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BIODIVERSITY AND BIOACTIVITY OF RED SEA SPONGE ASSOCIATED ENDOPHYTIC FUNGI

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Abstract: *Seven sponge species were collected from Red Sea in Ein El-soukhna, Egypt. Dextrose yeast extract medium (DYA) and the dilution technique were the most suitable condition for isolation. 95 endophytic fungal isolates constituting 22 species were screened from the sponge samples. The sponge species Cliona celate and Agelas citrine have been colonized by the highest endophytic fungal isolates. Aspergillus oryzae and Cladosporium cladosporioides were highly frequent endophytic fungal species in sponges with isolation rate 20% & 12% and colonized 4 sponge species with 57% colonization rate. However 50% of the endophytic fungi were specific to one sponge species. All endophytic fungal species showed variant spectrum of antimicrobial activity against different pathogenic bacteria and fungi. They also exhibited antioxidant activity. The highly prevalent A.oryzae and C.cladosporioides proved to be the potent fungal species in both biological activities.*

Key words: *Red Sea Sponge, Endophytic Fungi, Antioxidant Activity, Antibacterial Activity, Antifungal Activity*

INTRODUCTION

More than 70% of the planet's surface is covered with water which represents the most promising habitat to discover novel bioactive compounds. Endophytes are microorganisms (mostly bacteria and fungi) that inhabit plant and animal hosts as part of their life cycle without causing any apparent harm or symptomatic infection to their hosts (Strobel, 2003).

Endophytic fungi are an ecological polyphytic group of highly diverse fungi mostly belonging to Basidiomycetes, Ascomycetes and anamorphic fungi (Huang et al. 2001 . Arnold, 2007). Marine sponges are filter feeding organisms that efficiently obtain food from seawater by pumping water comprise over 50% of the sponge weight (passarini et al. 2014). Sponge is sessile filter-feeding with capacity to filter thousands of liters of water/day (Vacelet and Donadey, 1977; Taylor et al. 2007). They can be found in almost all of the 232 marine regions of the world (Spalding et al. 2007). Marine environment represent a unique combination of environmental factors such as salinity, pressure, low temperature and nutrition. However, marine sponge requires special metabolic capabilities in order to adapt and survive in such condition. So a large number of novel bioactive compounds have been isolated from marine organisms (Bose et al. 2015, Blunt et al. 2013, Lane and Moore, 2011). High salt concentration is integral for production of bioactive compounds by sponge associated filamentous fungi with new activity and mechanism of action (Wang et al. 2001, Ruger and Hentzschel et al. 1980). Such environment has been subsequently linked to the osmoregulatory mechanism that signals the production of polyol and amino compounds in combination with increasing concentration of cytoplasmic ions (Bugni and Ireland, 2004). Many of endophytes produce bioactive metabolites that may be involved in the host-endophyte relationship (Strobel, 2003). These metabolites may serve as a source of novel natural products for application in medicine, agriculture and industry (Bacon and White, 2000, Strobel and Daisy, 2003). More attention is now being given to study endophytic biodiversity, chemistry and bioactivity of their metabolites and the interaction between host and endophytic microorganisms (Tan and Zhou, 2001, Schulz et al. 2002). The difference in the produced endophytic metabolites and their biological activity might be related to the biochemical difference in their hosts (Paul et al. 2006). In this respect, sponges (phylum porifera) have been described as a rich source of bioactive compounds with promising biotechnological interest (Wang, 2006, Blunt et al. 2007). An interesting aspect in sponge is that these organisms maintain symbiotic relationships with microorganisms bear structural resemblance to metabolites produced from microbes (Moor, 1999, Schmidt et al. 2000). A growing number of studies indicate that sponge derived fungi can provide sources of novel bioactive secondary metabolites exhibiting anticancer, antibacterial, antiviral, anti-inflammatory, antifouling and antifungal activities (Compagnone et al. 1998 ; Rateb and

Ebel, 2011). Secondary metabolites in sponge associated marine fungi are chemically diverse and comprised unusual nucleosides (Sabie and Gadd, 1992) terpenes (Abraham, 2001), peptides (Kobyashi and Shibashi, 1993), alkaloids (Lyons et al. 1986), nonribosomal peptides (Henning et al. 2002) and polyketides (Liu et al. 2003). The biodiversity of microbial communites in sponge of Red Sea is poorly known and thus we focus, in this study, on the diversity and host specificity of endophytic mycobiota of some sponge species collected from Red Sea, Ein-El-Soukhna, Egypt. The biological activity of these fungal endophyte metabolites such as antimicrobial and antioxidant were assayed as well.

MATERIALS AND METHODS

Sponge collection:

Seven marine sponges belonging to seven families (*Agelasidae*, *Hemiasterellidae*, *Plakinidae*, *Coelosphaeridae*, *Astrocoeniidae*, *Clionaidae*, *Halichondriidae*) were collected from Ein El-Soukhna, Red Sea, Egypt, in October 2015. The sponges were collected at depth of 2-5m by scuba diving. Once excised, sponge specimens were transferred directly to sterilized jars containing sea water to prevent contact of sponge tissue with air, and cooled on ice. The samples were transported to the laboratory in ice box to process for fungal isolation.

Isolation of sponge-associated endophytic fungi

Sponges were rinsed with sterile distilled water prior to further analyses. After that, two parallel procedures for the processing of the sponges were used.

A- Impression method (Henriquez, et al. 2014)

Pieces of approximately $1cm^3$ of the inner tissues from each sponge species were excised under sterile conditions with scalpel and forceps, and directly spread onto petri plates containing different culture media (see below).

B- Dilution method

Compress the sponge samples ($1cm^3$) using a sterile mortar and pestle. Make different dilutions of sponge extract ($10^{-2,}$ 10^{-3}) then plating 200µl/dish from each dilution, on petri-dishes containing different culture media.

Culture media

Culture media used were potato dextrose agar (PDA, Techno pharmchem), GPYA(g/l) (1 glucose, 0.5 peptone, 0.1 yeast extract, 15 agar) and DYA(%) (1 dextrose, 1 yeast extract, 2 agar) all culture media prepared using sea water, and supplied with the antibiotic benzyl

penicillin (150 mg/liter) to prevent bacterial growth. The plates were incubated at 25-27^{o}c for one week. Each fungal isolates obtained was individually picked and transferred onto a new fresh DYA plates containing antibiotics and incubated at 25-27^{o}c for one week for purification and identification purposes. Stock cultures of purified fungal isolates were subcultured on DYA slants and preserved in refrigerator. The identification takes place following Moubasher (1993) and other reference books.

Identification of sponge species was performed with the kind help of Dr.Rewaida abd El-Hakim (Zoology department, Faculty of Science, Cairo University). They are *Agelas citrina*, *Stelligera rigida*, *Oscarella lobularis*, *Celtodoryx girardae*, *Madracis miriabilis*, *Cliona celata* and *Spongosorites difficilis*.

Biological activity of isolated fungi:

Pure fungal species were cultured in 250ml Erlenmeyer flasks containing 100ml of DYE broth medium. After incubation for 12 days at 27-30^{o}c and 200 rpm, the mycelium was separated from the culture medium by centrifugation. The culture supernatant was extracted with ethyl acetate (2×40ml) (Wiese et al. 2011). Then the filtrate was adjusted to pH 2 with 1N Hcl and extracted again with ethyl acetate. The organic phase was dried under vacuum using a rotary evaporator to give a solid or oily extract, which was used for bioassays. The aqueous fraction was discarded.

Antimicrobial assays of culture filtrate extract:

Antimicrobial assay was carried out by agar disc diffusion according to (Henriquez et al. 2014). Paper discs were impregnated with 5mg of the extracts dissolved in 10µl methanol. The methanol was allowed to evaporate then added 100µl of DMSO to increase diffusion of extracts in media. The discs were placed upon agar plates containing bacterial pathogens *Staphylococcus aureus, Bacillus subtilis, Streptococcus fecalis, Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeroginosa* and the fungal pathogen *Candida albicans*. The plates were incubated at 37^{o}c after 96hr of incubation, the inhibition zones diameters were measured. Negative controls were only treated with methanol and DMSO.

Antimicrobial assays of fresh mycelium:

The antimicrobial assay of mycelium was carried out by well diffusion method. Wells were made by sterile cork borer in agar plates seeded with bacterial and fungal pathogens. Then each well filled by the mycelium of isolated fungi separated from the culture medium by

centrifugation. The plates were incubated at 37°c. After 96hr of incubation, inhibition zone diameters were measured.

Antioxidant assays of isolated fungi using free radical scavenging (FRS) model:

The assay was performed according to (Hamed, 2009). One mg of extracts of each of the fungal species were dissolved in 1ml DMSO to prepare stock solution of 1000µg/ml. DPPH (2,2-diphenyl-1-picrylhydrazyl radical) 0.035g was dissolved in 100ml of methanol HPLC grade to prepare 0.035% solution (it must be stored in dark until use). 0.1ml of stock solution (samples)was added to 0.9ml of methanolic solution of DPPH (0.035%) to reach the final maximum concentration of tested samples 100µg/ml. The reaction mixture was incubated for 30 min, then the samples was measured at wave length 540nm by (JENWAY 6300) spectrophotometer. Blank was measured by replacing 0.1ml of samples by 0.1ml of dissolving agent (DMSO). All assays were run in triplicates. The free radicals scavenging activity of fungal extracts and calibrator can be calculated from the following equation:

% scavenging activity = $\{(A_{blank} - A_{sample}) / A_{blank}\} \times 100$

Where, A_{blank} (Absorbance of reaction mixture without test sample "DPPH" only)

A_{sample} (Absorbance of reaction mixture in presence of test samples)

Statistical analysis: Was performed by SPSS 15.0 for windows evaluation version.

RESULTS AND DISCUSSION

Atotal of 95 endophytic fungal isolates constituting 22 species were screened from the seven sponge samples.Two techniques and three nutrient media were used for isolation of sponge associated endophytic fungi (table 1). Dilution technique recorded 65 isolates representing 68.4% of the total count, whereas impression technique attained 30 isolates representing 31.5% of the total count.

Medium GPYA proved to be the most suitable culture in impression technique for isolation of sponge endophytic fungi (17.8%). Meanwhile, medium DYA was the most suitable medium for dilution technique (47.3%) so it used in the next experiment.

The sponge species *Cliona celata* and *Agelas citrina* were highly colonized with endophytic fungi representing 23.1% and 22.0% of the total isolates, respectively. Spongs *Oscarella lobularis* and *Stelligera rigida* followed representing 16.8% and 15.7% of the total endophytic fungal count (table 1 and figure 1). Biodiversity of sponge associated endophytic fungi (table 2 and figure 1) declaired that 11 isolates of aspergilli could be identified to the

species level with *Aspergillus oryzae* being the most frequent one (20% isolation rate). The genus *Cladosporium* followed in number of isolates from internal sponge tissues with 6 identified species. *C.cladosporioides* was the highly frequent species with 12% isolation rate. Five infrequent species were detected once with 2 colonies from sponge tissues. Those were *Fusarium neoceras*, *Harposporium diceracum*, *Penicillium rubrum*, *P.rugulosum* and *Rhizoctonia solani* (table 2 & figure 1).

Table (1) Endophytic fungal isolates screened from seven sponge samples collected from Red Sea El Ein El-Soukhna, Egypt

Sponge samples	Isolation Technique											
	Impression					Dilution					TC	F(%)
	PDA	GPYA	DYA	TC	F(%)	PDA	GPYA	DYA	TC	F(%)		
(S1) *A.citrina*	3	1	2	6	20	2	-	12	14	21.5	20	21.0
(S2) *S.rigida*	-	-	1	1	3.3	-	-	14	14	21.5	15	15.7
(S3) *O.lobularis*	-	1	2	3	10	-	3	10	13	20	16	16.8
(S4) *C.girardae*	-	-	3	3	10	1	-	2	3	4.6	6	6.3
(S5) *M.miriabilis*	-	3	-	3	10	7	-	1	8	12.3	11	11.5
(S6) *C.celata*	1	12	-	13	43.3	1	3	5	9	13.8	22	23.1
(S7) *S.difficilis*	-	-	1	1	3.3	3	-	1	4	6.1	5	5.2
Total isolates (cfu)	4	17	9	30		14	6	45	65		95	
Frequency (%)	4.2	17.8	9.4	31.5		14. 7	6.3	47.3	68.4			

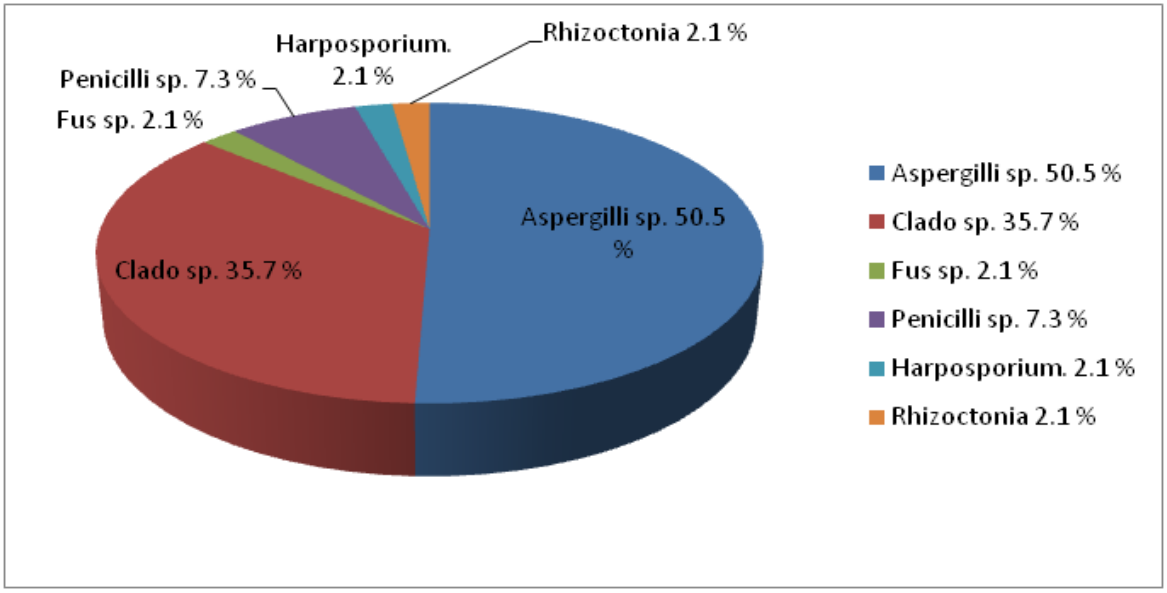

Fig (1) Relative frequencies of endophytic fungi isolated from 7 sponge species collected from Red Sea, Egypt

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Table (2) Total count (TC), relative density(RD%), colonization rate(%) and isolation rate(%) of endophytic fungi isolated from 7 sponge species collected from Red Sea El-Ein El-Soukhna, Egypt

Fungal SP.	*A.citrina*		*S.rigida*		*O.lobularis*		*C.girardae*		*M.miriabilis*		*C.celata*		*S.difficilis*		Total	
	TC	RD%	TC	RD%	TC	RD%	TC	RD%	TC	RD%	TC	RD%	TC	RD%	Colonization Rate %	Isolation Rate %
A.aculeatus	-	-	-	-	1	6.25	-	-	-	-	1	4.55	-	-	28.5	2.11
A.candidus	-	-	-	-	2	12.5	-	-	-	-	-	-	-	-	14.2	2.11
A.carbonarius	-	-	-	-	-	-	1	16.66	-	-	-	-	-	-	14.2	1.05
A.flavus	-	-	-	-	-	-	-	-	3	27.27	-	-	-	-	14.2	3.16
A.fumigatus	2	10	-	-	-	-	2	33.33	-	-	4	18.18	-	-	42.8	8.42
A.japonicus	2	10	-	-	1	6.25	-	-	-	-	-	-	-	-	28.5	3.16
A.oryzae	-	-	9	60	4	25	1	16.16	-	-	5	22.72	-	-	57.1	20
A.parasiticus	2	10	-	-	2	12.5	-	-	-	-	-	-	-	-	28.5	4.21
A.phoenicis	2	10	-	-	-	-	-	-	-	-	-	-	-	-	14.2	2.11
A.terreus	-	-	-	-	-	-	-	-	-	-	2	9.09	-	-	14.2	2.11
A.viride.nutans	2	10	-	-	-	-	-	-	-	-	-	-	-	-	14.2	2.11
C.cladosporioides	2	10	-	-	-	-	2	33.33	3	27.27	5	22.72	-	-	57.1	12.63
C.herbarum	-	-	3	20	-	-	-	-	-	-	-	-	-	-	14.2	3.16
C.macrocarpum	4	20	-	-	-	-	-	-	-	-	-	-	-	-	14.2	4.21
C.oxysporum	-	-	-	-	-	-	-	-	3	27.27	-	-	-	-	14.2	3.16
C.sphaerospermum	-	-	-	-	-	-	-	-	-	-	3	13.64	-	-	14.2	3.16
C.tenuissimum	2	10	-	-	2	12.5	-	-	-	-	-	-	5	100	42.8	9.47
F.neoceras	-	-	-	-	2	12.5	-	-	-	-	-	-	-	-	14.2	2.11
Harposporium diceracum	-	-	-	-	-	-	-	-	2	18.18	-	-	-	-	14.2	2.11
P.rubrum	-	-	3	20	1	6.25	-	-	-	-	2	9.09	-	-	42.8	6.32
P.rugulosum	-	-	-	-	1	6.25	-	-	-	-	-	-	-	-	14.2	1.05
Rhizoctonia solani	2	10	-	-	-	-	-	-	-	-	-	-	-	-	14.2	2.11
Colony count	20	21.05	15	15.79	16	16.84	6	6.32	11	11.58	22	23.16	5	5.26	-------	------
Species No.	9	----	3	----	9	----	4	----	4	----	7	----	1	----	22	100

In close relation. There were many undiscovered saprophytes and endophytes fungal species from sponge species. Only one or more endophytes were isolated from one sponge species. The diversity of sponge associated microorganisms has been poorly investigated in remote geographical areas like Red Sea (Strobel and Diasy,2003 and Huang et al. 2007). Sixty five isolates from 11 sponge samples were isolated from George Island. They are all belongs to the family Ascomycetes and the genera *Geomyces*, *Penicillium*, *Aspergillus*, *Epicoccum*, *Cladosporium*, *Aureobasidium*, *Phoma* and *Trichoderma* (Henriquez et al. 2014). Three orders from the phylum Basidiomycetes and seven orders from the phylum Ascomycetes were isolated from the sponge *Dragmacidon reticulatum* collected from Atlantic Ocean. Using ITS, rDNA and 18s gene these genera were *Penicillium*, *Fusarium*, *Pressia*, *Microsphaeropsis*, *Divriesia*, *Aurobasidium*, *Metaschnikowia*, *Gondema*, *Malassezia* and *Coprinellus* (Passarini et al. 2014).

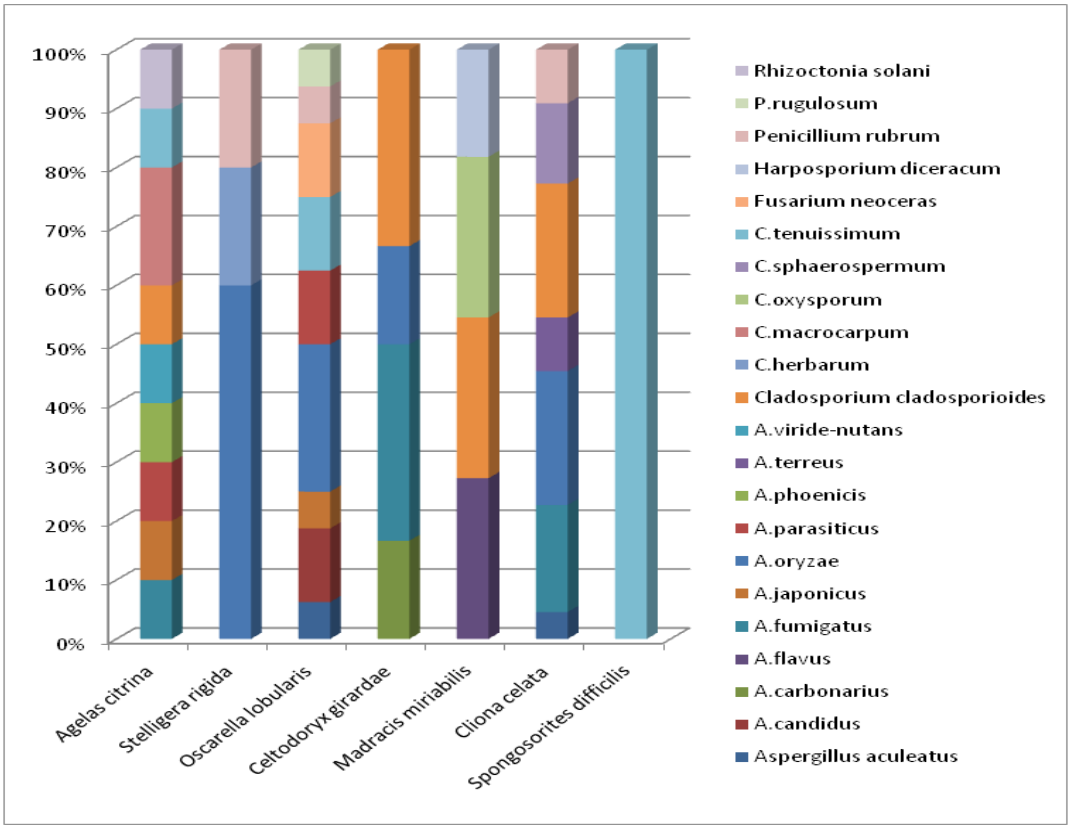

Fig (2) Colonization rate of sponge endophytic fungal taxa isolated from 7 sponge species collected from Red Sea, Egypt

Host specificity and occurrence of sponge associated endophytic fungi

All sponge species were found to be hosts to one/or more fungal species in their internal tissues (figure 2). *A.oryzae* and *Cladosporium cladosporioides* colonized four sponge species

out of seven with 57.1% colonization rate. *A.fumigates*, *C.tenuissimum* and *P.rubrum* followed and colonized 3 sponge species with colonization rate of 42.8% each.

A.aculeatus, *A.japonicus*, and *A.parasiticus* colonized 2 sponge species with 28.5% colonization rate. However, more than 50% of the endophytic fungi specified to one sponge only such as *A.phoenicus*, *A.viride-nutans*, *C.macrocarpum*, *Rhizoctonia solani*, were specified to sponge sample (*A.citrina*) only, *C.*herbarum sample (*S.rigida*), *A.candidus*, *Fusarium neoceras* and *P.rugulosum* sample (*O.lobularis*) *A.carbonarius* sample (*C.girardae*), *A.flavus*, *C.oxysporum* and *Harposporium diceracum* sample (*M.miriabilis*) and *A.terreus*, *C.sphaeospermum* were specified to sponge sample (*C.celata*). A variety of relationship can be exist between endophytes and their hosts, ranging from mutualism, symbiosis, antagonism, or slight pathogenesis (Schulz and Boyle 2005, Arnold 2007). Host-endophytes interaction can be described in terms of host specificity, host-recurrence, host-selectivity or host preference (Zhou and Hyde, 2001, Cohen, 2006). Host-specificity implies that complex biochemical interactions are occurring between the host and its specific endophyte . Host-recurrence refers to the frequent or predominant occurrence of endophyte in a particular host or range of hosts (Zhou and Hyde, 2001). Host preference is the occurrence of endophytic species in host or many host but there is a preference of one particular host (Cohen,2006).

Antimicrobial activity of sponge associated endophytic fungi

Antimicrobial activity is one of the most compounds investigated in marine sponge associated fungi. This is due to the increasing requirement of novel antibiotic drugs with new mechanism of action to which bacteria have no mechanism of resistance. Sponge represent a unique host capable of harboring different phyla of fungi with new biotechnological application and new antimicrobial activities (Santos et al. 2014).

The present data (table 3) declaired higher potentiality in antimicrobial activity of the fungal fresh mycelium than the culture filtrate extract against pathogenic bacteria and fungi. Furthermore the highly frequent sponge endophytes, *Aspergillus oryzae*, *Cladosporium cladosporioides* exhibited much more antimicrobial activity than other tested fungal endophytes.

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Table (3) Antimicrobial activity of culture filtrate extract & fresh mycelium of the most frequent endophytic fungi isolated from Red Sea sponge species

Fungal species	Inhibition zone diameter (mm)													
	Culture filtrate (96 h incubation)							Fresh mycelium (96 h incubation)						
	C.albicans	*E.coli*	*P.aerogin-osa*	*S.aureus*	*S.fecalis*	*B.subtilis*	*N.gonorr-oeae*	*C.albica-ns*	*E.coli*	*P.aero-ginosa*	*S.aureus*	*S.fecal-is*	*B.subti-lis*	*N.gono-rroeae*
A.fumigatus	$0^{a} \pm 0$	$8.3^{b} \pm 0.57$	$9.33^{b} \pm 0.57$	$9.33^{ab} \pm 0.57$	$9.33^{b} \pm 0.57$	$0^{a} \pm 0$	$7.66^{a} \pm 0.57$	$0^{a} \pm 0$	$17.33^{c} \pm 0.57$	$18^{cd} \pm 1$	$18.66^{c} \pm 0.57$	$17.33^{c} \pm 0.57$	$17.66^{c} \pm 0.57$	$17.33^{d} \pm 0.57$
A.oryzae	$11.33^{c} \pm 1.52$	$10.66^{c} \pm 1.52$	$12.66^{c} \pm 1.52$	$13.33^{d} \pm 1.15$	$10.66^{c} \pm 0.57$	$8.66^{c} \pm 0.57$	$11.66^{c} \pm 0.57$	$34.33^{b} \pm 1.15$	$14.33^{b} \pm 0.57$	$16.3^{bc} \pm 1.15$	$16.66^{c} \pm 1.52$	$13.66^{b} \pm 1.52$	$0^{a} \pm 0$	$13.33^{b} \pm 1.15$
C.cladosporioides	$7.33^{b} \pm 1.15$	$0^{a} \pm 0$	$9.33^{b} \pm 0.57$	$8.33^{a} \pm 0.57$	$7.33^{a} \pm 0.57$	$7.66^{b} \pm 0.57$	$8.66^{b} \pm 0.57$	$0^{a} \pm 0$	$20.66^{d} \pm 1.15$	$14.33^{b} \pm 2$	$12.66^{b} \pm 1.52$	$19.33^{d} \pm 0.57$	$12.66^{b} \pm 0.57$	$14.66^{c} \pm 0.57$
C.tenuissimum	$0^{a} \pm 0$	$8.33^{b} \pm 0.57$	$0^{a} \pm 0$	$10.66^{bc} \pm 0.57$	$8.33^{ab} \pm 0.57$	$8.66^{c} \pm 0.57$	$9^{b} \pm 0$	$0^{a} \pm 0$	$22.66^{e} \pm 1.52$	$19.33^{d} \pm 0.57$	$13.33^{b} \pm 1.15$	$20.66^{d} \pm 1.15$	$0^{a} \pm 0$	$0^{a} \pm 0$
P.rubrum	$0^{a} \pm 0$	$9.33^{bc} \pm 0.57$	$11.33^{c} \pm 1.15$	$11.33^{c} \pm 1.15$	$8.33^{ab} \pm 1.15$	$7.33^{b} \pm 0.57$	$8.66^{b} \pm 0.57$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$

Oppositely, the mycelium of *Pencillium rubrum* had no antimicrobial activity while the culture filtrate extract was active against all pathogenic microorganisms. Generally, the antimicrobial activities of all endophytes were a function of pathogen and extract. So, It was suggested that there may be a symbiotic relationship between sponge and its endophytic fungi. Endophytes obtain their nutrient requirements from sponge tissues, meanwhile it protect sponge against contamination by deteriorating microorganism by secreting the antimicrobials.

Similarly, Bugni and Ireland (2004) and Raghukumar (2008) claimed that sponge associated fungi are one of the major marine sources of antimicrobial compounds. Santos et al. (2014) reported that sponge associated microorganisms represent a potential source for production of antimicrobial substances against pathogens with medicinal importance. Mauikandan et al. (2014) tested filtrate of marine sponges and found that it exhibited antimicrobial activity against pathogenic bacteria and fungi, with novel mechanism of action.

Antioxidant activity of sponge associated endophytic fungi

The antioxidant activity of all endophytic fungal species isolated from the seven sponge samples were assayed. The results showed that they are all exhibited antioxidant activity (table 4). The mycelium have been exhibited stronger antioxidant activity than the culture filtrate extract. The descending arrangement of the most potent antioxidant endophytes in mycelium was *A.fumigatus*(59.7%) > *A.oryzae*(36.1%), *C.tenuissimum* (30.5%), *C.cladosporioides*(25.4%) > *P.rubrum*(22.5%), while in culture filtrate extract was *A.oryzae*(24.2%) > *A.fumigatus*(22.31%) > *C.cladosporioides*(21.67%) > *P.rubrum*(15.21%) > *C.tenuissimum*(14.6%).

This declaired that *A.fumigatus* and *A.oryzae*, the most prevalent species exhibited the strongest antioxidant activity. Similarly, fungi isolated from sponge in Antarctic water showed antioxidant, antimicrobial and antitumor activities (Henriquez et al. 2014). Active antioxidant metabolite was isolated from marine sponge-associated *Actinokineospora* sp. (Grkovic et al. 2014). Antioxidant compound Galanthamine hydrobromide was isolated from *Bacillus subtilis* isolated from the marine sponge *Fasciospongia cavernosa* collected from the Bay of Bengal on the East Coast of India. This compound is used in Alzheimer's disease treatment (Pandey et al. 2014)

Finally, we suggested that most of the biological activities exhibited by sponge species may be produced by the associated endophytic microorganisms or by the sponge in response to the stress of the microbial contamination. This suggestion enguorages investigators to search in the biological activities of these microbiota which lead to exploration of novel bioactive compounds applied in the medicinal and other fields.

Note: Using three media and two isolation techniques:

TC: total count, F(%): Frequency(%), PDA: Potato Dextrose Agar, GPYA: Glucose Peptone Yeast Extract Agar, DYA: Dextrose Yeast Extract Agar.

Table (4) Antioxidant activity of the culture filtrate and fresh mycelium of the most frequent endophytic fungi isolated from Red Sea sponge

Fungal species	Antioxidant activity (%)	
	Culture filtrate extract	Fresh mycelium
Aspergillus fumigates	22.31 %	59.7 %
A.oryzae	24.2 %	36.1 %
Cladosporium cladosporioides	21.67 %	25.4 %
C.tenuissimum	14.6 %	30.5 %
Penicillium rubrum	15.21 %	22.5 %

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