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Antagonistic effect of chosen endophytic fungus ASUF11 and ASUF23 **isolated from green seaweed *Ulva faciata***

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Abstract

Two endophytic fungi (ASUF11and ASUF23) were confined from the plump leafs of seaweed *Ulva faciata* collected from south peninsular bank of India and were removed by utilizing standard methodology. The antibacterial exercises of concentrates were completed against normal bacterial microbes. Both the concentrates displayed huge bactericidal action in all microorganisms. The methanolic concentrates of ASUF23 showed wide range antibacterial movement (70%) in both gram positive and gram-negative microorganisms than different gatherings.

Key words: Endophtic fungus, Antagonistic effect, Natural medicine

Introduction

The term advantageous interaction can be applied to various sort of affiliations. They can be generally arranged into mutualism, commensalism and parasitism as per benefits for the two accomplices (mutualistic advantageous interaction), microbial advantage not influencing the host plant (commensalistic beneficial interaction) or adverse consequence on have wellness (pathogenic or parasitic cooperation) (Rodriguez, et al., 2008). The lines between these classifications are, nonetheless, not fixed. For instance, mycorrhizal associations, the most known and far and wide earthbound plant-organism symbioses, are regular mutualistic (Li and Liu, 2004). Contingent upon ecological circumstances, notwithstanding, the organism can adversely affect plant advancement (Davis et al., 2003). Likewise, hereditary variables can switch this

connection (Redman et al., 1999). Then again, pathogenic organisms can colonize their hosts without causing any side effects (Omacini et al., 2001). Contagious endophytes colonize inner tissues without causing sickness side effects or plain tissue harm in their hosts (Musa et al., 2011). This gathering contains mycorrhizal growths along with the non-mycorrhizal organisms, of which the last option happen in over the ground plant tissues and in roots (Brundrett, 2004). The ongoing review was meant to seclude endophytic organism from broadened tomato plants and the separates were described to decide their ordered position and colonization design. This concentrate additionally focused to screen the antibacterial properties of endophytic parasitic metabolites secluded from the plump leafs of seaweed *Ulva faciata* gathered from south peninsular shore of India.

Materials and Methods

Isolation of Endophytic fungus

Green seaweed *Ulva faciata* samples were collected *in situ* at 0.52m depth during Nov2018 from the distinct sites of Mandapam coastal regions, south peninsular coast of India (latitude 9°17N, longitude 79°22E). The seaweed samples was picked with hand and immediately washed with seawater to remove the foreign particles, sand particles and epiphytes. The samples were transported to the laboratory on ice. The seaweed samples appeared to be healthy at the time of collection were used in this study.

In the laboratory, fleshy leaves of *Ulva faciata* were disinfected with 70% ethanol for one minute and random samples were directly transferred to 5% of a commercial sodium hypochlorite solution (2.5% NaOCl) for five or ten minutes (primary roots). After washing three times with sterile distilled water, thirty fragments of approximately 1-2 cm were cut per each disinfected leaf system and the effectiveness of surface sterilization was verified on potato dextrose agar (PDA; Hi- media, Mumbai) using the imprint technique (Swofford, 2000). Subsequently, each leaf fragment were subdivided into three pieces and each one was transferred on PDA, sabouraud dextrose agar (SDA; Hi- media, Mumbai) and 2% malt extract agar (MEA; Hi- media, Mumbai) supplemented with 0.5 mg ml-1 chloramphenicol (Merk, India) and incubated at 25^{o}C. Each fragment was checked weekly for eight weeks for mycelia emerging from the cut ends. Emergent mycelia were subcultured to new PDA plates and incubated in darkness at 25^{o}C to recover pure fungal colonies. Pure isolates were maintained on PDA covered with sterile oil at 4^{o}C.

Characterization of endophytic fungus

After recovering on agar, pure colonies of the selected endophytes were grown in different culture media such as PDA (Merck, India), corn meal agar (Sigma-Aldrich, Germany), SDA (Hi Media, Mumbai) and 1.5 % water agar (Hi Media, Mumbai) at 22o C or 25 oC. The fungus was grown in different pH (5.0, 5.5, 6.0, 6.5 and 7.0). Growth rate of the colonies was measured.

Isolation of secondary metabolites from endophytic fungus

The selected endophytes grown on PDA media was dried in room temperature and about 250 g of finely powdered material was refluxed three times in a 1 liter capacity round bottom flask in a water bath for about 6 h using 500 ml of appropriate solvents (methanol). The extracts were filtered and concentrated to recover the excess solvents in another distillation system. The concentrated extract (about 100ml) was again filtered through a Whatman no. 1 filter paper fitted with a Buchner funnel using suction pressure. Finally it was reduced to thick oily natured crude extract in a rotary vacuum evaporator (JSGW-Buchi type) at 40^{o}C, collected in air–tight plastic vials and stored in the refrigerator for further activity studies.

Antibacterial activity

The agar plate diffusion assay was used to evaluate the antimicrobial activity against four different bacterial strains such as *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC 1541), *Escherichia coli* (MTCC 443) and *Klebsiella pneumoniae* (MTCC 109). A 100 µl of bacterial liquid culture, in an exponential growth phase, was spread onto the surface of Muller Hinton agar plate. Immediately, 100 µl of crude extract of the fungal isolates were loaded onto the well. The culture was incubated at 30^{o}C for 18 h and the zone of inhibition was measured. Standard discs of Tetracycline (10 µg) and Chloramphenicol (10 µg) was used as positive control for antibacterial activity.

Results

Isolation and Identification of endophytic fungi

23 fungal isolates were initially obtained from 20 leaf fragments of green seaweed *Ulva faciata*. Among the 23 isolates, 21 do not show growth in culture after recovering from oil and were therefore not further characterized. After the isolation, a total of 2 different isolates were recovered from 20 disinfected seaweed leafs. The number of fungal isolates recovered from leaf fragments was 2 (8.6%). These isolates could be roughly categorized according to their morphological characteristics.

Characterization of fungal endophytes

The characterizatistic features of endophytes were ASUF11and ASUF23 were displayed in the table:1. These isolated produces branched mycelium with septate; hyaline, pale brown or grayish white. Conidiomata is acervular, separate or confluent, composed of hyaline to dark brown, thin or thick walled texture angularis; dehiscence irregular. Setae are sparse. Conidiogenous cells are enteroblastic to phialidic, hyaline. Conidia are fusiform, apices obtuse 9 – 24 X 3-4.5 µm. The textures of second type mycelium are spongy, cottony and flocculate. The colour of the colony is bright white at young stage and light pale orange at mature stage. Based

on the morphological characteristics of fungal colony were successfully identified as *Aspergillus flavus* (ASUF11) and *Aspergillus sp.* (ASUF23).

Growth in different mycological media

The radial growth of the fungal isolate was studied on different solid media like PDA, CMA, SDA and water agar. Among the various media, maximal growth was observed on PDA medium. The fungus was grown in different pH (5.0, 5.5, 6.0, 6.5 & 7.0) of PDA Broth medium for 21 days at 30^{o}C and the growth of mycelial growth was studied. (table. 2).

Table 1. The morphological description of isolated endophytic fungi

Sl. No	Code	Description	Species
1.	ASUF11	Colonies white or silvery white, reverse green to orange brown or reddish brown with thin or thick walled texture angularis. Conidial heads columnar in size. Vesicles globose to ovate, metulae fertile over entire vesicle, conidial heads splitting over age. Conidia smooth, irregular. Setae are sparse globose, 2-3cm in diameter. Conidia are fusiform	*Aspergillus flavus*
2.	ASUF23	The colony was pulvinate in appearance, green at the centre, with light yellow radial rays and white color edges. The diameter of the colony was 1.05 cm/day. The conidiophores were 500-530 cm inlength and 7.5 cm in width. The conidial heads were columnar, compact, about 15 cm in length and 13 cm in breadth from the line of the phyllade heads. The vesicles were flask shaped aseptate in nature.	*Aspergillus sp.*

Table 2. Growth of endosymbionts in different mycological media

Endosymbionts	PDA					CMA					SDA				
	5	5.5	6	6.5	7	5	5.5	6	6.5	7	5	5.5	6	6.5	7
ASUF11	+	+	+++	+	+	-	+	+	+	+	-	+	+	+	+
ASUF23	+	+	++	+	+	-	-	+	-	-	-	-	+	+	-

(- - No growth, + - minimum, ++ - moderate, +++ - maximum)

Antimicrobial potential of endophytic fungi with potential pathogens*B. Subtilis, M.luteus , E. coli* and *K. Pneumonia* are given in table 3. Both the fungus ASUF11 and ASUF23 showed significant antimicrobial activity at the rate of 28, 26, 24 and 16 mm and 26, 24, 22 and 16 respectively

Table 3. Antimicrobial potential of endophytic fungi with potential pathogens (in mm)

Endosymbionts	*B. subtilis*	*M. luteus*	*E. coli*	*K. pneumoniae*
ASUF11	28	26	24	16
ASUF23	26	24	22	16

Discussion

Two parasitic endophytes were disengaged and refined in the research facility. Morphological examinations, utilizing both naturally visible and tiny elements, have brought about the recognizable proof of four parasitic species: *Aspergillus flavus* and *Aspergillus sp.,* This study addresses the principal endeavor to confine and distinguish endophytes from ocean growth *U. faciata*leafs in Indian situation and in this way it gives an examination base to additional examinations. Among that the way of life media, maximal development was seen in PDA medium with pH 6.0 when contrasted and the development on other pH's of the medium. The antibacterial impact of the growths removes, the ASUF11 extricates delivered profoundly factor zones going between 16-37 mm

The yield of bioactive mixtures can some of the time be significantly expanded by the advancement of physical (temperature, saltiness, pH and light) and synthetic variables (media parts, antecedents, and inhibitors) for the development of microorganisms (Miao et al., 2006; Gautam et al., 2011; Bhattacharyya and Jha, 2011). Among the tried media, most extreme mycelial dry weight (74 mg/25 mL) was kept in potato dextrose mediummedium, trailed by malt separate medium (55 mg/25 mL) and czapek's dox medium (51 mg/mL) though sabouroud'smedium and supplement medium showed 28 mg/mL and 30 mg/mL separately. Additionally, greatest bioactive metabolite was created in potato dextrose medium (23 mm against *Klebsiella sps*) trailed by malt separate medium (17 mm against Staphylococcus aureus) and czapek's dox medium (19 mm against *Klebsiella sps*)(Gogoi et al., 2008; Ritchie et al., 2009).

Least creation of bioactive metabolite was seen in sabourod'smedium (5 mm against Klebsiella sps) and supplement medium (7 mm against Vibrio cholera). Perrone et al., (2008) announced the potato dextrose mediummedium as the best mode for the greatest development of *Drechslerahawaiiensis*, the foliar curse microorganism of *Marsilea minuta.* Comparably the marine inferred organism *Arthrinium c.f. saccharicola* was examined by Miao et al., (2006) and recommended that the way of life medium affected mycelial development and metabolite profile.

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