Diversity, Interaction and Biological properties of Endophytic fungi associated with *Cucumis dipsaceus* Ehrenb. ex Spach. (Cucurbitaceae)

Thesis

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By

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Summary

SUMMARY

More than centuries, plant based products have been widely used for medicinal and therapeutic purposes. Active compounds from plants were isolated and characterized even today. Many researchers identified that plants have mutualistic internal endophytes. Endophytes are microbes (Bacteria, Fungi and Actinomycetes) that reside in internal tissues of plants without causing any visible symptoms in the host. Only few plants have been completely studied for their endophyte ecology, therefore, handful of endophytes has been described. Knowledge on endophytes and their interaction with host plant were not in depth. Opportunities were available to find novel endophytic microbes, their interaction with host and their natural products. Hence, an attempt is made to identify novel endophytes associated with *Cucumis dipsaceus* which was summarized.

- Leaf and stem tissues of *Cucumis dipsaceus* were collected from foothills of Maruthamalai, Coimbatore district, Tamilnadu, India. Samples were collected aspetically in sterile bags. The plant samples were analyzed for its endophytic fungal diversity, interaction of endophytes with host and biological properties of selected endophytic fungi.
- The sterilization procedure was optimized for leaves and stem tissues to eliminate epiphytes. Effective sterilization was observed using of 80% ethanol for 30sec. and NaOCl for 5 min. Again rinsed with 80% ethanol and finally washed with sterile distilled water.
- Preliminary observation of endophytes in plant tissues were carried out using anatomical and maceration studies. The sections were stained with Lactophenol cotton blue and Tryphan blue which showed fugal mycelia between intercellular spaces of plant cells.
- Isolation of endophytic fungi from leaf and stem tissues were carried out using different media like Potato Dextrose Agar, Sabourad Dextrose Agar, Malt extract Agar and Czapeks Dox Agar. Fungi grown from plant tissues were separated and maintained as pure culture.

- Twenty two isolates namely *Aspergillus* sp., *Aspergillus aculeatus, Aspergillus flavus* 1, *Aspergillus flavus* 2, *Aspergillus fumigatus* 1, *Aspergillus fumigatus* 2, *Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus* 1, *Aspergillus terreus* 2, *Aspergillus terreus* 3, *Aspergillus ustus* 1, *Aspergillus ustus* 2, *Aspergillus ustus* 3, *Chaetomium* sp., *Chaetomium globosum, Melanospora zamiae, Nodulisporium gregarium, Penicillium javanicum, Talaromyces radicus* and *Purpureocillium lilacinum*. were isolated as endophytic fungi from *C.dipsaceus*.
- Host- endophyte interaction was studied using *in vivo* and HPLC studies. This study was carried out to find the true endophytes of *C.dipsaceus*. Under *in vivo* condition all the isolated endophytic fungi were reinoculated into host (*C.dipsaceus*) through their seeds, leaves and soil.
- Only five endophytes namely *Aspergillus* sp., *A.terreus, C.globosum, P.javanicum* and *T.radicus* promoted growth of the host plant. Therefore, these endophytes were considered as true endophytes of *C.dipsaceus*.
- Endophytes produced secondary metabolites similar to their host. To confirm this, presence of Cucurbitacin B produced by *C.dipsaceus* were analyzed in all true endophytes through HPLC method. Results confirmed that *Aspergillus* sp., *A.terreus, C.globosum, P.javanicum* and *T.radicus* also produced Cucurbitacin B.
- Genus and species of true endophytes were further confirmed using molecular identification.
- Biological properties of true endophytes were studied using liquid state fermentation. Suitable basal media was selected to enhance their growth and secondary metabolite production. As a result, Potato Dextrose Broth was found to be effective on their growth and metabolite production.
- The culture parameters like pH, temperature, carbon and nitrogen source were also optimized to enhance their growth and secondary metabolite production. Cultures produced high biomass and metabolite at pH 7, temperature 25˚C, dextrose as carbon source and Yeast extract as nitrogen source.

- The culture filtrates of true endophytes were subjected to preliminary phytochemical analysis. It was observed that all true endophytes possess proteins, flavonoids, phenols, glycosides, terpenoids, alkaloids and tannins.
- The functional groups of the bioactive compounds present in the culture filtrate of *Aspergillus* sp., *A.terreus*, *C.globosum*, *T.radicus* and *P.javanicum* were done using FTIR analysis. All the culture filtrates were found to possess some important groups like amine, alcohol, imine, nitro compounds etc., these group's help to find the nature of the compounds present in the filtrate.
- The culture filtrates were subjected to GCMS analysis, to find the bioactive compounds present in it. Twenty one different types of volatile compounds were present in the culture filtrates. Important compounds like Dibutyl phthalate, Diamyl phthalate, Benzenedicarboxylic acid, Octadecanoic acid were found to possess many biological properties which are useful to human beings.
- Endophytic fungi were subjected to qualitative and quantitative analysis of extracellular enzymes. Results confirmed that all endophytes have the capacity to produce Amylase, cellulase, protease and lipase. None of the endophytes produced Laccase.
- Isolated true endophytes have the capacity to produce organic acids. This is confirmed by the formation of yellow coloured zone around the culture in modified medium.
- Isolated endophytes were subjected to antimicrobial activity. Culture filtrates of endophytes were tested against bacterial and fungal pathogens. Isolates showed more activity towards pathogens but lesser than control (Chloramphenicol and Flucannozole).
- Antioxidant activity was tested on true endophytes using DPPH and Phosphomolybdenum method. All isolates showed about 80-88% antioxidant activity.
- All five endophytes significantly produced plant growth hormones like IAA and GA.