifera	-	01	-	-	01
		Total endophytes	**05**	**05**	**06**	**00**	**11**
5	Osmanabad	*Alovera*	-	02	-	-	02
		Catharanthus roseus	-	02	01	01	04
		Murraya koenigii	01	-	-	02	03
		Ocimum sanctum	-	02	01	-	03
		Withania somnifera	-	01	-	01	02
		Total endophytes	**01**	**07**	**02**	**04**	**14**
Total 73							

C) Biochemical characterization of isolated bacterial endophytes

All the bacterial endophytes isolated in this study were identified at genus level by using conventional biochemical methods. Different 73 bacterial endophytes were isolated, during biochemical characterization it was observed that maximum number of isolates showed similar activities as isolated from various locations. Observation table given below will show biochemical characterization of selected 10 isolates commonly isolated from selected plants from different locations with similar biochemical properties.

Table 3: Biochemical characterization of isolated bacterial endophytes

Strain no.	Grams nature	Motility	Biochemical characters													
			IMVIC I MR C VP				Sugar utilization Glu man lac sucrose				H_2S pro^n	Catalase	Oxidase	Urease	Nitraté réduction	Probable sp.
1	Gram positive	motile	-	+	+	-	AG	-	AG	A	+	+	+	+	+	*Bacillus sp*
2	Gram positive	Non-motile	-	+	-	-	A	A	A	A	-	-	-	-	-	*Streptococcus sp*
3	Gram negative	motile	-	-	+	+	AG	AG	AG	A	-	+	-	-	+	*Enterobacter sp*
4	Gram négative	motile	-	-	+	+	A	_	A	A	-	+	-	_	+	*Serratiasp.*
5	Gram négative	motile	+	-	+	+	A	-	_	_	-	+	+	+	+	*Pseudomonas sp*
6	Gram positive	Non-motile	-	-	-	+	-	-	-	A	-	-	-	+	+	*Micrococcus sp*
7	Gram negative	motile	+	+	_	_	AG	A	A	A	_	+	_	_	+	*Escherichia coli sp.*
8	Gram negative	Non-motile	_	_	+	+	AG	A	A	A	_	+		+	+	*Klebsiella sp.*
9	Gram négative	motile	+	+	_	_	A	-	A	A	-	+	-	_	+	*Serratiasp.*
10	Gram positive	Non-motile	-	-	+	-	AG	A	A	A	-	+	-	-	+	*Staphyllococci sp.*

4. Conclusion

Present study investigates bacterial endophytes from anticancer medicinal plants commonly available in Marathwada region. Based on the results, it was concluded that selected medicinal plants does contain diverse (10) types of culturable endophytic bacterial sp.among which *Bacillus* sp., *Serratia* sp. and *Pseudomonas* sp. were most common in all plants. Microbial endophytes reside in the internal plant tissues were stimulate phytochemicals properties of plants with wide clinical application. Our research findings could serve as a foundation for further research on these plants in determining potential roles of its bacterial endophytes in producing novel therapeutic compounds.

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