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Short communication

Screening of bacterial endophytes inhabiting *Mimosa pudica* L.

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ABSTRACT

Research on endophytes is burgeoning immense importance since recent years with almost all plants harboring untold number of microorganism as endophytes. Endophytic plethoras are reported to secrete unique novel metabolites bearing therapeutic properties which are being constantly exploited. The present study uncaps the bacterial endophytes inhabiting stem and roots of *Mimosa pudica* L located in southern part of India. The screening resulted in isolation of one forty one myriad bacterial endophytes with different morphological characteristics. The endophytes isolated in the present study will be exploited for further research which will be promising enough to extract any novel leads of pharmaceutical importance.

1. Introduction

Plants are known to serve man kind since ancient era as source of traditional medicine even still many parts of the globe, plants are being rapidly exploited against various diseases (Satish *et al*., 2008). Large number of the present medicines in the western world have been developed on the basis of traditional medicines records and till date plants are being explored for various therapeutic agents and pharmaceutical biology perceive plant as

biofactories of various bioactive compound with potent biological activity but harvesting of endangered species and slow growing rate of plants results in imbalance among plant diversity (Nicolaou *et al*., 1994). Hence research on alternative source of bioactive compounds has drastically expanded among which microorganism with their huge diversity can play an immense important role towards the haunt for bioactive compounds. Microorganisms are known to be bio factories of value added secondary metabolites hence they are being subject of interest across the globe (Berdy, 2005). Among the microbial diversity lay group of microorganisms harboring unique biological niches in plants called as endophytes. Endophytes can be defined as microorganism inhabiting in plants forming a symbiotic relation with its host without causing any inherent negative effects (Syed Baker *et al*., 2012). With improved scientific knowledge, association of plants and microorganisms are being clearly understood with different type of associations. These endophytes not only form symbiotic association with their host, they also secrete promising secondary metabolite which protects plants against the invading pathogenic microorganism and play an vital role in plant defense mechanism (Syed Baker *et al*., 2012). Research on endophytes is burgeoning importance in recent past as they produce plethora of metabolites bearing therapeutic properties. These endophytes can mimic the chemistry of their respective host plants and can secrete similar bioactive compound or derivatives that are more bioactive with respect to their host (Owen *et al*., 2004).

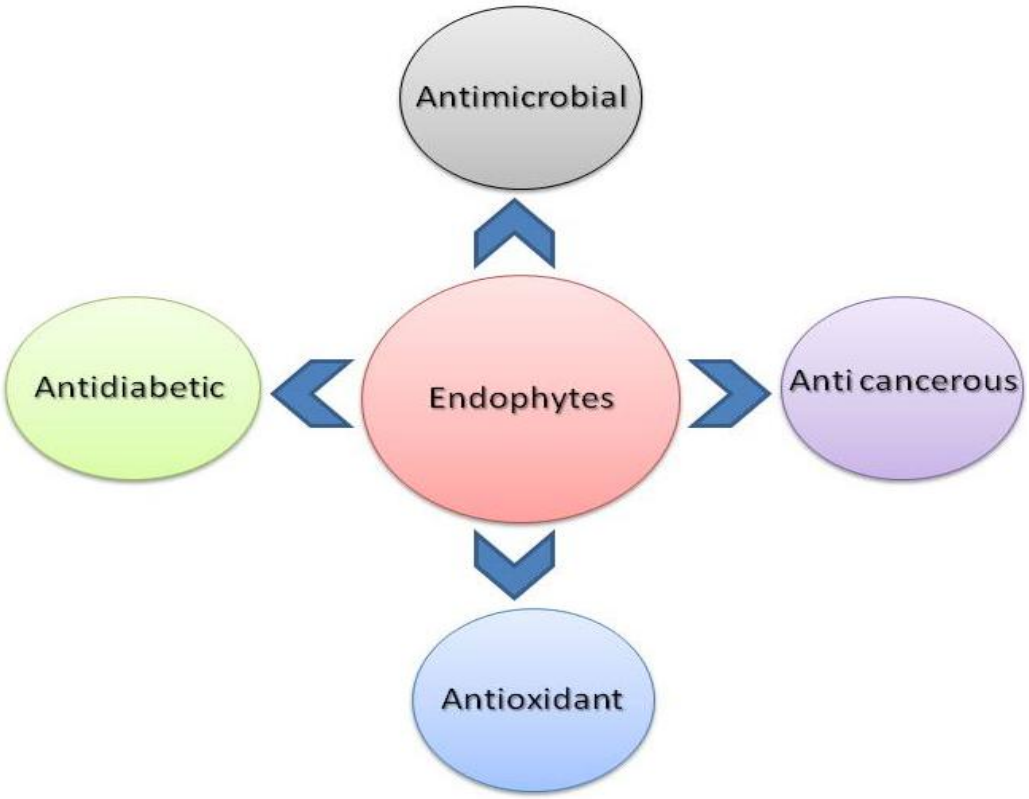

Fig. 1. Representing the pharmaceutical importance of compounds extracted from endophytes.

Till date studies on endophytes has exemplified to revealed that these endophytes isolated from unique niches are meant to secrete a large number of compounds with antimicrobial, antioxidant, anticancerous, immunosuppressant, Insecticidal (Fig. 1) etc., which belongs to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines, and flavonoids (Strobel, 2003). The present study envisions screening of bacterial endophytic plethora inhabiting *Mimosa pudica* L which has a unique characteristic of sensitive upon touching, it is also called as sleeping grass another fascinating properties of this plants are It can grow on soil with low nutrient concentrations and it is shade intolerant and does not compete with tall vegetation or grow under forest canopies. Hence due to its unique properties *Mimosa pudica* L was selected as subject of interest in the present investigation for screening of bacterial endophytes.

2. Materials and methods

2.1. Collection of plant material

Plant material such as stem, root of *Mimosa pudica* L were collected from ten healthy mature plants per site from southern parts of India. Individual plant materials were collected aseptically 3 cm above the soil level, and the stalks were stripped and placed into sterilized plastic bags and carried to laboratory.

2.2. Surface sterilization and isolation of endophytes

Stem and roots were subjected to surface sterilization under aseptic washed thoroughly with tap water followed by distill water to remove adhering soil and debris Later the surface sterilization was carried by sequential steps initially by immersing in 3.15% sodium hypochlorite for 5 mins and then followed by ethanol 70% for 30 seconds (Syed Baker *et al*., 2012). In every step of the surface sterilization procedures the plant materials were washed in sterile double distilled. Outer tissue of surface sterilized plant material were removed with sterilized scissor scalpel, later carefully excise into 0.5-1.0 cm tissue blocks of plant material and placed on surface of nutrient agar supplemented with 250 µg/ml of cycloheximide and incubated for 48hrs (hours) to observe bacterial colonies (Webster *et al*., 2001 and Zin *et al*., 2010). To confirm that the surface disinfestations process was successful and to verify that no biological contamination from the surface of stem and roots, sterility checks were carried out for each sample to monitor the effectiveness by impressions and 0.1 ml from the final rinse was plated out on nutrient agar as control plate. Endophytic bacterial colonies emerging from surfaced sterilized tissues of stem and roots were sub cultured with accession number for further studies.

2.3. Primary screening of endophytic bacterial isolates for antimicrobial activity

Endophytic bacterial colonies obtained from surfaced sterilized tissues were subjected to primary screening for antimicrobial activity by dual culture method for fungi (Raubitschek et al., 1950) and agar over lay method for bacteria where in endophytic isolate were point-inoculated and incubated for 3-days. Later inactivated by placing (1.5-2.0ml) chloroform containing sterile blotter in the upper lid for 40 mins. The inactivated colonies were overlaid with 5 ml of sloppy soft nutrient agar containing 0.65% agar that had been inoculated with the test pathogens. Zones of inhibition around the colonies were recorded after 24 hrs at 30^{o}C (Hayakava *et al*., 2004).

3. Results and discussion

The present investigation resulted in isolation of one forty one bacterial endophytes emerging from stem and roots of surface sterilization tissue and isolates obtained exhibited myriad morphological characteristics. The plant *Mimosa pudica* L is known to display various medicinal properties and used in treatment of leprosy, inflammation, asthma, burning sensation, blood impurities and it is also very useful in diarrhea , amoebic dysentery (Varnika *et al*., 2012).

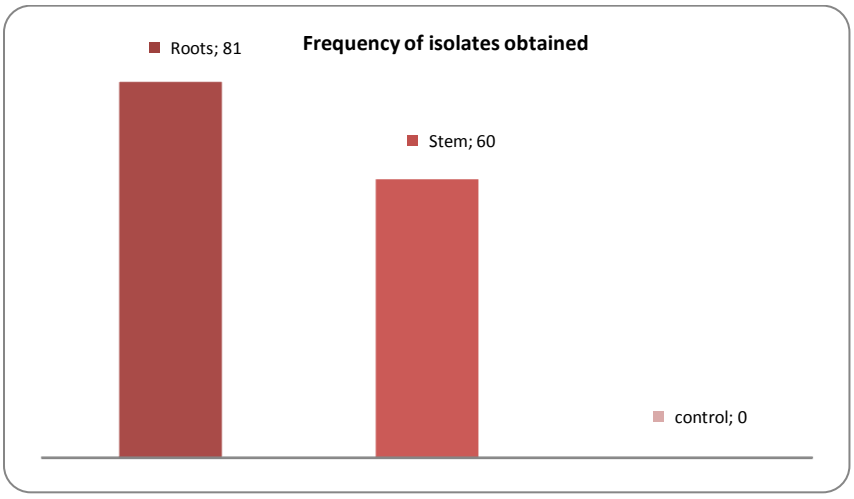

Fig. 2. Colony frequency emerging from surfaced sterilized roots stems and control plate.

Screening endophytes from such plants bearing medicinal property can yield a novel isolates which can secrete compound similar to its host which can reduce the harvesting of plant species which in turn can maintain the biodiversity among plant kingdom. The sterility of surface sterilization displayed no colonies on control plate which was a clear indication of successful surface sterilization which has eliminated the surface flora of the plant material and the bacterial endophytes were sub cultured and maintained with different accession number preserved at herbal drug technological laboratory for further studies. Among the isolates obtained in presents investigation endophytes emerging from the root were more dominant compared to stem (Fig-2), apart from which Gram negative bacterial species predominant when compared to the Gram positive (Fig-3).

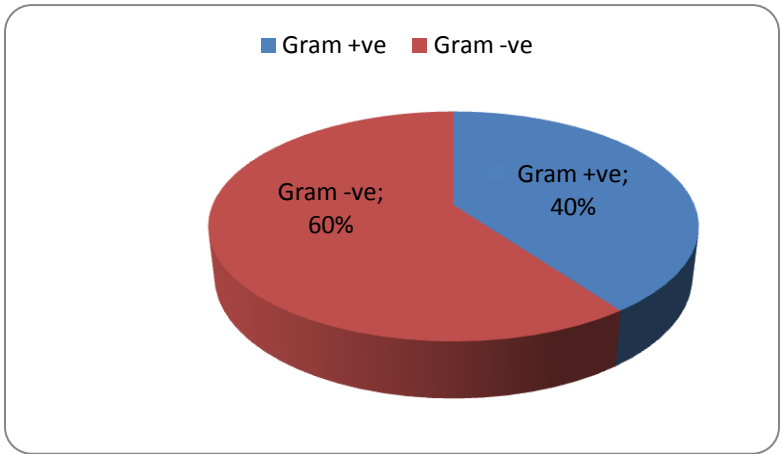

Fig. 3. Percentage of bacterial endophytes isolated based on Gram staining.

But when preliminary screening for antimicrobial activity related that Gram positive bacterial species exhibited antimicrobial activity which was confirmed as a clear zone of inhibition of the test pathogens. In the present study both human and phytopathogens *viz*., *Escherichia coli (MTCC 7410), Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 7443), *Staphylococcus epidermidis* (MTCC 435), *Bacillus subtilis* (MTCC 121), *Xanthomonas campestris (7908), Vibrio cholera, Shigella, Candida albican (MTCC 183), Proteus mirabilis (MTCC 425), Fusarium Oxysporum, Aspergillus niger* and *Aspergillus flavus* were employed to evaluate antimicrobial property of bacterial endophytic isolate. All the test cultures were procured from IMTECH-MTCC Chandigarh India. The results obtained were promising enough and displayed a positive preliminary indication of an isolate bearing antimicrobial property. Further study in this area will be promising enough to reveal any novel compound of interest which can combat the multi drug resistant microorganisms.

4. Conclusion

Research on endophytes have expanded rapidly since recent past as it mimic plant chemistry and secret various novel metabolite and these bioactive metabolites have a broad range of biological activities and could be the starting materials for pharmaceuticals or novel lead structures for the development of pharmaceutical or agrochemical products (Schulz *et al* ., 2002). Screening of bacterial endophytes from *Mimosa pudica* L in the present study is a preliminary report which has revealed one forty one bacterial endophytes which may envision towards the discovering a novel lead compound in future hence further research will be promising enough.

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