Rajesh Butti et al. / Journal of Pharmacy Research 2014,8(8),1059-1066

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Antimicrobial Profile of *Stachybotrys chlorohalonata* Isolated from the Marine Habitats

Rajesh Butti, Sunita Guntagani and Vijayalakshmi Muvva*
Department of Botany & Microbiology, Acharya Nagarjuna University, Nagarjunanagar – 522 510, Guntur, Andhra Pradesh, India.

Received on:19-05-2014; Revised on: 28-06-2014; Accepted on:31-07-2014

ABSTRACT

Background: The main objective of this work was to study the antimicrobial profile of *Stachybotrys chlorohalonata* isolated from marine habitats of Nizampatnam, East coast of India. **Methods**: Soil samples were collected from marine habitats of Nizampatnam and serial dilution plate method was employed for isolation of fungi. All the fungal strains were screened for antimicrobial activity and the potent strain was identified through cultural, morphological and molecular approaches. Antimicrobial activity of the strain was tested against Gram +ve and Gram –ve bacteria as well as fungi. Attempts were made to optimize the cultural conditions to get high yield of the metabolite. Antimicrobial spectrum as well as cytotoxic activities of the strain was studied by using disc diffusion method and MTT proliferation assay against *HeLa* cells respectively. **Results**: A total of 56 marine fungal strains were isolated and 7 of them showed antimicrobial activity. MVR 14 exhibited broad spectrum antimicrobial activity and was identified as *Stachybotrys chlorohalonata*. When the strain was grown on different culture media, it produces green halo around the colony on Czapek-Yeast autolysate agar media. The metabolite extracted from *S. chlorohalonata* cultured on optimized Czapek-Dox broth exhibited enhanced antimicrobial activity along with cytotoxicity. **Conclusions**: This is the first report of *S. chlorohalonata* from marine habitats. In this study, the strain was grown on different culture media to know its cultural and morphological characteristics. The strain grew luxuriously on Corn Meal agar and it exhibited good antimicrobial activity when cultured on Czapek-Dox agar. As *S. chlorohalonata* failed to grow on the culture media without sodium chloride, it is considered that it is a marine strain. This study revealed that the strain possessed broad spectrum antimicrobial activity along with cytotoxicity. Hence further research was recommended to identify the secondary metabolites produced by *S. chlorohalonata*.

KEYWORDS: Antimicrobial activity, Cytotoxicity, marine fungi, *Stachybotrys chlorohalonata*, 18S rRNA

1. INTRODUCTION

The rapid spread of infections as well as the emergence of drug resistant pathogens has become a life-threatening and serious problem now-a-days. Hence replacement of existing antibiotics with novel bioactive compounds isolated from various sources is necessary. As one of the untapped resources, marine ecosystems serve to be a potential source for production of diverse bioactive compounds. 80% of the world's plant and animal species are found in the marine environment[1]. Marine fungi are believed to be fruitful resources of natural products [2, 3, 4]. The biological activities of marine-derived fungal metabolites include antibiotic, anticancer, antiviral, nueritogenitic activity along with properties like cell cycle inhibition, antagonism of platelet activating factor, phosphatase inhibition, kinase inhibition etc.[5, 6]. Due to their peculiar living conditions (salinity, nutrition, high pressure, temperature variations, competition with other microbes) marine fungi may have developed specific secondary metabolic pathways compared with terrestrial fungi[7]. Generally, the production of secondary metabolites was decreased at high salt concentrations in many terrestrial fungi while increase in the production of secondary metabolites with the increase of salt concentrations or addition of seawater to the media was reported for marine-derived fungi[8].

In view of the importance of marine fungi, in the present study an attempt has been made to isolate fungi from marine habitats of Nizampatnam, Andhra Pradesh, India and to screen them for the production of potent bioactive metabolites.

***Corresponding author.**
Prof. M. Vijayalakshmi
Department of Botany and Microbiology,
Acharya Nagarjuna University,
Nagarjunanagar – 522 510, Guntur,
Andhra Pradesh, India.
E mail: muvvavl@yahoo.co.in

2. MATERIALS AND METHODS

2.1 Soil sample collection

Soil samples collected from marine habitats of Nizampatnam were initially analyzed for physico-chemical properties such as moisture content (%), pH, organic carbon (mg/g) and total nitrogen (µg/g) following standard protocols[9, 10].

2.2 Isolation of fungi

Soil samples collected were shade dried and used for the isolation of fungi by serial dilution plate technique. Czapek-Dox Agar (CDA) medium amended with sodium chloride (3%) and streptomycin (40µg/ml) was used for the isolation of marine fungi[11]. Serial dilutions were prepared and 0.1ml of 10^{-3} and 10^{-4} dilutions were plated on media with the help of a spreader. The inoculated plates incubated at 30 ± 2°C for 10 days were observed for fungal colonies. The colonies were picked up and maintained as pure cultures on CDA slants and stored at 4°C for further study.

2.3 Identification of fungi

The fungal strains were identified based on colony characteristics (Colony size, color, shape, appearance, pigment production) and micro morphological (Mycelium, conidiophores and conidia) characteristics[12, 13, 14]. The isolates were mounted in lactophenol using tape preparations and examined under microscope at various magnifications[15].

2.4 Screening for bioactive metabolites

Antifungal and antibacterial assay

Dual culture assay[16] and Perpendicular streak method[17] were employed for antifungal and antibacterial assays respectively. The plates with test fungi and test bacteria alone served as control. Bacteria employed for testing include *Escherichia coli* (ATCC 35218), *Bacillus subtilis* (ATCC 6633), *B. megaterium* (ATCC 14581), *Shigella flexneri* (ATCC 12025), *Staphylococcus aureus* (MTCC 3160), *Xanthomonas campestris* (MTCC 2286) and *Serratia marcescens* (MTCC 8708) while *Aspergillus ustus* (ATCC 1033), *A. flavus* (ATCC 9643), *A. terreus* (ATCC 1012), *A. versicolor* (ATCC 9577), *Rhizoctonia* sp. and *Penicillium* sp. were used as test fungi. Growth inhibition zones against test fungi and test bacteria were recorded.

As the strain MVR14 exhibited high antimicrobial activity against bacteria and fungi tested, attempts were made for its identification using cultural, morphological and molecular approaches.

2.5 Cultural and morphological characteristics of MVR14

The strain was grown on several culture media like CDA, Corn Meal Agar (CMA)[18], Czapek Yeast Autolysate Agar (CYA)[19], Potato Dextrose Agar (PDA)[18], Malt Extract Agar (MEA)[20, 21], Potato Sucrose Agar (PSA)[19] and Yeast Extract Malt Extract Dextrose Agar (YMD)[19] for one week to study the colony characteristics (Colony size, color, shape, appearance, pigment production) and micro morphological (Mycelium, conidiophores and conidia) characteristics[14].

2.6 Molecular identification of strain MVR 14

Molecular identification was done using 18s rRNA sequence analysis. Phylogenetic and molecular evolutionary analysis was conducted using Molecular Evolutionary Genetic Analysis (*MEGA*) version 5.0 [22].

2.7 Growth pattern of the strain MVR 14

The strain was inoculated into CD broth amended with sodium chloride @ 3% and incubated at 30 ± 2°C on a rotary shaker at 180 rpm. At every 24 h interval up to 25 days, the flasks were harvested and growth of the strain was measured in terms of dry weight of biomass. The antimicrobial metabolite production was determined in terms of its antimicrobial spectrum. The culture filtrate extracted with ethyl acetate was tested for antimicrobial activity by agar well diffusion method[10].

2.8 Fermentation, Extraction and Antimicrobial assay of bioactive compounds produced by MVR 14

The pure culture of the strain was transferred aseptically into the seed medium (CD broth). After 7 days of incubation, the seed culture at a rate of 10% was inoculated into the production medium of the same composition. Fermentation was carried out at 30 ± 2°C for 18 days under agitation at 180 rpm. After incubation, the dry weight of the biomass was recorded and expressed as mg/100ml. The secondary metabolites produced by the strain were extracted by the method of Ellaiah *et al.* (2005)[23]. The culture filtrate was extracted twice with ethyl acetate and the pooled solvent extracts were concentrated under vacuum to yield a crude residue. The residue dissolved in ethyl acetate was used for testing antimicrobial activity.

2.8.1 Antimicrobial assay

Nutrient agar (NA) and CDA media were used for culturing the test bacteria and fungi respectively. The antimicrobial activity of metabolites produced by the strain was determined by agar well diffusion method[10]. Ethyl acetate extract (50 ppm) was added to each well while the addition of only ethyl acetate served as control. The inoculated plates were incubated at 30 ± 2°C and the diameter of inhibition zone was measured after 24 h of incubation for bacteria and 4-7 days for fungi.

2.9 Effect of Culture media on antifungal activity of *S. chlorohalonata*

To determine the culture medium suitable for the enhanced production of antifungal metabolites, six culture media including CDA, CMA, CYA, PDA, PSA and YMD were employed. The media sterilized at 15 lbs pressure (121°C) for 15 minutes were inoculated with *S. chlorohalonata* and incubated at 30 ± 2°C for 18 days. After incuba-

tion, the metabolites were extracted with ethyl acetate and antifungal activity was tested against test fungi.

2.10 Optimization of physiological and cultural parameters for enhanced production of secondary metabolites by *S. chlorohalonata*

Salt concentration has a profound effect on the production of antimicrobial metabolites by marine microorganisms[24]. To determine the impact of sodium chloride on growth and antimicrobial activity, different concentrations of NaCl (0 - 8%) were added to the CD medium. After 18 days of incubation, the metabolite extracted with ethyl acetate was used for antimicrobial activity against test bacteria and fungi. The biomass and antimicrobial activity were measured at each concentration of NaCl.

To study the influence of pH on growth and antimicrobial metabolite production, the strain was inoculated into sterilized CD broth with pH levels ranging from 5-9. To study the influence of temperature, CD broth with optimized pH was inoculated and incubated at different temperatures ranging from 20° – 40° C. Both the biomass and bioactive metabolite production by the strain was measured and recorded.

To determine the effect of carbon sources on growth and bioactive metabolite production, different carbon sources such as D-glucose, fructose, lactose, sucrose and cellulose each at a concentration of 3% were added to the CD medium. Influence of different levels of the best carbon source (1.0 - 5.0%) among the carbon sources tested on growth and bioactive metabolite production was also examined.

The influence of various nitrogen sources such as sodium nitrate, ammonium nitrate, ammonium sulphate and ammonium chloride was studied by adding nitrogen source (0.2%) to the medium with optimized carbon source. Further, the optimal levels of the best nitrogen source (0.1 – 0.5%) supporting optimal yields of bioactive metabolites was also recorded. The optimal concentration of carbon and nitrogen sources supporting the maximum production of metabolite was selected for further studies.

2.11 Antimicrobial Spectrum of *S. chlorohalonata* on optimized medium

The culture inoculated into the optimized medium was incubated at 30°C with shaking at 180 rpm for 18 days. The broth was then harvested and growth of the strain was measured in terms of dry weight of biomass and antimicrobial metabolite production was determined in terms of its antimicrobial spectrum.

2.12 Cytotoxicity of the bioactive compounds produced by *S. chlorohalonata*

The ethyl acetate extracts of *S. chlorohalonata* were tested against *HeLa* (Human cervical cancer cell line) cells using MTT cell proliferation assay[25]. *HeLa* cell lines were obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10% fetal bovine serum (FBS). The cells (2000 cells per well) were seeded in a 96 – well microplate containing 100 µl of DMEM + 10% FBS medium per well and incubated at 37°C with 5% CO_2. The cells were treated with different concentration of extracts up to 72 h at every 24h interval. Controls were maintained with 0.5% DMSO. After 72 h treatment, 5µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazolium bromide) reagent (R&D Systems, USA) along with 45 µl of phenol red free DMEM (Sigma Life Science, USA) without FBS was added to each well and plates were incubated at 37°C with 5% CO_2 for 4 h. Thereafter, 50 µl of solubilization buffer (R&D Systems, USA) was added to each well to dissolve the colored formazan crystals produced by the reduction of MTT. After 24 h, the optical density was measured at 550 nm using microplate reader (Bio-Rad, USA).

2.13 Statistical Analysis

Results obtained are statistically analyzed by using AGRISTAT and MINITAB 16 software.

3. RESULTS AND DISCUSSION

The characteristics of soil samples collected from Nizampatnam include - Moisture content – 8%, pH – 7.6, Organic carbon – 7.15 mg/g and total nitrogen - 5.64 µg/g.

A total of 56 fungal strains designated as MVR1 to MVR 56 were isolated from the soil samples of Nizampatnam. All the fungal strains were screened for bioactive metabolites. Among the 56 isolates, MVR 6, 14, 26, 35, 40, 46 and MVR 50 showed antimicrobial activity (Table 1). Among the 7 isolates, MVR 14 was found potent against test bacteria and fungi. Hence an attempt was made to identify the MVR 14 strain.

3.1 Cultural and Morphological characteristics of MVR 14

Cultural characteristics of MVR 14 were studied on 6 different media viz. CDA, CMA, CYA, PDA, PSA and YMD and the results are shown in Table 2. MVR 14 grew luxuriously on CMA followed by PDA medium and produced green pigment around the colony on CYA medium (Plate 1) and light green pigment was produced on PDA and CDA media. The dark green pigment produced on CYA extending up to 5-6 mm around the edge of the colonies is a characteristic feature of *Stachybotrys chlorohalonata* (*chlorohalonata refers to green halo of extracellular pigment around the colonies on CYA media*[26]). Brown

Table 1. Antimicrobial activity of the strains MVR 6, 14, 26, 35, 40, 46 and MVR 50 against test bacteria and fungi

Test Organism	Diameter of inhibition zone (mm)						
Bacteria	**MVR6**	**MVR14**	**MVR26**	**MVR35**	**MVR40**	**MVR46**	**MVR50**
Escherichia coli	12	23	3	8	2	18	8
Bacillus subtilis	6	19	2	8	5	6	4
Bacillus megaterium	2	20	5	7	6	4	4
Staphylococcus aureus	3	13	10	4	7	10	5
Xanthomonas campestris	6	14	8	5	2	7	3
Serratia marcescens	2	20	3	6	7	4	2
Shigella flexneri	2	11	10	4	3	3	3
Fungi							
Aspergillus flavus	3	19	8	2	5	5	1
Aspergillus ustus	8	18	6	3	4	7	5
Aspergillus terreus	7	20	4	8	12	6	4
Aspergillus versicolor	10	18	10	3	6	8	3
Rhizoctonia sp.	6	10	5	8	4	2	2
Penicillium sp.	5	15	6	12	6	6	1

The results are statistically analyzed and found to be significant at 5% level.

Table 2. Cultural characteristics of MVR 14 recorded after 7 days of incubation on different growth media.

Media	Colony color	Colony size (mm)	Texture	Pigmentation	Pigment color
CDA	Light green	11	Smooth	+	Light green
CMA	Light pink	25	Smooth	-	-
CYA	Grayish green to dull green	17	Smooth	+	Dark green
PDA	Dark brown	20	Smooth	+	Light green
PSA	Black	15	Smooth	+	Dark brown
YMD	Dark brown	18	Smooth	+	Light brown

+ Presence - Absence

CDA: Czapek-Dox Agar,**CMA**: Corn Meal Agar ,**CYA**: Czapek-Yeast Autolysate Agar,**PDA**: Potato Dextrose Agar,**PSA**: Potato Sucrose Agar,**YMD**: Yeast extract Malt extract Dextrose Agar

color pigment was formed on PSA and YMD but there was no pigmentation on CMA. Morphological characteristics like morphology of mycelium, conidiophore and conidia were assessed by using slide culture technique. Mycelium was hyaline and cobweb-like with black – slime heads. Conidiophores are erect, slightly flexuous and branched twice with septa. Conidia are smooth and hyaline at the base and slightly black towards the apex.

Plate 1. Isolate MVR 14 on CYA medium

Identification of the strain based on molecular approach was also carried out using Phylogenetic study based on 18s rRNA analysis. The phylogenetic tree was constructed based on Neighbor-joining method using boot strap analysis (Fig 1) and the strain was identified as *Stachybotrys chlorohalonata*.

Figure 1. Phylogenetic tree of strain MVR 14

3.2 Growth pattern of *Stachybotrys chlorohalonata*

Analysis of growth pattern revealed that the culture entered into log phase on 6th day of incubation which extended up to 14th day followed

by stationary phase from 15-18 days and then entered into decline phase (Fig 2).

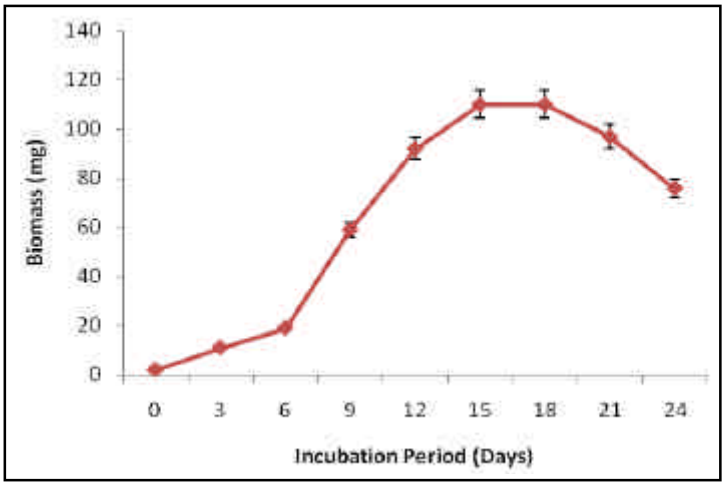

Figure 2. Growth pattern of *Stachybotrys chlorohalonata*

3.3 Effect of culture media on antifungal activity of *S. chlorohalonata*

Among the 6 culture media tested, CD broth was found to support good antifungal activity followed by CYA broth (Table 3).

Table 3. Influence of culture media on antifungal activity of *Stachybotrys chlorohalonata* against test fungi

	Diameter of inhibition zone (mm)					
Fungi	**CDB**	**CMB**	**CYB**	**PDB**	**PSB**	**YMB**
Aspergillus ustus	20	0	15	14	9	3
A. flavus	20	0	15	16	9	2
A. terreus	22	0	16	15	7	2
A. versicolor	23	0	15	14	6	5
Rhizoctonia sp.	18	0	14	13	10	4
Penicillium sp.	16	0	17	12	6	3

CDB: Czapek-Dox Broth, **PDB**: Potato Dextrose Broth,**CMB** : Corn Meal Broth, **PSB**: Potato Sucrose Broth,**CYB**: Czapek-Yeast Autolysate Broth,**YMB**: Yeast extract Malt extract Broth

3.4 Optimization of physiological and cultural parameters for enhanced production of secondary metabolites by *S. chlorohalonata*

NaCl @ 3% proved to be optimum for both the growth and antimicrobial activities of *S. chlorohalonata*. The strain failed to grow without NaCl on culture media (Table 4). CD broth with neutral pH (7.0) favored growth as well as metabolite production of *S. chlorohalonata*, whereas acidic and alkaline pH levels adversely affected the growth and antimicrobial activities (Table 5). At 30°C temperature *S. chlorohalonata* exhibited good growth as well as antimicrobial activity followed by 25°C (Table 6). Among the various carbon sources tested, *S. chlorohalonata* exhibited good growth and antimicrobial activity in cellulose supplemented CD broth (Table 7). Cellulose level @ 3% promoted good growth and antimicrobial activity (Table 8). Among the nitrogen sources tested, sodium nitrate was found to promote good growth and antimicrobial activity of the strain (Table 9). Sodium nitrate @ 0.2% was found to support good growth and antimicrobial metabolite production (Table 10).

3.5 Antimicrobial spectrum of *S. chlorohalonata* grown on optimized culture medium

S. chlorohalonata was cultured on modified CD broth (Sodium Nitrate: 0.20%; Potassium dihydrogen ortho phosphate: 0.10%; Potassium chloride: 0.05%; Magnessium Sulphate: 0.05%; Ferrous Sulfate : 0.001%; Cellulose : 3.0%; Soidum Chloride: 3.0%; pH: 7; Temperature : 30°C) at optimal conditions for 18 days and the metabolite was harvested and tested for antimicrobial activity against test bacteria and fungi. High antimicrobial activity was recorded when cultured on modified CD broth at optimum conditions compared with normal CD broth (Table 11).

3.6 Cytotoxicity of metabolite produced by the *S. chlorohalonata*

The cytotoxic activity of the metabolites produced by *S. chlorohalonata* against Human cervical cancer cell lines (*HeLa*) was

Table 4. Effect of NaCl on biomass and antimicrobial activity of *Stachybotrys chlorohalonata*

NaCl concentration (%)	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	**Bs**	**Bm**	**Sa**	**Xc**	**Sm**	**Sf**	**Af**	**Au**	**At**	**Av**	**R**	**P**
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	225	18	16	14	6	5	13	6	12	8	9	10	5	8
2	268	22	18	17	10	9	17	10	17	12	15	14	7	11
3	305	25	19	21	13	14	19	10	20	18	20	19	12	16
4	246	23	17	19	12	12	17	7	18	18	17	14	8	12
5	228	20	14	17	10	10	15	5	16	14	15	10	5	8
6	192	21	13	15	7	8	14	3	10	12	13	8	3	6
7	150	18	9	10	4	5	8	0	6	8	9	5	0	3
8	80	10	6	4	0	3	5	0	3	4	5	2	0	0

Ec - *Escherichia coli*, **Bs** - *Bacillus subtilis*, **Bm** - *Bacillus megaterium*, **Sa** - *Staphylococcus aureus*, **Xc** - *Xanthomonas campestris*, **Sm** - *Serratia marcescens*, **Sf** - *Shigella flexneri*, **Af** - *Aspergillus flavus*, **Au** - *Aspergillus ustus*, **At** - *Aspergillus terreus*, **Av** - *Aspergillus versicolor*, **R** – *Rhizoctonia sp.*, **P** – *Penicillium sp.**The results are statistically analyzed and found to be significant at 5% level.

Table 5. Effect of pH on growth and antimicrobial activity of *Stachybotrys chlorohalonata*

pH	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	Bs	Bm	Sa	Xc	Sm	Sf	Af	Au	At	Av	R	P
5	120	14	10	12	8	6	12	7	16	12	13	10	6	10
6	276	27	17	18	13	14	18	10	19	17	15	14	9	4
7	325	27	20	22	15	17	21	13	22	20	20	19	14	17
8	285	20	16	18	13	10	14	9	18	16	14	13	8	13
9	125	13	10	11	8	5	9	6	7	4	7	2	6	6

Table 6. Effect of Temperature on growth and antimicrobial activity of *Stachybotrys chlorohalonata*

Temperature (^{o}C)	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	Bs	Bm	Sa	Xc	Sm	Sf	Af	Au	At	Av	R	P
20	150	18	13	14	8	10	7	8	7	5	10	3	6	8
25	300	25	15	17	13	14	13	12	12	13	17	6	9	14
30	340	29	21	23	17	19	20	15	20	21	22	20	14	18
35	130	14	8	8	0	7	5	3	6	3	0	5	2	0
40	20	0	0	2	0	1	0	0	1	0	0	0	0	0

Table 7. Effect of Carbon source on growth and antimicrobial activity of *Stachybotrys chlorohalonata*

Carbon sources (3%)	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	Bs	Bm	Sa	Xc	Sm	Sf	Af	Au	At	Av	R	P
Glucose	350	20	16	17	14	15	14	18	15	22	16	15	17	16
Sucrose	350	29	22	22	18	19	19	16	20	21	20	16	16	17
Lactose	400	27	20	21	15	16	17	18	17	20	14	16	17	17
Cellulose	450	30	23	22	18	21	20	16	23	23	21	21	17	18
Fructose	320	24	20	8	15	16	15	17	21	20	16	17	15	15

Table 8. Effect of cellulose levels on growth and antimicrobial activity of *Stachybotrys chlorohalonata*

Cellulose concentrations (%)	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	Bs	Bm	Sa	Xc	Sm	Sf	Af	Au	At	Av	R	P
1	275	21	14	12	10	14	12	10	12	17	18	12	10	16
2	385	25	18	17	16	18	18	14	16	20	20	14	12	16
3	450	30	23	22	18	21	20	16	23	23	22	20	18	18
4	400	27	21	23	18	17	16	12	16	18	16	14	16	14
5	350	12	18	17	12	14	12	21	12	14	12	10	12	12

Ec - *Escherichia coli*, **Bs** - *Bacillus subtilis*, **Bm** - *Bacillus megaterium*, **Sa** - *Staphylococcus aureus*, **Xc** - *Xanthomonas campestris*, **Sm** - *Serratia marcescens*, **Sf** - *Shigella flexneri*, **Af** - *Aspergillus flavus*, **Au** - *Aspergillus ustus*, **At** - *Aspergillus terreus*, **Av** - *Aspergillus versicolor*, **R** – *Rhizoctonia sp*., **P** – *Penicillium sp*.*The results are statistically analyzed and found to be significant at 5% level.

Table 9. Effect of nitrogen sources on growth and antimicrobial activity of *Stachybotrys chlorohalonata*

Nitrogen sources (0.2%)	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	Bs	Bm	Sa	Xc	Sm	Sf	Af	Au	At	Av	R	P
Sodium Nitrate	500	31	24	23	21	22	23	19	25	25	23	21	21	22
Ammonium Nitrate	420	25	20	20	18	19	21	17	18	21	18	15	14	18
Ammonium Sulphate	430	23	18	17	15	20	18	17	16	21	20	17	15	16
Ammonium Chloride	400	27	21	18	17	19	19	16	18	22	19	16	14	18

Table 10. Effect of $NaNo_3$ levels on growth and antimicrobial activity of *Stachybotrys chlorohalonata*

Conc. of $NaNo_3$ (%)	Biomass weight (mg/100ml)	Antimicrobial activity in terms of zone of inhibition (mm)												
		Ec	Bs	Bm	Sa	Xc	Sm	Sf	Af	Au	At	Av	R	P
0.1	430	26	23	20	18	18	19	16	18	20	18	18	14	15
0.2	500	30	23	24	21	23	23	19	25	25	23	23	22	22
0.3	400	23	18	17	15	18	20	14	17	22	18	16	15	18
0.4	370	18	16	14	12	16	14	13	14	18	14	16	13	15
0.5	200	11	12	10	9	12	10	11	11	12	10	10	9	11

Ec – *Escherichia coli* ,**Bs**- *Bacillus subtilis*,**Bm** – *Bacillus megaterium*,**Sa** – *Staphylococcus aureus* ,**Xc**–*Xanthomonas campestris* ,**Sm** – *Serratia marcescens*,**Sf** – *Shigella flexneri*,**Af** – *Aspergillus flavus*,**Au** – *Aspergillus ustus*,**At** – *Aspergillus terreus*,**Av** – *Aspergillus versicolor*,,**R** – *Rhizoctonia* sp.,**P** – *Penicillium* sp,.*The results are statistically analyzed and found to be significant at 5% level.

Table 11. Antimicrobial activity of metabolites produced by *Stachybotrys chlorohalonata* on CD broth and modified CD broth

Test organism	Diameter of inhibition zone (mm)	
	CD broth	Modified CD broth
Bacteria		
Escherichia coli	23	30
Bacillus subtilis	19	23
Bacillus megaterium	20	24
Staphylococcus aureus	13	21
Xanthomonas campestris	14	23
Serratia marcescens	20	23
Shigella flexneri	11	19
Fungi		
Aspergillus flavus	19	25
Aspergillus ustus	18	25
Aspergillus terreus	20	23
Aspergillus versicolor	18	23
Rhizoctonia sp.	10	22
Penicillium sp.	15	22

*The results are statistically analyzed and found to be significant at 5% level.

high. The IC_{50} value recorded for the strain was 52µg/ml. Attempts are in progress for the identification of bioactive metabolites produced by *S. chlorohalonata*.

4. CONCLUSION

This is the first report of *Stachybotrys chlorohalonata* isolated from marine habitats. In this study, *S. chlorohalonata* was cultivated on CDA, PDA, PSA, CYA, SA, MEA and YMD culture media. When the strain was grown on different culture media, the CMA promotes good growth than others but the antimicrobial activity was high on CDA. Modified CD broth promoted good growth and high metabolite yield reflected by high antimicrobial activity. Hence, *S. chlorohalonata* is considered to be the potent strain as it exhibited good antimicrobial and cytotoxic activities.

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Source of support: Nil, Conflict of interest: None Declared