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Endophytic fungi of marine algae and seagrasses: a novel source of chitin modifying enzymes

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Abstract

Endophytic fungi (which infect living tissues of plants and reside in them without causing any visible disease symptoms) were isolated from 19 seaweed and 10 seagrass species growing in Mandapam (Palk Bay, 9°16′N, 79°7′E), Keezhakarai (Palk Bay, 9°13′N, 78°46′E), Kodiyakkarai (Palk Strait, 10°16′N, 9°49′E) and Kovalam (Bay of Bengal, 8°22′N, 76°59′E) along the eastern coast of Tamilnadu state, southern India and screened for the production of chitinase and chitosanase enzymes. This study was done during July 2012- December 2012. Of the 117 fungi screened, 14% was positive for chitinase, 41% was positive for chitosanase acting on chitosan of 56% degree of acetylation, 66% was positive for chitosanase acting on chitosan of degree of acetylation 38% and 56% was positive for chitosanase acting on chitosan of degree of acetylation 1.6%. Among the isolates, a *Penicillium* sp. and a *Cladosporium* sp. showed high chitinase activity. Presence of NaCl in the medium influenced the production and activity of chitinase and chitosanase. This study identifies for the first time endophytic fungi of marine plants as a novel source of chitin modifying enzymes which find use in food, cosmetics, agriculture and pharmaceutical industries.

Key words – chitinase – chitosanase – marine-derived fungi – marine angiosperms – seaweeds

Introduction

Chitin, a polymer of β-1,4 linked N-acetylglucosamine, is an abundant but under-utilized natural resource obtained mostly from shells of marine crustaceans. Both chitin and chitosan, a partially deacetylated chitin derivative, are non-toxic, biodegradable and biocompatible and hence find use in food, cosmetics, agriculture and pharmaceutical applications (Dutta et al. 2004). Chitooligosaccharides are oligomers derived from chitin or chitosan with potential to be used as drugs, antibacterial agents, wound-healing chemicals and vectors in gene therapy (Aam et al. 2010, Park & Kim 2010). The process of obtaining chitin and chitosan from the shells of crustaceans involves treatment with strong alkali; similarly, chitooligosaccharides are generated by acid hydrolysis of chitosans. Here, the use of chitin modifying enzymes such as chitinase and chitosanase to obtain functional chitin derivatives including biofunctional chitooligosaccharides

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appears to be a better option as it is environmentally friendly and would result in products with known characteristics such as molecular weight, molecular weight distribution, and pattern of N-acetylation (Aam et al. 2010). In this context, bacterial chitin modifying enzymes have received more attention than those of fungi (Kielak et al. 2013, Frederiksen et al. 2013). Fungal chitin modifying enzymes, apart from modulating their growth, branching, spore formation and spore germination (El Gueddari et al. 2002, Karlsson & Stenlid 2008), play a major role in recycling nitrogen and carbon trapped in chitin (Kellner et al. 2010). Of the different ecological groups of fungi, endophytes appear to possess a repertoire of novel enzymes (Nagarajan et al. 2014, Thriunavukkarasu et al. 2011) including chitin modifying enzymes (Govinda Rajulu et al. 2011; Nagaraju et al. 2009). Endophytes are fungi which infect living tissues of plants and survive in them without producing any apparent disease symptoms. Although a few recent studies have shown that infection by endophytes increases the host plant's tolerance to abiotic (Vesterlund et al. 2011) and biotic (Rocha et al. 2011) stress and results in the up-regulation of many defense-related genes of the host plant (Mejía et al. 2014), many questions regarding endophyte biology remain unanswered (Suryanarayanan 2013). We screened in this study endophytes (which were marine-derived fungi) isolated from marine algae and seagrasses for their chitin modifying enzymes. Fungi occurring in marine ecosystems are broadly classified as true or obligate marine fungi and marine-derived fungi; the former grow and sporulate only in marine environment while the latter are terrestrial fungi but also occur in marine environments (Kohlmeyer & Kohlmeyer 1979).

Materials & Methods

Isolation of endophytes from seaweeds and seagrasses

Endophytes isolated from 19 seaweed and 10 seagrass species were screened in this study. The seaweeds included seven brown algae [*Lobophora variegata* (LVA), *Padina tetrastromatica* (PTE), *Sargassum ilicifolium* (SIL), *S. wightii* (SWI), *Sargassum* sp. (SAR), *Stoechospermum marginatum* (SMA) and *Turbinaria* sp. (TUR)], seven green algae [*Caulerpa racemosa* (CRA), *C. scalpelliformis* (CSC), *C. sertularioides* (CSE), *Chaetomorpha* sp. (CHA), *Halimeda macroloba* (HMA), *Ulva fasciata* (UFA) and *U. lactuca* (ULA)], and five red algae [*Gracilaria edulis* (GED), *Grateloupia lithophila* (GLI), *Halymenia* sp. (HAY), *Jania adharens* (JAD) and *Portieria hornemanii* (PHO)]. The seagrasses included *Cymodocea serrulata* (CYS), *Halodule beaudettei* (HAB) , *H. uninervis* (HAU), *Halodule* sp.1 (HAL), *Syringodium* sp. (SYR), belonging to the family Cymodoceaceae and *Enhalus acoroides* (ENA), *Halophila ovalis* (HAO), *Thalassia* sp. 1(TH1), *Thallasia* sp. 2 (TH2) and *Thalassia* sp. 3 (TH3) belonging to the family Hydrocharitaceae. Seaweeds and seagrasses were collected from Mandapam (Palk Bay, 9°16′N, 79°7′E), Keezhakarai (Palk Bay, 9°13′N, 78°46′E), Kodiyakkarai (Palk Strait, 10°16′N, 9°49′E) and Kovalam (Bay of Bengal, 8°22′N, 76°59′E) along the eastern coast of Tamil nadu state, southern India.

Fresh algal thalli and healthy, mature seagrasses having undamaged leaves and growing in shallow seawater were collected and brought to the laboratory in sterile polythene bags and processed within 24 h. The surface sterilization protocol for isolating endophytes was as follows. The seaweeds were washed in running tap water and cut into segments of approximately 0.5 cm^2; these tissue segments were dipped in 70% ethanol for 5 s followed by immersion in sterile distilled water for 10 s (modified after Zhang et al. 2009). Seagrasses were washed in running tap water and tissue segments (0.5 cm^2) were cut from the mid portion of mature leaves and rhizomes and surface sterilized as follows. Segments were rinsed in 70% ethanol for 5 s, immersed in 4% sodium hypochlorite for 60 s and finally rinsed in sterile distilled water (Devarajan et al. 2002).

One hundred sterilized segments from each seaweed or seagrass were plated on potato dextrose agar medium amended with an antibiotic (chloramphenicol 150 mg l^{-1}) in Petri dishes and incubated in a light chamber for four weeks at 26^{o}C; the light regimen was 12 h of light followed by 12 h of darkness (Suryanarayanan 1992). The Petri dishes were observed periodically, and the

fungi that grew out from the tissues were isolated and identified based on spore morphology. The identity of five of the isolates was further corroborated by 5.8S rRNA analysis - 542- *Chaetomium spirochaete* (GenBank Accession No. **JN209921),** 548 - *Chaetomium globosum* **(JF826006),** 568 - *Trichoderma harzianum* **(KC330218)**, 580 - *Fusarium oxysporum* **(KC254033),** 590 - *Alternaria brassicae* **(JX984695)** [Kaushik et al. 2014].

Preparation of chitinase and chitosanase from fungal culture filtrates

An endophyte was grown in Potato Dextrose medium (Potato 200 g, Dextrose 20 g, distilled water 1000ml, pH 6) for 5 days as static culture at 26°C and the mycelium was removed by filtering through Whatman No. 1 filter paper. One hundred ml of the culture filtrate was dialyzed for 15 h against distilled water. The dialyzed culture filtrate was lyophilized and used as crude enzyme source.

Dot blot assay and visualization of enzyme activity

A composite gel consisting of stacks of glycol chitin, chitosans of 56%, 38% or 1.6% degree of acetylation was layered as follows (Govinda Rajulu et al. 2011).

A gel was prepared by mixing a solution of 1 ml of 30% Acrylamide/Bisacrylamide, 0.1 ml substrate (1 % glycol chitin), 1.9 ml of 50 mM sodium acetate buffer (pH 5.5), 0.003 ml of 100 % tetraethylmethylenediamide (TEMED) and 0.003 ml of 40 % ammonium persulphate, poured in a gel cassette and allowed to polymerize. A few drops of butanol was added to the top of the solidifying gel to help polymerization. After 20 min, the butanol was decanted and the gel was topped with a solution of 1 ml of 30 % Acrylamide/Bis acrylamide, 0.3 ml substrate (0.1% chitosan 56% Degree of Acetylation, 1.7 ml of sodium acetate buffer (pH 5.5), 0.003 ml of 100% TEMED, 0.003 ml of 40% ammonium persulphate. The surface of this gel was layered with a few drops of butanol to facilitate polymerization. After 20 min, the butanol was removed and this process was repeated with (0.1% chitosan degree of acetylation 38% or 0.1% chitosan degree of acetylation 1.6%). Thus a compound gel consisting of chitin (100% degree of acetylation), and chitosans of 56% degree of acetylation 38% degree of acetylation or 1.6% degree of acetylation was obtained.

5 mg of the lyophilized culture filtrate of each endophyte isolate (enzyme source) was mixed in 1 ml of 50 mM sodium acetate buffer (pH 5.2) and centrifuged at 14000 rpm for 5 min (20 ˚C). 10 µl of the supernatant was loaded on the gel and incubated at 37 ˚C for 24 h; gels were stained with 0.1 % calcofluor white for 5 min and washed with distilled water for 1 h and observed under UV transilluminator to detect zones of darkness which indicated enzyme activity. A 50 mM Sodium acetate buffer, pH 5.2, (10 µl) spotted on the gel served as control.

Effect of salt on chitin modifying enzymes

Four representative isolates which were positive for all the chitin substrates screened isolates [*Aspergillus* sp. (507), *Humicola* (509), Sterile form (515) and *Penicillium* sp. (645)] were selected and cultured in PD medium or PD medium amended with 0, 0.3 or 0.6M NaCl and their culture filtrate was screened by dot blot assay for chitinase and chitosanase acting on chitosan of degree of acetylation 1.6% as mentioned above.

Spectrophotometric assay of chitinase *(Wirth & Wolf 1990)*

A reaction mixture consisting of 0.6 ml of 0.1 M sodium acetate buffer (pH 5.2), 0.2 ml of CM-chitin-RBV (Remazol Brilliant Violet-dye labelled chitin, Löwe Biochemica, Germany), and 0.02 ml of enzyme preparation was made. The blank tube contained all these except the enzyme which was replaced by 0.02 ml of buffer. The reaction mixture was mixed well and incubated at 37 ˚C for 30 min allowing the substrate to be digested. The reaction was terminated by adding 0.2 ml of 2 N HCl, incubated in 0 ˚C for 10 min, centrifuged at 14000 rpm for 5 min and the supernatant used. The absorbance of the dye labelled *N*-acetylglucosamine monomers and/or oligomers released from the violet dye labelled chitin was estimated at 550 nm.

Results

A total of 117 endophyte isolates (73 isolated from seaweeds and 44 from seagrasses) were screened for chitin modifying enzymes using glycol chitin (for chitinase) and chitosans with different degree of acetylations (DA) (for chitosanase) as substrates (Table 1). *Aspergillus flavus* (625), *A. nidulans* (560), *Aspergillus* sp. (507), *Aureobasidium pullulans* (585), *Chaetomium* sp. (680), *Cladosporium* sp. (624), *Humicola* sp. (509), and *Penicillium* spp. (552, 645, 642) produced enzymes which acted on chitin and all the three chitosan substrates tried.

Table 1 Chitinase and chitosanases enzyme activity of marine derived endophytic fungi (dot blot assay).

				Chitosans		
Fungus	**Isolate**	**Host code**	**Glycol chitin**	**DA 56%**	**DA 38 %**	**DA 1.6 %**
Alternaria brassicae	590	TUR	-	-	++	-
Alternaria sp.	603	SAR	-	-	++	++
Alternaria sp.	572	SWI	-	-	-	++
Alternaria sp.	626	SMA	-	-	-	-
Alternaria sp.	592	TUR	-	-	++	++
Alternaria sp.	590	TUR	-	-	++	-
Aphanocladium sp.	508	TH1	-	-	-	-
Aspergillus flavus	625	TH1	+	++	+++	+++
Aspergillus sp.	591	TUR	-	+++	+++	+++
Aspergillus sp.	597	TUR	-	-	+++	+++
Aspergillus sp.	545	ULA	-	+	++	+++
Aspergillus sp.	524	CRA	-	-	-	-
Aspergillus sp.	533	CSC	-	+	++	-
Aspergillus sp.	525	CSE	-	-	-	-
Aspergillus sp.	551	HMA	-	++	+++	+++
Aspergillus sp.	600	SAR	-	+	++	-
Aspergillus sp.	569	SWI	-	-	-	+
Aspergillus sp.	570	SWI	-	+++	+++	-
Aspergillus sp.	636	ENA	-	++	+++	++
Aspergillus sp.	637	ENA	-	++	+++	+
Aspergillus sp.	507	TH1	+	+	++	++
Aspergillus sp.	643	CYS	+	+	-	+
A. janus	581	SWI	-	+++	+++	++
A. nidulans	541	PHO	-	++	++	-
A. nidulans	567	SWI	-	+++	+++	-
A. nidulans	560	HAY	+	+++	+	+
A. niger	523	CRA	-	-	-	-
A. niger	531	CSC	-	-	-	-
A. niger	544	ULA	-	-	-	+
A. niger	526	CSE	-	-	-	-
A. terreus	532	CSC	-	-	++	-
A. terreus	539	GED	-	-	++	+++
A. terreus	550	HMA	-	++	+++	-
A. terreus	598	SAR	-	-	++	+++
A. terreus	537	SIL	-	-	++	+++
A. terreus	611	TUR	-	+	+++	+++
A. terreus	615	UFA	-	++	+	+++
A. terreus	543	ULA	-	++	++	++
A. terreus	644	CYS	-	-	++	-
A. terreus	639	HAB	-	++	+++	++
A. terreus	640	SYR	-	++	+++	++
A. terreus	641	SYR	+	+++	+++	-
A. terreus	635	ENA	-	-	-	+++
Aureobasidium pullulans	585	TH1	+	+	+	-
Chaetomium globosum	548	ULA	-	-	+++	+
Chaetomium spirochaete	542	GED	-	+	++	+++

Fungus	Isolate	Host code	Glycol chitin	Chitosans		
				DA 56%	DA 38 %	DA 1.6 %
Chaetomium sp.	559	HAY	-	-	-	-
Chaetomium sp.	558	PTE	-	+	-	-
Chaetomium sp.	576	SMA	-	+++	+++	-
Chaetomium sp.	680	TH3	++	+	++	+
Cladosporium sp.	575	GLI	-	-	+++	+++
Cladosporium sp.	564	PHO	-	-	-	++
Cladosporium sp.	579	SWI	-	-	-	-
Cladosporium sp.	547	ULA	-	-	+	+
Cladosporium sp.	512	HAO	+	-	+	-
Cladosporium sp.	624	TH1	+	+	++	++
Cladosporium sp.	520	CRA	-	+++	+++	+++
Cladosporium sp.	601	SAR	-	-	++	-
Cladosporium sp.	505	TH1	-	-	-	-
Cladosporium sp.	506	TH1	-	-	-	-
Colletotrichum sp.	513	HAO	-	-	-	-
Curvularia lunata	608	SAR	-	-	-	-
Curvularia sp.	535	CSC	-	-	-	-
Curvularia sp.	566	SWI	-	-	+	+++
Curvularia sp.	577	SWI	-	-	-	+
Curvularia sp.	593	TUR	-	-	++	+
Curvularia sp.	638	ENA	-	-	+	-
Curvularia sp.	582	TH1	-	-	-	-
Curvularia tuberculata	606	TUR	-	-	-	+
C. tuberculata	516	SYR	-	-	-	-
C. tuberculata	503	TH1	-	+	+++	++
Drechslera papendorfii	589	TUR	-	-	-	-
Drechslera sp.	610	TUR	-	++	+++	-
Drechslera sp.	583	TH1	-	-	-	+++
Fusarium oxysporum	580	SWI	-	+++	+++	+++
Fusarium sp.	595	TUR	-	-	+++	-
Fusarium sp.	623	TH1	-	-	-	-
Gonatophragmium mori	622	TH1	-	-	+	+++
Humicola sp.	509	TH2	+	++	++	+++
Memnoniella sp.	647	CYS	-	-	++	++
Nigrospora sp.	573	GLI	-	-	-	++
Nigrospora sp.	538	JAD	-	-	-	-
Nigrospora sp.	594	TUR	-	+++	+++	+
Nigrospora sp.	627	UFA	-	-	++	-
Nigrospora sp.	546	ULA	-	-	-	++
Nigrospora sp.	621	HAU	-	-	+	-
Nigrospora sp.	646	CYS	-	-	+	-
Nigrospora sp.	685	CYS	-	+	++	-
Nodulisporium sp.	684	SYR	-	-	+	-
Nodulisporium sp.	679	TH3	-	++	++	++
Paecilomyces sp.	534	CSC	-	-	-	-
Paecilomyces sp.	584	TH1	-	+	-	++
Paecilomyces sp.	681	TH1	-	-	+++	+++
Penicillium sp.	527	CSE	-	+++	+++	+++
Penicillium sp.	563	PHO	-	-	-	-
Penicillium sp.	511	HAO	-	-	-	-
Penicillium sp.	521	CRA	-	+++	+++	+++
Penicillium sp.	536	CSC	+++	+++	++	-
Penicillium sp.	552	HMA	+	+++	+++	+++
Penicillium sp.	645	CYS	+	+	++	+++
Penicillium sp.	642	HAB	+++	+++	+++	+++
Penicillium sp.	504	TH1	-	+	+++	+++
Pestalotiopsis sp.	574	SWI	-	-	-	+
Phoma sp.	605	SAR	-	-	+	++
Phomopsis sp.	562	PHO	-	-	-	-

Fungus	Isolate	Host code	Glycol chitin	Chitosans DA 56%	Chitosans DA 38 %	Chitosans DA 1.6 %
Pithomyces sp.	604	SAR	-	-	+	-
Pithomyces sp.	682	HAL	+++	-	++	++
Sterile form	515	HAO	+	+++	+++	+++
Sterile form	530	CRA	-	-	-	-
Sterile form	565	PHO	-	-	-	++
Sterile form	614	ULA	-	-	+++	++
Sterile form	619	CHA	-	-	++	-
Sterile form	561	LVA	-	-	-	-
Sterile form	686	CYS	-	-	+	+++
Sterile form	687	CYS	-	+	+++	++
Torulomyces sp.	683	SYR	-	+++	+++	+
Trichoderma harzianum	568	SWI	-	-	+++	-
Xylariaceous form	678	TH3	-	++	++	++

- = No activity, + = Low activity, ++ = Medium activity, +++ = High activity

Chitinase

Of the 117 endophyte isolates screened by dot blot assay, 16 isolates (14%) were positive for chitinase enzyme (Table 1, Figs. 1-2); these included *Aspergillus* spp., *Aureobasidium pullulans*, *Chaetomium* sp., *Cladosporium* sp., *Humicola* sp. and *Penicillium* spp. These isolates were assayed spectrophtometrically for chitinase. Of these, a *Penicillium* sp. (isolate 645) endophytic in the seagrass *C. serrulata* and a *Cladosporium* sp. (isolate 512) isolated from the seagrass *H. ovalis* showed high activity (Fig. 3). *Humicola* sp. (509) and sterile form (515) which produced chitinase in normal medium failed to elaborate this enzyme in the presence of NaCl (Table 2); *Aspergillus* sp. (507) produced chitinase in normal and 0.3 M NaCl medium but not in 0.6 M NaCl medium. *Penicillium* sp. (645) elaborated chitinase in medium with 0, 0.3 and 0.6 M NaCl (Table 2).

Table 2 Chitinase and chitosanases enzyme activity of marine derived fungi isolated from Seagrasses with different molarity(M) of NaCl amended medium by dot blot method.

Isolate	Host	Fungus	Glycol chitin 0 M	Glycol chitin 0 .3 M	Glycol chitin 0 .6 M	Chitosan DA 1.6% 0 M	Chitosan DA 1.6% 0 .3 M	Chitosan DA 1.6% 0 .6 M
507	*Thalassia* sp. 1	*Aspergillus* sp.	+	+	-	++	++	+++
509	*Thalassia* sp. 2	*Humicola* sp.	+	-	-	+++	-	-
515	*Halophila ovalis*	Sterile form	+	-	-	+++	++	++
645	*Cymodocea serrulata*	*Penicillium* sp.	+	+	+	+++	++	-

- = No activity, + = Low activity, ++ = Medium activity, +++ = High activity

Chitosanase

Forty-eight isolates (41% of the 117 isolates screened) including *Aspergillus* spp., *Aureobasidium pullulans*, *Chaetomium* spp., *Cladosporium* spp., *Curvularia tuberculata*, *Drechslera* sp., *Fusarium oxysporum*, *Humicola* sp., *Nigrospora* spp., *Nodulisporium* sp., *Paecilomyces* sp., *Penicillium* spp., *Torulomyces* sp. and xylariaceous form were positive for chitosanase acting on chitosan degree of acetylation 56%. With chitosan degree of acetylation 38% as substrate, *Alternaria* spp., *Aspergillus* spp., *Aureobasidium pullulans*, *Chaetomium* spp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* sp., *Fusarium* spp., *Gonatophragmium mori*, *Humicola* sp., *Memnoniella* sp., *Nigrospora* spp., *Nodulisporium* spp., *Paecilomyces* sp., *Penicillium* spp., *Phoma* sp., *Pithomyces* sp., *Tourlomyces* sp., *Trichoderma harzianum* and Xylariaceous form (77 isolates representing 66%) were positive for the enzyme. With chitosan degree of acetylation 1.6% as substrate, *Alternaria* spp., *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* sp., *Fusarium oxysporum, Gonatophragmium*

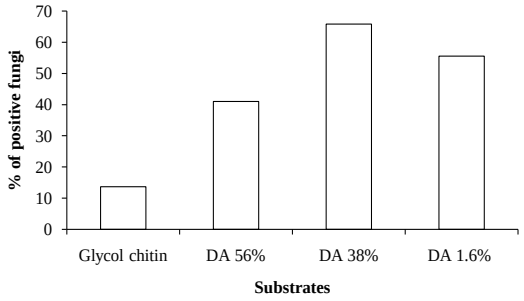

Fig. 1 – Percentage of marine derived fungal endophyte isolates from **seaweeds and seagrasses** positive for chitin modifying enzymes (dot blot method). Total number of isolates tested =117, DA = Degree of acetylation

mori, *Humicola* sp., *Memnoniella* sp., *Nigrospora* spp., *Nodulisporium* sp., *Paecilomyes* spp., *Penicillium* spp., *Pestalotiopsis* sp., *Phoma* sp., *Pithomyces* sp., *Torulomyces* sp. and Xylariaceous form (65 isolates representing 56%) were positive (Figs. 1-2 & Table 1). More isolates of *Aspergillus* and *Penicillium* produced chitosanases acting on chitosans with different % of degree of acetylation (Table 1). Chitosanase activity of *Humicola* sp. (509) was absent when the fungus was cultured in salt amended (Table 2); in the case of sterile form (515) and *Penicillium* sp. (645), chitosanase activity reduced with the presence of salt in the medium. Interestingly, in the case of *Aspergillus* sp. (507), chitosanase activity was stronger (producing darker spots on dot blot gels) with higher salt concentration in the medium (Table 2).

Discussion

Many fungi including several species of *Aspergillus* (Narayanan et al. 2013), *Beauveria bassiana*, *Trichoderma harzianum*, *Lecanicillium lecanii*, *Metarhizium anisopliae*, *Fusarium* spp. (Duo-chuan 2006, Matsumoto 2006) produce chitinase enzymes. Fungi produce 25 different types of chitinases (Seidl 2008); the products of chitinases are useful in control of tumours and microbes, wound healing, drug delivery and wastewater treatment (Aoyagi et al. 2007, Dai et al. 2009, Da Sacco & Masotti 2010, Nam et al. 2010). Furthermore, chitooligosaccharides have been reported to exhibit antimicrobial, hypo-cholesterolemic, immune-stimulating, and anti- cancer activities (Aam et al. 2010). Although there has been an increased interest on microbial chitinases for this reason, various aspects regarding their expression and regulation are still unknown (Seidl 2008). One option to overcome this bottleneck in realizing fully the technological potential of chitinase is to explore as many different ecological groups of fungi for their chitin modifying enzymes (Govinda Rajulu et al. 2011, Hartl et al. 2012). Govinda Rajulu et al. (2011) reported for the first time that endophytes of terrestrial plants are a good source of chitin modifying enzymes. In the present study, we show that endophytes of marine angiosperm and seaweeds could be a novel source of chitin modifying enzymes. We observed that species of *Aspergillus* which are known to dominate the endophytes assemblages of both seagrasses (Venkatachalam et al. 2015) and seaweeds (Suryanarayanan et al. 2010) could elaborate different chitin modifying enzymes when compared to the other genera of endophytes isolated (Table 1). The same species of endophyte harboured by different plant host differ in their chitin modifying enzymes profile indicating the

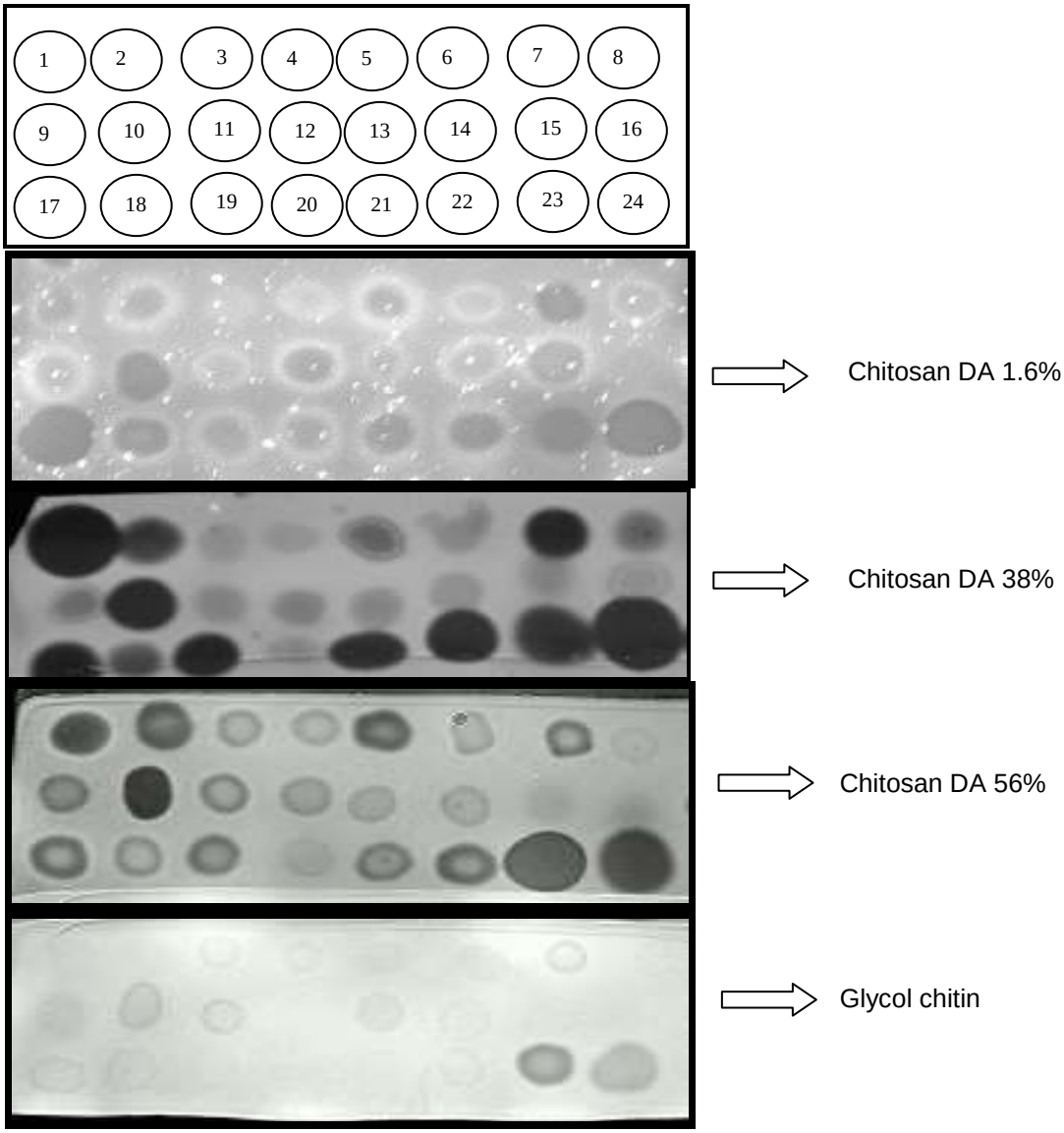

Fig. 2 – Dot blot assay for chitinase and chitosanase activity of marine-derived endophytes from seagrasses

Dot no.	Isolate	Dot no.	Isolate
1.	*Curvularia tuberculata* (503)	13.	*Aureobasidium pullulans* (585)
2.	*Penicillium* sp. (504)	14.	*Nigrospora* sp. (621)
3.	*Cladosporium* sp. (505)	15.	*Gonatophragmium mori* (622)
4.	*Cladosporium* sp. (506)	16.	*Fusarium* sp. (623)
5.	*Aspergillus* sp. (507)	17.	*Aspergillus flavus* (625)
6.	*Aphanocladium* sp. (508)	18.	*Aspergillus* sp. (636)
7.	*Humicola* sp. (509)	19.	*Aspergillus* sp. (637)
8.	*Penicillium* sp. (511)	20.	*Curvularia* sp. (638)
9.	*Colletotrichum* sp. (513)	21.	*Aspergillus terreus* (639)
10.	Sterile form (515)	22.	*Aspergillus terreus* (640)
11.	*Curvularia tuberculata* (516)	23.	*Aspergillus terreus* (641)
12.	*Paecilomyces* sp. (584)	24.	*Penicillium* sp. (642)

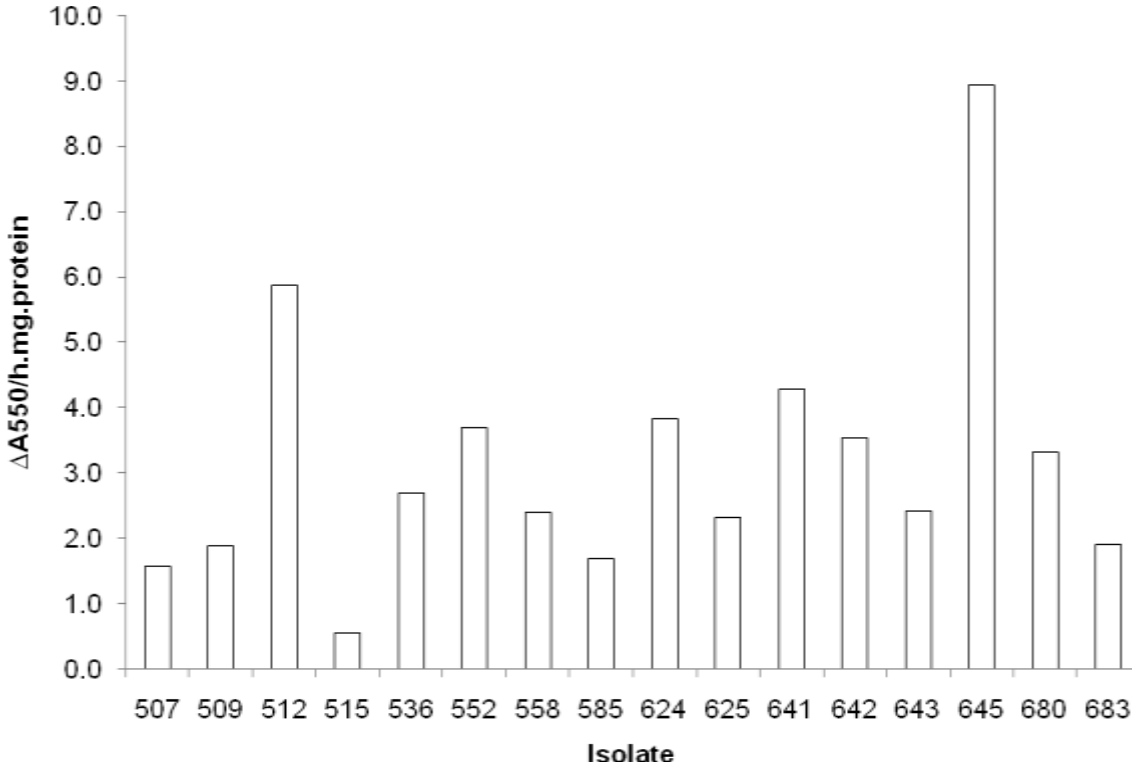

Fig. 3 – Spectrophotometric assay of chitinases of marine derived fungi isolated from seaweeds and seagrasses. Refer isolate code Table 1

high diversity of these enzymes in these fungi. For instance, *A. terreus* and *C. tuberculata* isolated from different hosts (or even from the same host) showed different degrees of activity on chitin/chitosans (Table 1). This result is similar to that obtained by Govinda Rajulu et al. (2011) for endophytes of terrestrial plants and substantiates their conclusion that endophytes need to be explored vigorously for their chitin modifying enzymes. The observation that many endophytes of marine plants elaborate different chitosanases (Fig. 1 & Table 1) is of significance since more detailed studies are likely to identify a variety of this enzyme from these fungi. It is important to identify a variety of microbial chitin modifying enzymes since unlike production of chitooligosaccharides form chitosan by acid hydrolysis, enzyme action on chitosan would result in production of defined chitooligosaccharides with known fraction of N-acetylated residues, molecular weight distribution, and pattern of N-acetylation – a highly desirable attribute for the many pharmaceutical applications of chitooligosaccharides (Aam et al. 2010). It appears that action of chitin modifying enzymes of marine-derived endophytes is influenced by salt. This preliminary observation need to be explored in detail to understand the role of salt in the induction and action of chitin modifying enzymes of this ecological group of fungi. It is pertinent to mention here that the presence of salt induces chitinase in plants (Hong & Hwang 2002) and alters the composition of cell wall destructuring enzymes produced by a mangrove fungus (Arfi et al. 2013).
The need for studies on fungal endophytes of seaweeds for technological exploitation has been stressed recently (Suryanarayanan & Johnson 2014). Focused studies on chitin modifying enzymes of marine-derived endophytes to understand their regulation and expression (homologous and heterologous) and features such as substrate-binding properties and functional overlaps would help in choosing appropriate enzymes for various technological applications.

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