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Extracellular enzymatic potential and antimicrobial activity of endophytic fungal isolates from *Operculina turpethum*-an endangered medicinal plant

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Abstract

Twenty eight fungal isolates of endophytic nature were isolated from different plant parts (leaf and root) of *Operculina turpethum*-an endangered medicinal plant by inoculating them on different medium (Malt Extract, Sabouraud Dextrose, Czapek Dox and Potato Dextrose Agar medium).Screening of these fungal isolates for different enzymes (extracellular) showed maximum number of amylase, protease, lipase and l-asparaginase producers.57% of isolates showed good phosphate solubilisation potential. All the fungal endophytes were also evaluated for the inhibitory activity against three fusarial pathogen. Data recorded on percent growth inhibition exhibited good scoring index of leaf 4 and root 4 against *Fusarium chlamydosporum* and *Fusarium tricinctum.* Results indicated the occurrence of good number of endophytic fungi having lot of potential to be exploited for the industrial purpose.

Key words: Endophytic Fungi, Extracellular Enzymes, Antagonism, Medicinal Plants

Introduction

Operculina turpethum (syn. *Ipomoea turpethum* L,) commonly known as Trivrit which belongs to the family Convolvuceacea is large evergreen perennial climber which blooms with fragrant flowers. This plant is known to be native to Bangladesh and India which can also be found in the Pacific Islands, China and Australia. [1]. It is popularly known as "transparent wood rose" and is found to be beneficial for the treatment of various ailments such as ulcers, tumors, neurological disorders, constipation, dysmenorrhea, and inflammation. In addition to this, the plant also possesses anticancer, antiproliferative, antimicrobial, antidiabetic, anti-inflammatory and antihepatotoxic activities. [2]

In this plant, most of its parts have several medicinal properties. The root part of plant contains a glycoside resin, concentrated in the root

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bark known asturpethin is a mild purgative which is used during debility, toxicity, etc [3]. It also contains other glycosides such as Scopoletin, turpethinic acid A, B, C, D, and E etc. The root is made into pasteand is used as an external application in hemorrhoids, chancres and ulcerations. Even oil which is extracted from the root bark is used as cure against skin diseases of a scaly nature. The fresh juice obtained from leaves is being droppedinto the eyes for inducing lachrymation in ophthalmia. [1].Stem part of the plant also contains triterpenes (betulinic acid, betulin, and lupeol) and sitosterol. [2].

Microbes that inhabit inside plant parts of their host without causing any harm to them are called "Endophytes". There are increasing reports on the isolation of different biomolecules from these endophytes therefore, these microbes can be used as a suitable alternate for the plant species which are on verge of extinction and considered to be endangered in the present status.

The common trait of endophytic fungi is penetration and colonization of selected cell of host plant through production of extracellular enzymes. In literature, studies on endophytic fungi are only restricted to screening for secondary metabolites along with antimicrobial and antioxidant properties. In present context, the capability of endophytic fungi as a source of industrially important enzymes has not been explored by many researchers. Hence they still remain unexplored and can serve as a new dimension in obtaining different enzymes with strong potentialities [4]. Hence new sources of valuable extracellular enzymes from endophytic fungi should be discovered in order to understand their functional role in the host plant.

In the present study, we have isolated endophytic fungi from *Operculina turpethum*an endangered plant species and characterized them for useful extracellular enzymatic and antagonistic activity. The objective behind the study is to conserve the fungal population residing in the rare and endangered plants also.

Materials and methods

1. Collection of source material and Isolation of endophytic fungi

The plant *Operculina turpethum* was collected from botanic garden of Regional Plant Resource Centre, Bhubaneswar, Odisha. Various parts of the plant which include leaf, root etc. were washed thoroughly under tap water to remove dirt and then with distilled water. The samples were then surface sterilized in the following sequence first treated with 90% ethanol for 1 min, then 0.5% Sodium hypochlorite for 5 minutes followed by 90% ethanol for 1 min and finally washed with distilled water for 2 times. The root and leaf were cut into thin sections about 3-4mm and inoculated into inoculated in different medium plates that includes Malt extract, Sabouraud dextrose, Czapekdox, PDA in duplicates for each type of medium. The plates were incubated in dark at 28°C for 6-7 days. When colonies appeared, they were sub cultured into freshly prepared Sabouraud dextrose medium plates and allowed to grow for 10 days. Fungal cultures were maintained in slants of Sabouraud dextrose medium slants at 4°C for routine use and at -20°C for long term storage.[5].

2. Extracellular enzymatic activity

a) Amylase Activity (Starch hydrolysis)

All the endophytic fungal isolates were screened for starch hydrolysis on starch agar medium containing 2% starch. All the isolates were spot inoculated on starch agar media and incubated at 28°C for 5-7 days. After incubation, Results were noted by using reagent 1% iodine. The appearance of clear zone surrounding the colony was considered positive for amylase enzyme.

b) Protease Activity

All the endophytic fungal isolates were screened for proteolytic activity on Gelatin agar medium containing 2% gelatin. All the isolates were spot inoculated on gelatin agar media and incubated at 28°C for 5-7 days. After incubation, Results were noted by using reagent 15% $HgCl_2$ and 20% HCl.

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c) Cellulase Activity:

The medium containing 0.5% Carboxy-methylcellulose was used to determine cellulolytic activity. After 3-5days of fungal colony growth, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M NaCl for 15minutes. Appearance of yellow zone around the fungal colony in an otherwise red medium indicated positive cellulase activity.

d) Lipolytic Activity:

For lipase activity, the fungi were grown on Peptone Agar medium supplemented with Tween 20 separately sterilized and added 1% to the medium. At the end of the incubation period, a visible precipitate around the colony indicated positive lipase activity.

e) L-Asparaginase activity

The medium containing 1% L-asparagine was used to determine l-asparaginase activity. After 3-5days of fungal colony growth, the plates were flooded with Nesler's reagent. Strains were screened on the basis of good pink zone formed around microbial colonies in medium
containing phenol red as colour.

3. Phosphate Solubilization activity

Phosphate solubilising activity of endophytic fungi was studied where all the isolates were spot inoculated on Pikovaskaya's agar plates containing Tricalcium phosphate and incubated at 28°C for 3-5 days. After incubation, all the plates were observed for clear halo zone formation around the fungal growth.

4. Antagonistic activity in vitro

The antagonistic activity assay was performed on SDA medium plates by dual culture method [6]. The pathogens selected for the study were species of Fusarium – *Fusarium chlamydosporum, Fusarium graminearum, Fusarium tricinctum.* The mycelial plugs of pathogens and fungal antagonists were placed 2cm from the plate boundary on either side. The plates were incubated at 25ºC for 5 days. Dishes were inoculated with test pathogens were served as control. The Percent growth inhibition (PGI) was calculated by following the method given by Korsten et al., 1995. [7,8]

Results and Discussion

A good number of fungal endophytes (13 no. in leaf) and (15 no. in root) from *Operculina turpethum* plants were obtained. Fungi of endophytic nature from different medicinal plants have been reported well [4]. The medicinal plants are reported rarely as far as fungal association of endophytic type is concerned. Our observation showed that almost all fungi showed extracellular enzyme production except fungal isolate R 8. Overall 43% fungi exhibited amylase production followed by protease, lipase and l-asparaginase production (39%) and cellulose production (36%). fungal isolates L 4 and R 15 showed good potential of extracellular enzymes. (Table-2)

The present study indicated that the roots are more colonized with amylase producing fungi than the leaf. It confirms the rhizosphere as good source of cellulosic material and in turn became the residence of amylase producers.

Among all the fungal isolates screened, 57% (16 isolates) showed clear zone for phosphate solubilisation activity. 6 leaf isolates (46%) and 10 root isolates (67%) were able to solubilise tricalcium phosphate present in the solid medium. R 6 showed greater clear zone as compared to other isolates which is considered to be best phosphate solubilizer in the list. Similiarly, L 11 showed good phosphate solubilisation activity among all the isolates.

Several medicinal plants are reported for antimicrobial properties. Endophytic fungal residents are also confirmed as antagonistic towards many bacterial and fungal strains [9]. Fungal isolates obtained from *Operculina turpethum* were also tested for antifungal activity against three pathogens of *Fusarium* such as *Fusarium chlamydosporum, Fusarium graminearum, Fusarium tricinctum.* Antimicrobial activity of all fungal

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isolates for leaf and roots was depicted in table 5 and 6. It is observed that leaf isolates have shown good relative inhibition against pathogen *Fusarium chlamydosporum* and *Fusarium tricinctum* whereas fungal isolates from root of the medicinal plants exhibited good antagonistic behaviour against all the Fusarial pathogen tested. The score index pertaining to present growth inhibition also has been calculated and it was found 3-4 in root fungal isolates against *Fusarium chlamydosporum* and 2-3 against *Fusarium tricinctum*. The root fungal endophyte R 4 and L4 exhibited maximum percent growth inhibition against *Fusarium chlamydosporum* and *Fusarium tricinctum*. Results confirm that endophytes associated with medicinal plants are potential agents for antimicrobial and extracellular industrial enzymes [9].

Table 1 Extracellular enzymatic activity of fungal isolates from different parts of *Operculina turpethum*

SL.NO.	ISOLATES	AMYLASE	CELLULASE	L-ASPARAGINASE	LIPASE	PROTEASE
1	L 1	-	+	+	-	-
2	L 2	-	+	++	-	+
3	L 3	++	-	+	-	-
4	L 4	++	++	++	-	++
5	L 5	-	+	-	+	-
6	L 6	-	-	-	++	-
7	L 7	-	+	-	++	-
8	L 8	-	-	-	+	-
9	L 9	-	-	-	-	++
10	L 10	-	-	++	-	+
11	L 11	+	-	++	++	-
12	L 12	-	-	-	++	+
13	L 13	-	-	-	++++	+
14	R 1	++	-	+	-	-
15	R 2	+	-	-	-	-
16	R 3	+	++	-	-	-
17	R 4	-	++	-	-	-
18	R 5	-	-	-	-	+
19	R 6	++	+	-	++	-
20	R 7	++	++	-	-	-
21	R 8	-	-	-	-	-
22	R 9	++	-	+	-	+
23	R 10	-	-	++	-	-
24	R 11	-	-	-	-	++
25	R 12	-	-	++	+	++
26	R 13	++	-	+	-	-
27	R 14	++	-	-	+++	-
28	R 15	++	+	-	+++	+

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Table 2 Phosphate solubilisation potential of fungal isolates from different parts of *Operculina turpethum*

SL.NO.	ISOLATES	PHOSPHATE SOLUBILISATION
1	L 1	-
2	L 2	++
3	L 3	-
4	L 4	++
5	L 5	-
6	L 6	+
7	L 7	++
8	L 8	-
9	L 9	-
10	L 10	+
11	L 11	+++
12	L 12	+
13	L 13	+
14	R 1	-
15	R 2	-
16	R 3	+
17	R 4	-
18	R 5	-
19	R 6	++++
20	R 7	-
21	R 8	+
22	R 9	-
23	R 10	+
24	R 11	+
25	R 12	++
26	R 13	-
27	R 14	++
28	R 15	+++

(++++)-High activity, (+++)- Good activity, (++)- Medium activity, (+)- Low activity, (-) – No activity.

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Table 3 Evaluation of Antifungal properties of of fungal isolates from different parts of *Operculina turpethum*

		Fusarium chlamydosporum			*Fusarium graminearum*			*Fusarium tricinctum*		
SL.NO.	Organism	R1 (in mm)	%PGI	SCORING INDEX	R1(in mm)	%PGI	SCORING INDEX	R1 (in mm)	%PGI	SCORING INDEX
1	L 1	8	60	3	27	40	2	11	56	3
2	L 2	11	45	2	41	9	1	16	36	2
3	L 3	10	50	2	28	38	2	14	44	2
4	L 4	3	85	4	34	24.4	1	4	84	4
5	L 5	6	70	3	38	16	1	19	24	1
6	L 6	18	10	1	39	13.3	1	15	40	2
7	L 7	10	50	2	41	9	1	19	13.3	1
8	L 8	12	40	2	45	0	0	16	36	2
9	L 9	8	60	3	40	11.1	1	20	20	1
10	L 10	8	60	3	36	20	1	9	64	3
11	L 11	13	35	2	45	0	0	22	12	1
12	L 12	12	40	2	42	6.7	1	17	32	2
13	L 13	9	55	3	38	16	1	14	44	2
14	R 1	7	65	3	31	31.1	2	11	56	3
15	R 2	5	75	3	42	6.7	1	13	48	2
16	R 3	8	60	3	27	40	2	12	52	3
17	R 4	2	90	4	15	67	3	3	88	4
18	R 5	9	55	3	43	4	1	18	28	2
19	R 6	5	75	3	45	0	0	15	40	2
20	R 7	4	80	4	24	47	2	14	44	2
21	R 8	4	80	4	23	49	2	9	64	3
22	R 9	6	70	3	36	20	1	8	68	3
23	R 10	9	55	3	33	27	2	10	60	3
24	R 11	9	55	3	41	9	1	11	56	3
25	R 12	11	45	2	45	0	0	12	52	3
26	R 13	10	50	2	28	38	2	14	44	2
27	R 14	12	40	2	43	4.4	1	13	48	2
28	R 15	12	40	2	14	69	3	8	68	3

R1= Relative inhibition
% PGI=Percent growth inhibition

SI= Scoring index

Conclusion

Operculina turpethum was found to be a good source of endophytic organism with exploitable potential of industrial enzymes production and antimicrobial behaviour. Since it is rare and endangered medicinal plant, studies on isolation and conservation of associated endophytes is also a good approach along with the mass propagation and conservation of the medicinal plants towards the enrichment of biodiversity conservation programme worldwide.

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