Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

ENDOPHYTIC FUNGI FROM *JATROPHA CURCUS*: A PRELIMINARY STUDY

Santosh Kumar Singh*
Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005. India
E-mail: singhsk71@yahoo.com
DOI: 10.7897/2277-4572.02209
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 25/01/13 Revised on: 21/03/13 Accepted on: 16/04/13

ABSTRACT
Fungal endophytes are ubiquitously reported from the living tissues of healthy plant parts from every host studied so far. These microbes attributed significantly in upraising the caliber of the host to counteract against the different stresses and herbivores, and also some times to improve the host fitness. This study presenting here the endophytic mycoflora of *Jatropha curcus*, which remain less explored. A total of eighteen species of fungi were isolated from leaf, stem, and roots of *Jatropha curcus*. The root was heavily colonized by the genera like *Alternaria, Cladosporium*, and *Aspergillus* spp. The leaf tissues however showed somewhat greater diversity of endophytic colonization. *Drechslera, Curvularia, Bipolaris, Alternaria*, and *Aspergillus* sp. were dominant in to the leaf tissues with strong presence of an unidentified genus. The species richness as well as frequency of colonization of endophytic fungi was more pronounced in the leaf tissues rather than the root and stem. This study reaffirms the fact that endophytes are host and tissues specific. In this regard, the endophytic fungi received in this study, may represent a unique source of one or more of the interesting and useful bioactive compounds similar to those of vinca alkaloid group.
Key Words: Bioactive compounds, *Jatropha curcus,* endophytic fungi, *Alternaria, Cladosporium*

INTRODUCTION

Medicinal plants and their therapeutic potential have always drawn the curiosity and attention to search some new bioactive compounds over many millennia. Many plants to-date has been recognized for providing effective medicines[1]. Some potential medicines are quinine, digitalin, precursor compounds for birth control pills, taxol® and many more[2-5]. But almost all-medicinal plant with therapeutic potential has extensively explored for their natural products, which ultimately forced us to think about some novel source of natural products. Microorganisms especially fungi have long been looking as an important source of novel metabolites with promising anti-bacterial, anti-mycotic, and anti-viral activity. Thus, fungi are the most probably one of the major source of natural bioactive compounds. Approximately 4000 bioactive metabolites of fungal origin have been described to have biological activity[6]. Despite the existence of potent antibiotics and antimycotic agents available in the market, there is a continuous search for novel drug compounds, as many of the microbes pathogenic to the human, animals and plants acquired resistance against these existing 'first generation drugs'[7]. Early searches for bioactive compounds were focused around 'soil fungi', but very soon the recovery of interesting new compounds from the soil fungi have got diminished. So researches have turned to exploration of niches that have not yet been explored[8] for finding of novel pharmacologically active compounds under industrial screening program. One such niche is the healthy green tissue of living plants, which are known to harbor a rich and diverse fungal microbial community 'fungal endophytes' that is distinct from the soil microbial community[9]. In this context it is thought that endophytic fungi isolated from plants of medicinal value may be more promising. The surge of interest in endophytic fungi as a source of novel bioactive compounds is mainly because the difficulty to find new bioactive compound from already extensively investigated organisms[10].

Since *Jatropha curcus* plant is so well documented for its various medicinal properties, therefore, it was selected for the study of endophytes. The aim of this study was to isolate the maximum possible numbers of endophytes from this well-known medicinal plant, to observe the diversity of fungal endophytes in different part of this host and also to explore the possibilities to identify new taxa as well as novel bioactive compounds in future.

MATERIALS AND METHODS

Plant sample collection

The *Jatropha curcus* is a cosmopolitan evergreen herb. In this study we have collected specimen plant from different localities exposed to different ecological conditions. The specimen plants in this study were collected from the agricultural field experimental unit of Banaras Hindu University, Varanasi [25.5º N 82.9º E, elevation 279 ft / 85 m] India. The prevailing conditions at this location support luxuriant growth to the local herbs and trees. Another specimen plant was collected from the Rajeev Gandhi south campus, Barkacchha, of the University, which was located nearly 75 km away. What was unique to the location is the composition of soil, which was brick red with somewhat higher loamy character, supports the growth of local weeds and spiny xerophytic vegetation. The identity was authenticated at the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, by the expert of the medicinal plant. Herbarium voucher specimen reference number is 307 for selected plant (*Jatropha curcus*), preserved in our department.

Isolation, Identification, and preservation of endophytes

The samples were thoroughly washed in running tap water and then were surface sterilized to effectively remove the debris, and epiphytic mycelia adhere to the surface of the sample tissues. A total of 300 segments were taken 50 each

from leaf, stem and root of the two locations. The surface sterilization is a routine task while studying endophytes and this can be achieved by submerging the sample tissues in disinfectant, usually sodium hypochlorite of varying concentration or H_2O_2 of varying concentration. In this experiment I have followed the reported protocol[11]. The sample tissues were first immersed in 75 % ethanol for 2 minutes and then further sterilized in to 5.0 % NaOCl (v/v) for one minute and there after dipped in to 75 % ethanol for 10 seconds. After drying in sterile condition small discs were cut under sterile condition and placed on PDA amended by 50-mg/l chloramphenicol to suppress bacterial contaminations. The Parafilm wrapped Petries were incubated for 25 days on 25 ± 2 º C in BOD cum humidity incubator (L K Scientific Inc. New Delhi).

Macroscopic and microscopic morphological characters of the endophytes were used to identify the recovered endophytic fungal taxa. All isolated and identified endophytic fungi were maintained in cryo-vials on PDA layered with glycerol (15 %, v/v), and also in a lyophilized form at – 20 º C in a deep freezer (Blue Star). All the samples were deposited in the Department of Botany, Banaras Hindu University, India.

Analysis of data

The relative frequency (%CF) of colonization of endophytic species was calculated as number of segments colonized by a single endophyte divided by total number of segments observed X 100. % CF = (Ncol / Nt) X 100

Where, N_{col} = Number of segments colonized by each fungus, and N_t = Total number of segments. One-way ANOVA and paired sample't' test were done to analyze that whether location, site and tissues specific differences in endophyte assemblage, were significant or not.

RESULTS AND DISCUSSION

The endophytic fungi reside into a specific niche (the healthy internal living tissues of the plants) attracts curiosity since its discovery in 1904 in Darnel, Germany. Many initial workers started isolation of endophytic mycoflora of individual host and thus the hidden repertoire of an unexplored microbial diversity came to know. Up to 1980s workers rapidly screened many host plants for their endophytic microbial community; many new taxa were identified and established during that period. The spatial distribution, tissue specificity, environmental variability, tissue age, as well as the positions on to the canopies, sterilization and incubation, were the point of study during this period.

In 1990s, the bioactivity and isolation of secondary metabolites and search for new natural products from endophytic fungi were started. The researches in this particular area, got momentum after the discovery of 'Taxol' a potent anti-cancerous drug, from Taxomyces andreanae an endophytic fungus of Pacific Yew plant (*Taxus brevifolia*)[3], and it was designated as ever most promising natural bioactive molecule discovered against cancer from this new source. This discovery significantly established the fact that the endophytic fungus which resides in the living healthy tissues of the plant, having medicinal or therapeutic potential, may produce one or more bioactive principle or natural products which was earlier known to produce by the host plant. This may happen, only if during course of evolution of symbiosis between fungus and host plant, the reciprocal gene transfer were occurred. As it was observed in case of *Agrobacterium tumefaciens*, the T-DNA of bacterial plasmid transferred and incorporated in to the genome of host plant. This is a fine example of the transaction of genetic materials between prokaryote to eukaryote[12]. However, in case of endophytes host relationship, it's purely eukaryot to eukaryot transaction.

Table 1 the endophytic fungal isolates recovered from the leaf, stem and root tissues of *Jatropha curcus*

Endophytic Fungi	BHU Main Campus			BHU South Campus		
	Leaf	Stem	Root	Leaf	Stem	Root
Aspergillus fumigatous	0	2	3	2	1	2
A. terrreus	1	0	4	0	0	1
Alternaia alternata	3	2	2	4	2	3
A.longipace	0	0	1	0	1	2
Chloridium virescenc	1	0	2	1	4	1
Chloridium cladosporiodes	5	4	1	3	2	1
Curvularia lunata	0	0	2	1	2	0
Humicola sp.	1	0	3	0	3	2
Drechslera sp.	3	0	4	1	1	2
Fusarium moniliformae	0	0	0	2	0	1
F.roseum	2	3	4	1	3	2
Nigrospora oryzae	0	3	0	2	1	
Penicillium crysogenum	0	2	1	3	3	2
Collitotrichum sp.	0	1	2	1	0	2
Penicillium citrinum	4	3	1	3	3	2

Initially, the isolation and documentation of endophytes were taken in this experiment. A total of 15 fungal species were recovered from the leaf, stem, and root tissues of the host (Table 1). The dominant endophytic genera recovered were *Alternaria alternata, Fusarium roseum, Curvularia, Cladosporium,* and *Aspergillus* spp. Three unidentified fungal taxa were placed under the unidentified group, except one of them none was found to produce fruiting bodies. The leaf tissues were having maximum colonization frequency (32.25%) from Loc1 (Location) (BHU Main Campus), while the colonization frequency from the Loc2 (BHU, South Campus) (34.74%) was quite higher than from Loc1. In Loc1 the colonization frequency of root tissues were significantly (38.64%) higher than the leaf tissues (32.25%), while the stem tissues harbor (28.41%). This result confirms the assumption that the soil fungi, rhizospheric fungi, and root pathogenic fungi appear more symbiotic to successfully establish the endophytic relation with the host plant. Although, root endophytes are apparently quite common with wide host and geographic regions, and the ecological role of most species is still to be defined. Alternaria, Cladosporium, Penicillium, and Fusarium sp. were amongst frequently

recovered genera from the root samples. Unidentified genera from root sample were also recovered, however, these days the new biochemical, molecular and genetic markers are being used to distinguish host or site specific strains. This approach was exemplified by the RFLP analysis of sterile *Penicillium fortinii*, isolated from various alpine hosts[13]. Among 18 endophytic fungi, Cladosporium cladosporioides had maximum (11.36%) colonization frequency from loc1, of which leaf tissues were highly colonized (3.3%) while root sample were least colonized (0.7%). Contrast to loc1, loc2 represented 6.31% of total colonization (Table. 2). Leaf again showed maximum (2.0%) colonization, while root sample were least colonized (0.7%). Thus the leaf tissues harbor rich biodiversity of endophytic community and this result corroborates the previous results of some earlier study of the same line. According to many workers, the variation of species isolation could be because of different climatic conditions such as site moisture, rainfall, and wind exposure, which may influence endophytic infestation[14-15]. The locations wise (Loc1 and Loc2) differences in endophytic assemblage were not found significant by paired't' test (P = 0.587). The differences in extent of colonization of the two locations were also not found significant as analyzed by the one-way ANOVA. The colonization frequency of endophytes between Loc1 (P = 1.00) and Loc2 (P= 0.587) could also not show any significant variation (Table 2). So, I can conclude safely that the two locations despite of slight differences in to their environmental condition, having a constant 'core species' of endophyte. The tissues specificity was also observed in certain genera like Aspergillus terreus received dominantly from root tissues. Many earlier workers also exemplified the tissues as well as the host specificity for endophytes[16], however most of the received genera were isolated from both locations and tissues. The potential endophytes received in this study, has its previous records of producing some natural products of host origin like Chloridium virescence. Chloridium virescence was endophytically recovered from stem tissues of loc 2. This fungus has also earlier reported as an endophyte from Terminalia arjuna[17] and Azadirachta indica[18]. A new 12-membered macrolide (chloriolide; **1**) was obtained from solid-substrate fermentation cultures of Chloridium virescens var. chlamydosporum that was originally isolated from decayed wood[19].

Table 2 the relative colonization of endophytic fungi from leaf, stem, and root of Jatropha curcus L. from two different locations

Percentage Colonization Frequency								
BHU Main Campus					BHU South Campus			
Leaf	Stem	Root	Total	Endophytic Fungi	Leaf	Stem	Root	Total
-	1.3	2.0	5.68	*Aspergillus fumigatous*	1.3	0.7	1.3	5.26
0.7	-	2.7	5.68	*A. terreus*	-	-	0.7	1.05
2.0	1.3	1.3	7.95	*Alternaia alternata*	2.7	1.3	2.0	9.47
-	-	0.7	1.14	*A. longipes*	-	0.7	1.3	3.15
0.7	-	1.3	3.41	Chloridium virescence	0.7	2.7	0.7	6.31
3.31	2.7	0.7	11.36	*Cladosporium cladosporiodes*	2.0	1.3	0.7	6.31
-	-	1.3	2.27	Curvularia lunata	0.7	1.3	-	3.15
0.7	-	2.0	4.54	*Humicola sp.*	-	2.0	1.3	5.26
2.0	-	2.7	7.95	*Drechslera sp.*	0.7	0.7	1.3	4.21
-	-	-	-	*Fusarium moniliformae*	1.3	-	0.7	3.15
1.3	2.0	2.7	10.22	*F. roseum*	0.7	2.0	1.3	6.31
-	2.0	-	3.41	*Nigrospora oryzae*	1.3	0.7	-	3.15
-	1.3	0.7	3.41	*Penicillium crysogenum*	2.0	2.0	1.3	8.42
-	0.7	1.3	3.41	*Colletotrichum sp.*	0.7	-	2.0	3.15
2.7	2.0	0.7	9.09	Penicillium citrinum	2.0	2.0	1.3	8.42

So the potential of this endophytic fungus must again be considered for production of bioactive molecules of host origin. Drechslera also recovered from many hosts. *Petasol, an eremophilanes* was recovered from endophytic *Drechslera gingantica* in liquid culture[20]. The results obtained in this work are in agreement with many reports on endophytes from other host in which generally a large number of species can be isolated from a given host, but a few 'rare or incidental' species are present[21]. The scope and future of researches in endophytes constantly and rapidly increasing, as this unique mutual symbiosis enabled host plant to defend against not only insects herbivorey but also from some severe stress conditions[22]. This co-evolution of plant endophyte symbiosis improves the evolutionary fitness of the host to make it compatible for future generation against its contemporary. Keeping this in mind, strategies are to be evaluated to utilize these endophytic fungi not only as biocontrol agents but also as potential 'factories' for the production of secondary metabolites. This might be the alternative source for the bioactive natural products, as our concern is to search few novel antibiotics, agrochemicals and other bioactive molecules to save and secure the human life from threats of novel incurable human and plant diseases. Finally, endophytic research has potential to revolutionize our agricultural, pharmaceutical, and biotechnological research in near future.

REFERENCES

1. Klein E R, Klein P D. A selected bibliography of biomedical and environmental applications of stable isotopes. Biomed Mass Spectrom1979; 6: 515-545. http://dx.doi.org/10.1002/bms.1200061202 PMid:393317
2. Wani MC, Taylor WH, Wall ME, Coggon P, Mc Phail AT. Plant anti tumor agents VI, The isolation, and structure of Taxol- a novel antileukemic and anti tumor agent from Taxus brevifolia. J. Am. Chem. Soc, 1971; 93: 2325-2327. http://dx.doi.org/10.1021/ja00738a045 PMid:5553076
3. Stierle A, Strobel, G, Stierle D. Taxol and taxane production by Taxomyces andreanae , an entophytic fungus of pacific yew. Science. 1993; 260: 214-216. http://dx.doi.org/10.1126/science.8097061 PMid:8097061
4. Hoffman A, Khan W, Worapong J, Strobel G, Griffin D, Arbogast B, Borofsky D, Boone RB, Ning L, Zheng P, Daley L. Bioprospecting for taxol in angiosperms plant extract using HPLC thermo spray mass spectrometry to detect the anticancer agents and its related metabolites in filbert trees. Spectroscopy. 1998; 13: 22-32.
5. Hoffman, A. Poster at American chemical society, San Francisco, April 2000.
6. Dryfuss, MM, Chapela I.H. Potential of fungi in the discovery of novel low molecular weight pharmaceuticals: in the discovery of natural

products with therapeutic potential (Gullo VP, Edt) Butter worth-Heinemann, Boston, MA.1974;49-80.
7. Pinner R, Teutsch S, Simonsen L, Klug H, Graber J, Clarke M, Berkelman R. Trends in infectious disease mortality in united State. J. Am. Med. Soc, 1996; 275:189-193.
8. Pelaez F, Collado J, Arenal F, Basilio, A, Cabello A, Diez matas M T, Garcia J B, Gonzalez Del val A, Gonzalez V, Gorrochategui J, Hernandez P, Martin I, Platas G, Vicente F. Endophytic fungi from plants living on Gypsum soil as a source of secondary metabolites with antimicrobial activity. Mycol. Res.1998; 102: 755-761. http://dx.doi.org/ 10.1017/S0953756297005662
9. Cannon PF, Simmons CM. Diversity and host preference of leaf endophytic fungi in the Iwokrama forest reserve, Guyana. Mycologia.2002; 94: 210-220. http://dx.doi.org/ 10.2307/3761797
10. Corthout J, Pieter L, Clays M, Vanden Berghe A A, VlietinckAJ.Phytochem.1991;30:1129.http://dx.doi.org/ 10.1016/S0031-9422(00)95187-2
11. Petrini, O. Taxonomy of endophytic fungi of aerial plant tissues. J. Fokkema and J. van den Heuvel, ed., Cambridge University Press, Cambridge: Microbiology of the Phyllosphere; 1986: 175-187.
12. Bundock P, den Dulk-Ras A, Beijerbergen A, Hooykaas PJJ. Trans-kingdom T-DNA transfers from Agrobacterium tumefaciens to Saccharomyces cerevisiae. EMBOJ.,1995;14:3206-3214. PMid:7621833 PMCid:394382
13. Stoyke G, Egger K N, Currah R S. Characterization of endophytic fungi from the mycorrhizae of sub alpine plants. Can. J. Bot., 1992;71:2009-2016.http:// dx.doi.org /10.1139/b92-250
14. Holdenrieder O, Sieber TN. Fungal associations of serially washed healthy nonmycorrhizal roots of Picea abies. Mycol. Res., 1992 96: 151-156. http://dx.doi.org/ 10.1016/S0953-7562(09)80932-5
15. Petrini O, Sieber TN, Toti L, Viret O. Ecology metabolite production and substrate utilization in endophytic fungi. Nat. Tox. 1992; 1: 185-196. http://dx.doi.org/10.1002/nt.2620010306 PMid:1344919
16. Toofanee SB, Dulymamode R. Fungal endophytes associated with Cordemoya integrefolia. Fungal Divers. 2002; 11: 169-175.
17. Tejesvi MV, Mahesh B, Nalini M S, Prakash H S, Kini KR, Subbiah V, Hunthrike S S. Endophytic fungal assemblages from inner bark and twig of Terminalia arjuna W. &A. (Combretaceae). World. J. Micro. Biotech.,2005;21:1535–1540. http://dx.doi.org/10.1007 /s11274-005-7579-5
18. Verma V C, Gond SK, Kumar A, Kharwar RN, Strobel GA. Endophytic mycoflora from leaf stem and bark tissues of Azadirachta indica A.Juss from Varanasi India. Microb.ecol.2006;54:119-125.http://dx.doi.org/ 10.1007/s00248-006-9179-9 PMid:17394041
19. Bunkers GJ, Kenfield D, Strobel GA. Production of 'Petasol' by Drechslera gigantea in liquid culture. Mycol. Res., 1991; 95(3): 347-351 http://dx.doi.org/10.1016/ S0953-7562(09)81246-X
20. Kogel K H, Franken P, Huckelhoven R. Endophyte or parasite? What decides? Current Opinian in Plant Biology. 2006; 9: 358-363. http://dx.doi.org/10.1016/ j.pbi.2006.05.001 PMid:16713330
21. Jiao P, Swenson D C, Gloer J B, Wicklow D T. Chloriolide, a 12-membered Macrolide from Chloridium Wirescens var. chlamydosporum. (NRRL37636). J. Nat. Prod., 2006; 69: 636-639. http://dx.doi.org/10.1021/ np0504964 PMid:16643041

Source of Support: Nil, Conflict of Interest: None

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Santosh Kumar Singh. Endophytic fungi from *Jatropha curcus*: A preliminary study. *J Pharm Sci Innov*. 2013; 2(2): 26-29.