

Caerulomycin A—An Antifungal Compound Isolated from Marine Actinomycetes

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Abstract

Actinomycetes have been prolific sources of novel secondary metabolites with a range of biological activities that may ultimately find application as therapeutic compounds. Hence several drug discovery companies are engaged in isolation of novel bioactive metabolites from these microbial sources. Antibiotics form the major class of such bioactive metabolites and have been widely used for treating infectious diseases. One of the most critical problems in clinical practice is the increase of prevalence of drug resistant strains, especially azole resistance among fungi. Due to this, there is a constant need for development of new antifungal antibiotics having novel scaffolds and/or mechanism of action. In our in-house screening program in the quest of novel and superior antifungal compounds, an actinomycetes strain PM0525875 was isolated from a marine invertebrate. The extracts of this microbe showed potent *in-vitro* antifungal activity against drug resistant fungal strains. The antifungal active peak from the extract obtained by shake flask fermentation was identified by chromatographic and other analytical techniques during bioactivity guided isolation. Later the fermentation conditions were optimized in 30 L fermentor for the production of sufficient amount antifungal compound for complete structural characterization. Consequently the fermented broth extract was subjected to bioactivity-guided fractionation, to isolate the active principle using different preparative chromatographic techniques followed by its characterization. The active principle was characterized to be Caerulomycin A. Minimum inhibitory concentration (MIC) of the compound was found in the range of 0.39 - 1.56 µg/ml against pathogenic fungal test strains. The phylogenetic analysis of producer strain using 16S rRNA sequence showed closest match with *Actinoalloteichus cyanogriseus*. Herewith we report the isolation of Caerulomycin A from marine invertebrate-associated *Actinoalloteichus* sp. using optimized medium and fermentation conditions.

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Keywords

Caerulomycin A, Antifungal, Non-Polyene, Actinoalloateichus cyanogriseus, Marine Actinomycetes

1. Introduction

The incidences of invasive fungal infection are increasing due to number of immune compromised host and occurrence of antibiotic resistant strains [1]-[3]. The number of antifungal agent available for the treatment is limited and their use has been restricted because of toxicity or unfavorable pharmacokinetics profile. Hence, research interest has focused on discovery of safe and potent antifungal agent. One of the most successful approaches is to screen microbial resources to extract novel antifungal antibiotics.

Drug discovery from natural products has been traditionally focused on empirical exploitation of the most prolific microbial groups: actinomycetes and filamentous fungi. The literature vividly displays that actinomycetes have been the origin of the largest number of new antibiotic drug candidates and also lead molecules with applications in many other therapeutic areas [4] [5]. Access to the microbial diversity of actinomycetes in the environment has traditionally been focused on intensive sampling from a wide diversity of geographical locations and habitats [6]. One of the approaches is to explore untapped actinomycetes communities that might be associated with rhizospheres, plant endophytes, lichens, and entophytic bacteria [7] [8], as well as marine sediments and marine invertebrate-associated actinomycetes, which might facilitate isolation of novel microbial communities potentially producing novel chemical scaffolds [6].

Oceans, making up 70.8% of the surface of planet earth, contain untapped actinobacterial resources of bioactive compounds [9]. The salinity, low temperature, pressure and stress conditions in marine environments provoke a set of different metabolic pathways and defense system compared to their terrestrial counterparts, ensuring the potential ability to produce novel antibiotics [10]. According to recent findings, actinobacteria are widely distributed on the surface and inside animals and plants, in seawater and marine sediments. Marine-derived actinomycetes are rich sources of novel secondary metabolites having diverse bioactivity such as antimicrobial, antitumor and immunosuppressive [11]-[13]. The novel structure, promising biological properties and prospects of fermentation-based mass production have led to the renewed interest in the search for potential drug from marine microorganism.

During the ongoing screening program for the discovery of novel and superior antifungal compounds, an actinomycetes strain PM0525875 was isolated from a marine invertebrate. It showed potent *in-vitro* antifungal activity encompassing resistant fungal strains. The present work deals with the isolation and characterization of antifungal compound, Caerulomycin A from a marine-derived actinomycetes species.

2. Materials and Methods

2.1. Isolation of Marine Actinomycetes

Marine invertebrate sample (Sample ID: **NIO_SA_An_15**) was collected from deep sea (Anjuna Beach, Goa, India) in sterile polypropylene bag and stored at 4°C. The invertebrate (~10 g) sample was washed thrice with sterile demineralized water to remove the adhering particles like sand, sea weeds and then crushed in the sterile mortar and pestle. The crushed sample was serially diluted to 10⁻⁸. Last three dilutions viz. 10⁻⁶, 10⁻⁷ and 10⁻⁸ were surface spreaded on the actinomycetes isolation agar (AIA) medium (Himedia, India) prepared in 75% (v/v) artificial sea water (AS-AIA). The plates were incubated at 30°C till 30 days and observed periodically for the growth of actinomycetes colonies. The well isolated colonies were picked up, purified and grown on AS-AIA slants (made in 75% ASW). The well grown isolates on the slants were preserved at 4°C.

2.2. Primary Screening of Marine Actinomycetes for Antimicrobial Activity

The isolated marine actinomycetes were screened for their antimicrobial activity. The loopful of the growth from the slant was inoculated into 50 ml of seed medium; **ASW-274(1)** [glucose 15 g/L, corn steep liquor 5 g/L, sodium chloride 5 g/L, calcium carbonate 2 g/L, peptone 7.5 g/L, yeast extract 7.5 g/L, made in 75% ASW, pH 7.0 to 7.5] distributed in 250 ml Erlenmeyer flask and incubated for 96 h at 30°C at 200 rpm on a rotary shaker.

A 3 ml of well grown pure seed was transferred to the 100 ml of each media in 500 ml Erlenmeyer flask. Three different production media used were as followed: **ASW-SM12(1)** [glucose 50 g/L, yeast extract 11 g/L, peptone 4 g/L, beef extract 4 g/L, sodium chloride 2.5 g/L, calcium carbonate 5 g/L, made in 75% ASW, pH 7.4 - 7.6]; **ASW-36P(1)** [soluble starch 20 g/L, glucose 15 g/L, yeast extract 2 g/L, peptone 3 g/L, calcium carbonate 2 g/L, ammonium sulphate 0.5 g/L, corn steep liquor 2 g/L, sodium chloride 2 g/L, magnesium phosphate 5 g/L, cobalt chloride 0.001 g/L and TSS 1 ml/L (copper sulphate 7 g, ferrous sulphate 1 g, manganese chloride 8 g, zinc sulphate 2 g and demineralized water 1 L), made in 75% ASW pH 7.3 - 7.6]; **ASW-1M** [glycerol 30 g/L, glucose 3 g/L, yeast extract 2 g/L, sodium chloride 3 g/L, sodium nitrate 1 g/L, calcium carbonate 3 g/L, peptone 3 g/L and TSS 1 ml/L, made in 75% ASW, pH 7.0 - 7.2]. The flasks were incubated for 96 h at 30°C on rotary shaker at 200 rpm. The 1 ml of harvested whole broth was extracted with equal amount of methanol and centrifuged at 4000 rpm for 10 min. The supernatant was tested for antimicrobial activity by agar well diffusion method using different pathogenic strains [14]. LR grade chemicals were used in the study.

2.3. Secondary Screening of Active Isolate and Antifungal Activity

The isolate PM0525875 which exhibited exclusive antifungal activity was screened for its reproducibility as per the procedure mentioned in 2.2. The methanolic extract of the whole culture broth, cell mass extract and cell free culture filtrate were tested against *Candida* sp. to confirm the antifungal activity. To rule out the presence of polyene in the active extract, the UV-visible spectrum of extract was compared with the UV-visible spectrum of standard polyenes classes from the literature [15].

2.4. Selection of Natural Variant and Screening in Shake Flask

The 5 ml of sterile tween 80 (0.1%) was added to fully sporulated slant. The spores were scraped with sterile cell scraper. The spore suspension was filtered through sterile cotton to remove the mycelial residues. The spore suspension was spread on AS-AIA. The plates were incubated at 30°C for 30 days. The colonies having different morphology and sporulation pattern were picked up and transferred on AS-AIA slants. These variants were screened in shake flask as mentioned in 2.3. The harvested broth was extracted with equal amount of methanol and then serially diluted up to 1:1024 dilutions. Antifungal activities of this extract were checked by agar well diffusion assay [14].

2.5. Phylogenetic Identification of PM0525875

DNA was isolated from the active strain (PM0525875) grown in ASW-ISP2 (International Streptomyces Project Medium 2) containing [dextrose 4 g/L, yeast extract 4 g/L, malt extract 10 g/L, made in 75% ASW, pH 7.0 to 7.5]. DNA from washed cell suspension was extracted using Ultraclean™ Tissue and Cells DNA Isolation kit, Mo Bio Laboratories, Inc. PCR-mediated amplification of 16S rRNA gene was carried out using 16 S universal primers: 8 F (AGAGTTTGATCCTGGCTCAG), 1492 R (ACGGCTACCTTGTTACGACTT) and BDTv3.1 Cycle sequencing kit on ABI 3730x1 Genetic Analyzer. The near complete 16S rRNA sequence (1377 bp) was desired and aligned with corresponding first ten matching sequences. The program CLUSTALW2 (www.ebi.ac.uk/Tools/msa/clustalw2/) was used for both multiple alignment and phylogenetic analyses. Based on maximum identity score, first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and phylogenetic tree was constructed using MEGA5.

2.6. Large Scale Fermentation

The higher yielding variant (PM0525875-V4) was selected for cultivation in 30 L fermenter. The seed was prepared from 96 h grown culture in 200 ml ASW-274(1) medium in 1000 ml Erlenmeyer flask. The fermenter was sterilized *in-situ* at 121°C for 25 min along with the ASW-36P(1) medium containing 0.04% antifoam. A 3% of mature seed was aseptically inoculated into a fermenter. The fermentation process was carried out at 30°C, 180 rpm, and 0.6 vvm with a backpressure 0.5 bar and for 96 h. Periodically 200 ml of sample was aseptically withdrawn for determination of packed cell volume (PCV) and total sugar content [16]. The antifungal activity of extract was performed as mentioned in 2.4.

2.7. Bioactivity Guided Isolation and Purification

The 8.5 L fermented broth was centrifuged at 4000 rpm for 30 min to separate the biomass. The 400 g of biomass was extracted with methanol and clear solvent layer was obtained by filtration. Three subsequent extractions were performed. The extracts were pooled and concentrated to make it solvent free. The dried crude extract was suspended in demineralized water and further passed through a glass column packed with diaion HP-20 resin having bed volume 225 ml. The resin was washed twice with 3 L of demineralized water to remove the unbounded components and media particles. The resin column was further eluted with water and methanol gradient with varying polarity. The bioactive fractions were pooled together and evaporated till dryness with a rotary evaporator. The methanolic extract was further fractionated with petroleum ether followed by dichloromethane. The active dichloromethane extract (500 mg) was subjected to silica gel column chromatography and eluted with gradient of chloroform-methanol. The fractions were tested for bioactivity against *Candida* sp. (disc diffusion assay). The active fractions were pooled together and concentrated till dryness to get semi pure sample. The semi-pure material (100 mg) was further purified by preparative chromatography using the following conditions: C-18 Eurospher column (20 mm × 250 mm, 10 µm) in isocratic mode and eluted with acetonitrile and 0.01 M phosphate buffer (35:65) at a flow rate 20 ml/min with UV detection wave length at 220 nm. The pure active fraction (eluted at 11 - 13 min) was evaporated under reduced pressure and desalted using HP-20 resin followed by elution with methanol. The eluted solvent was concentrated to get pure compound (20 mg). The purified compound was characterized by ¹H NMR, MS and IR spectroscopy. All the solvents used for extraction were of LR grade, where as HPLC grade solvents were used for analytical and preparative HPLC. Analytical HPLC purity was determined in Water's Empower software LC using Kromasil RP-18, SN: 39673 (150 mm × 4.6 mm), 3.5 µm column. Normal column chromatography (CC) was performed on Silica gel (60 - 120 #) and TLC on Silica Gel 60F₂₅₄ (20 × 20 cm) aluminum sheets from Merck. NMR Spectra were recorded in DMSO-d₆ on Bruker 300 MHz. Chemical shifts are expressed in ppm and tetramethylsilane was used as an internal standard. Mass spectra were taken on Bruker Daltonics system. IR spectra were recorded in pressed KBr discs with Perkin Elmer PARAGON1000 spectrometer.

2.8. Determination of MIC of Isolated Bioactive Compound

The MIC was determined by the NCCLS (CLSI) Macrobroth dilution method [17] [18]. The cultures used for the assay were *Candida albicans*, *Candida albicans* CO9, *Candida glabrata*^{Fluc^R} HO5 and *Candida krusei*^{Fluc^R} GO3. The inoculum was prepared in sterile saline adjusted at an optical density (OD) of 1.00 at 600 nm comprising of 10⁸ cfu/ml. The final working inoculum was 0.5 × 10⁴ cfu/ml. The bioactive compound was diluted in the concentration range of 0.019 - 10 µg/ml. The concentration range for amphotericin B used was 0.031 - 16 µg/ml and for fluconazole was 0.5 - 256 µg/ml. The assay tubes were incubated at 37°C for 48 h following which the visible growth was noted and the MIC value determined.

3. Results

3.1. Isolation and Screening of Marine Actinomycetes

Few actinomycetes colonies isolated from marine invertebrate sample (Sample ID: **NIO_SA_An_15**) were found to grow on ASW-AIA plate. The white sporulating slow growing colony showing black diffusible pigment on the agar was picked up and referred to as PM0525875. The extracts of PM0525875 showed exclusive antifungal activity, specific towards *Candida albicans* in **Table 1**. The medium ASW-36P(1) supported better activity than other two media. During the secondary screening, the antifungal activity against *Candida albicans* was determined and results are shown in **Table 2**. The culture filtrate obtained from all the three media did not show any active zone. The antifungal activity was found to be confined to mycelia, indicating its intracellular concentration. The medium ASW-36P(1) was shortlisted for large scale cultivation in fermenter. Based on the UV profile displayed the extract we ruled out presence of any polyene type of compound in it. Prior to it, total four of the morphological variants were selected (shown in **Figure 1**) and their antifungal activity was compared with the parent strain and results are showed in **Table 3**. The variant 4 (PM0525875-V4) displayed best antifungal activity as it showed the antifungal activity at the lowest dilution of broth (1:64). So PM0525875-V4 was selected for further studies.



Figure 1. Natural variants of PM0525875 on ASW-AIA plate.

Table 1. Primary screening: antimicrobial activity of PM0525875 using agar well diffusion method.

Seed medium	Production medium	Test cultures					
		<i>Aspergillus fumigatus</i>	<i>Candida albicans</i>	<i>Staphylococcus aureus</i> 209P	<i>Escherichia coli</i> ATCC 25922	<i>Escherichia coli</i> ATCC 20732	<i>Escherichia coli</i> ATCC 35218
(Zone of inhibition in mm)							
ASW-274(1)	ASW-SM12(1)	8	9	Nil	Nil	Nil	Nil
ASW-274(1)	ASW-36P(1)	8	14	Nil	Nil	Nil	Nil
ASW-274(1)	ASW-1M	Nil	9	Nil	Nil	Nil	Nil
Standard antibiotics							
	Amphotericin B (20 µg/ml)	18	20	NA	NA	NA	NA
	Vancomycin (50 µg/ml)	NA	NA	14	NA	NA	NA
	Gentamicin (100 µg/ml)	NA	NA	NA	14	14	15

Nil—No zone of inhibition seen, NA—Not Applicable.

Table 2. Secondary screening of PM0525875: bioactivity of extracts against *Candida albicans* using agar well diffusion method.

Seed medium	Production medium	Extraction method	Zone of inhibition (mm) against <i>Candida albicans</i>
ASW-274(1)	ASW-SM12(1)	Whole Broth Extract	11
		Mycelial Extract	10
		Culture Filtrate	Nil
ASW-274(1)	ASW-36P(1)	Whole Broth Extract	12
		Mycelial Extract	11
		Culture Filtrate	Nil
ASW-274(1)	ASW-1M	Whole Broth Extract	9
		Mycelial Extract	9
		Culture Filtrate	Nil
	Amphotericin B (20 µg/ml)		20

Nil—No zone of inhibition seen.

Table 3. Comparison between antifungal activity of original isolate PM0525875 and its variants (serial dilution of whole broth extract, by agar well diffusion assay).

Dilution of broth	Original isolate	Variant 1	Variant 2	Variant 3	Variant 4
	Zone of inhibition in (mm) against <i>Candida albicans</i>				
1:02	11.9	13.1	13.1	10.6	14.7
1:04	9.1	12.23	12.3	8.4	13.8
1:08	7	10.8	11	Nil	11.1
1:16	Nil	9.2	9.9	Nil	9.9
1:32	Nil	Nil	Nil	Nil	8.7
1:64	Nil	Nil	Nil	Nil	Nil
Amphotericin B (20 µg/ml)		20	20	20	20

Nil—No zone of inhibition seen.

3.2. Large Scale Fermentation of PM0525875-V4

Large scale fermentation was carried out using PM0525875-V4 and fermentation medium ASW-36P(1). The fermentation was carried out till 96 h. The time course of fermentation batch is shown in **Table 4** and expressed in graphical form as shown in **Figure 2**. During fermentation, the pH was dropped with the time and later maintained to 6.5 till end of the fermentation. The rise in biomass (PCV) was slow initially and was picked up after 24 h. After 48 h, the growth rate was steady and the stationary phase was achieved. Dissolve oxygen (DO) dropped significantly till 24 h and was maintained at around 40% till end of the process. The total sugar consumption was slow at beginning and it was reduced to half of the concentration at 48 h. Based on the bioactivity pattern obtained by the broth dilution method (as mentioned in 2.4), the maximum activity was attended at 72 h and maintained steady till end of the fermentation process. Hence, the fermentation cycle was terminated at 96 h.

3.3. Isolation and Characterization of Bioactive Compound

The active compound was found to be intracellular, hence mycelial extract was subjected to bioactivity guided isolation and purification as mentioned in 2.7. The active compound was able to adsorb on HP-20 resins. Further fractionation of crude material using petroleum ether helped removing the impurities and subsequent dichloromethane fraction was found to be active. The pure compound was finally isolated by silica gel chromatography followed by preparative HPLC using RP-18 resin. The characterization of isolated compound was carried out based on data obtained from mass, IR and ^1H NMR spectra (**Figures 3-5**). In ^1H NMR spectra, the signals at δ 7.8 and 7.35 were assigned for protons attached at C-3 and C-5 respectively. Proton at unsaturated C-7 appeared at δ 8.13 due to nitrogen and OH group being in proximity. The aromatic protons associated with other pyridine ring were assigned in the range of δ 7.4 - 8.3. The O-methyl group showed a singlet for 3 H at δ 3.9. Structural confirmation was done by comparing the spectral values with the published data [19]. Based on the data, the active compound was characterized as Caerulomycin A. **Figure 6** and its chemical properties are shown in **Table 5**. From the final yield of the isolated compound (20 mg from 8.5 L broth), the back calculated titer in the harvested broth was estimated as 2.3 mg/L. In order to simplify the extraction method for scale-up purification, the whole broth extraction with equal quantity of ethyl acetate was attempted. Caerulomycin A was found to be completely extractable in ethyl acetate hence this method could be used for large scale extraction batches.

3.4. Phylogenetic Analysis of PM0525875

The 16S rRNA gene from PM0525875 was sequenced. The partial sequence containing 1377 nucleotide base pairs showed close similarity with *Actinoalloteichus cyanogriseus*. The phylogenetic tree constructed using ten matching sequences of 16S rRNA from NCBI data-base is shown in **Figure 7**. The evolutionary history was inferred using the Neighbor-Joining method [20]. The bootstrap consensus tree inferred from 500 replicates was represented in the evolutionary history of the taxa [21] and the branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The evolutionary distances were computed using the Kimura 2-parameter method [22]. The analysis involved 11 nucleotide sequences and the codon positions included

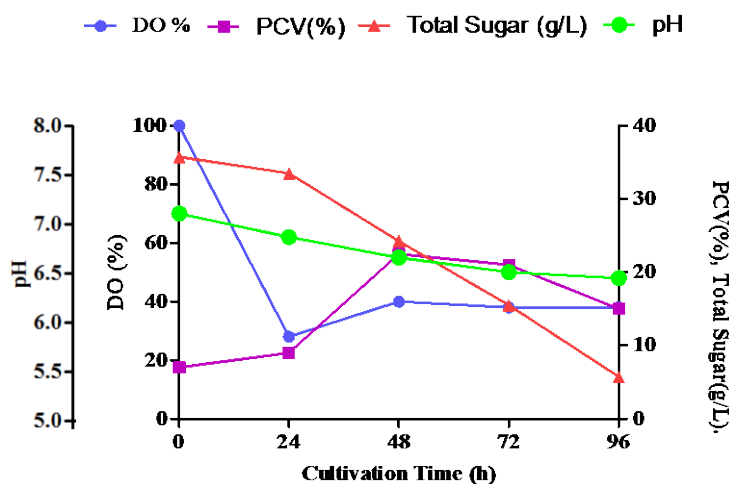


Figure 2. Time course of fermentation process showing pH, packed cell volume (PCV), dissolved oxygen (DO), and total sugar consumed.

Table 4. Fermentor batch parameters.

Cultivation time (h)	pH	PCV (%)	DO (%)	Total sugar (g/L)	Bioactivity against <i>Candida albicans</i> (lowest dilution of broth showing bioactive zones)*
0	7.02	7	100	35.75	NA
24	6.90	9	28	33.50	NA
48	6.65	22.5	40	24.25	1:16
72	6.55	21	38	15.50	1:32
96	6.54	15	38	5.75	1:32

*Agar well diffusion method, NA—Not Applicable.

Table 5. Physico-chemical and spectroscopic properties of isolated compound—Caerulomycin A.

Appearance	White powder
Molecular weight	230.23 [M + H] ⁺ , 252.19 [M + Na] ⁺
IR (KBr)	3498.09, 3193.70, 2848.56, 1605.37 1573.50, 1588.91, 1430.68, 1360.77 cm ⁻¹
¹ H NMR (DMSO-d ₆ 300 MHz)	δ 7.8(H-3), 7.35(H-5), 8.13(H-7), 8.3(H-3'), 7.9(H-4'), 7.4(H-5'), 8.6(H-6'), 3.93(4-OCH ₃), 11.69(OH)

were 1st + 2nd + 3rd + Noncoding. There were a total of 1381 positions in the final datasheet. Evolutionary analyses were conducted on computer software MEGA5 [23]. Based on this results the isolated actinomycetes strain was identified as *Actinoalloteichus cyanogriseus*. The gene sequence was deposited in NCBI GenBank under accession no **KF861694.1**.

3.5. MIC of Caerulomycin A

MIC of the isolated compound Caerulomycin A and two standard antifungal compounds was determined using NCCLS Macrobroth dilution method and results are shown in **Table 6**. Caerulomycin A showed potent activity against four *Candida* strains, including two fluconazole resistant strains. The MIC of the compound was in the range of 0.39 - 1.256 µg/ml. The MIC values obtained of Caerulomycin A against fluconazole resistant *Candida glabrata* were comparable with the MIC values obtained for Amphotericin B. This result indicates the potential of Caerulomycin A as a potent and broad spectrum antifungal agent.

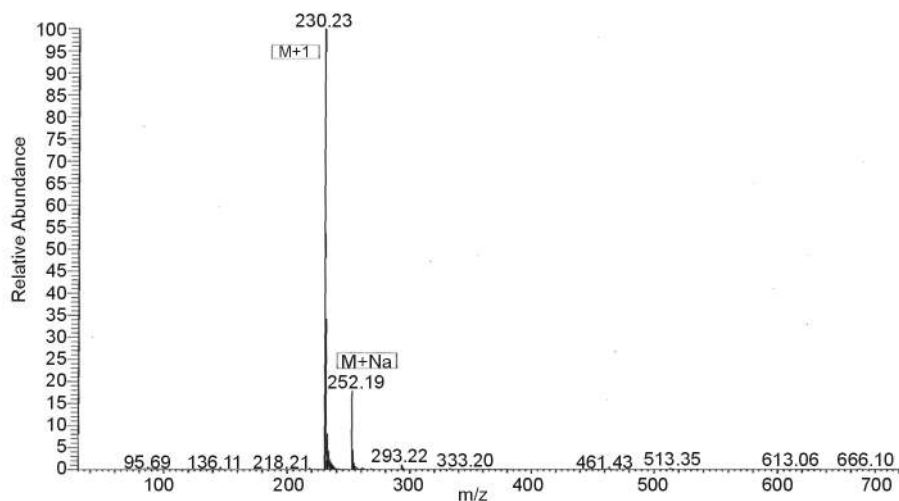


Figure 3. ESI-MS spectrum of Caerulomycin A.

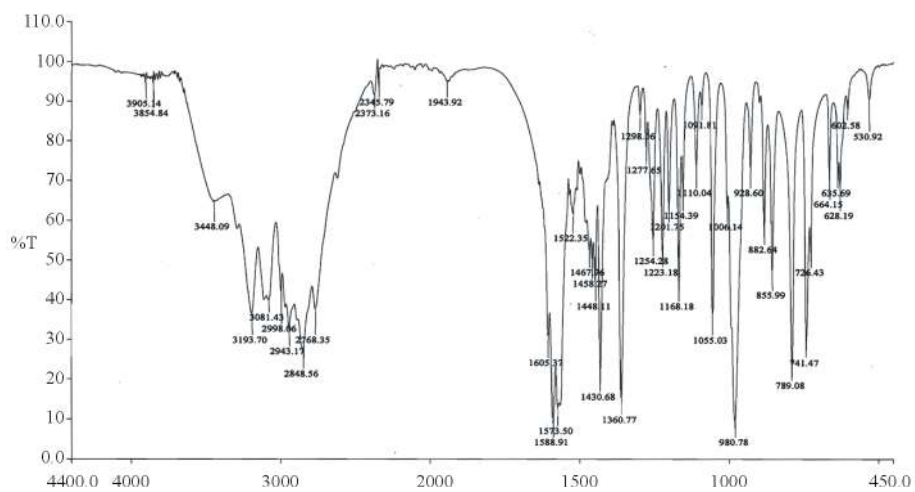


Figure 4. IR spectrum of Caerulomycin A.

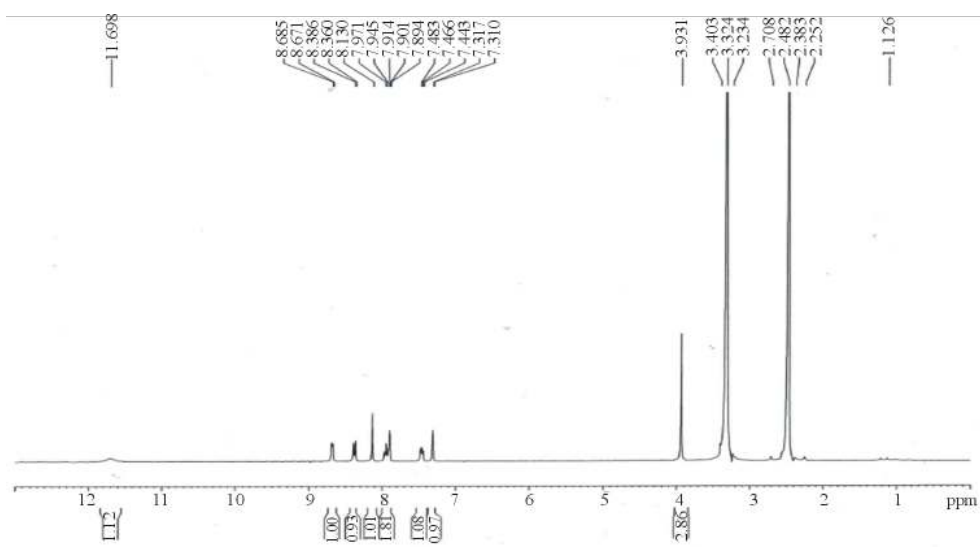


Figure 5. ¹H NMR spectrum of Caerulomycin A.

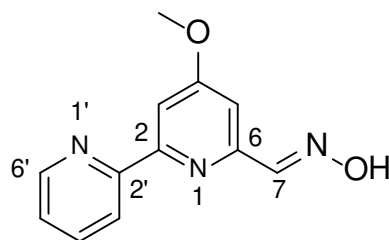


Figure 6. Structure of Caerulomycin A.

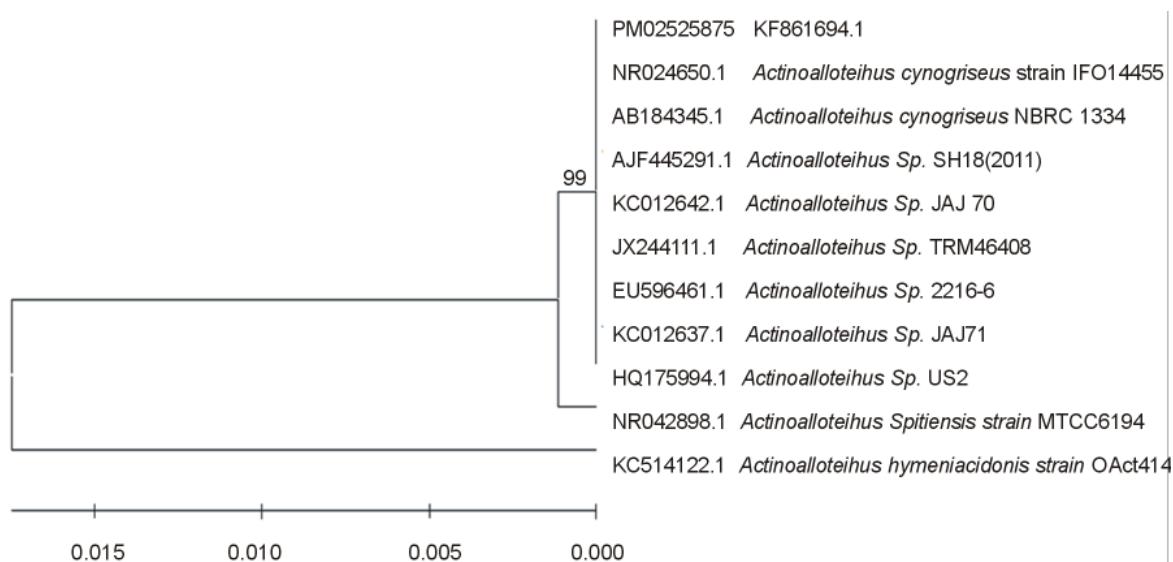


Figure 7. Phylogenetic tree showing relationship between PM0525875 and *Actinoalloteichus* sp. based on clustering of the sequences of the 16S rRNA gene (coefficient of neighbour-joining) by using MEGA5 software.

Table 6. MIC of Caerulomycin A and standard antifungal compounds against selected fungal strains.

Sr. No	Test Organism	MIC ($\mu\text{g/ml}$)		
		Caerulomycin A	Amphotericin B	Fluconazole
1	<i>Candida albicans</i>	0.78 - 1.56	0.39 - 0.78	>256
2	<i>Candida albicans</i> CO9	0.78 - 1.56	0.195 - 0.39	NT
3	<i>Candida glabrata</i> HO5 ^{FlucR}	0.39 - 0.78	0.313 - 0.625	>256
4	<i>Candida krusei</i> GO3 ^{FlucR}	0.78 - 1.56	0.313 - 0.625	64

NT: Not Tested.

4. Discussion

The global antifungal market was estimated at \$9.4 billion in 2010 and is expected to grow at a rate of 1.9% during 2010-2017. The major class of antifungal compound includes azoles, polyenes, pyrimidines, allyamines and echinodians. The antifungal therapies intend eradicating fungi through several action mechanisms, mainly involving destruction of cell wall and inhibition of cell division. There is a significant unmet need of novel antifungal compounds, because the present portfolio of treatments interact unfavorably with other medication, have resistance problem, low spectrum of activity, are fungistatic as opposed to fungicidal and are often toxic. Hence research has been focused on development of potent and safe antifungal compounds, either synthetic or from microbial sources [24]. In the present study, we exploited the marine resources for isolation of actinomycetes strains producing antifungal compounds. In the course of study, the marine invertebrate associated active actinomycetes strain PM0525875 was isolated and identified as *Actinoalloteichus cyanogriseus*. The antifungal principle from this strain was characterized as Caerulomycin A that showed potent *in-vitro* activity against pa-

thogenic and drug resistant *Candida* strains.

Caerulomycin A was first isolated from *S. caeruleus* [25] [26] as an antibiotic. Later it was reported to have anti-cancer activity [27]. *In-vitro* and *in-vivo* activity of Caerulomycin A against *Entamoeba histolytica* has been reported [28]. Natural derivatives of Caerulomycin A has also been reported [29]. Caerulomycin B and C, a new 2,2'-dipyridyl derivatives were isolated from *Streptomyces caeruleus* supplemented with 1 mM L-tryptophan. This group also reported the isolation of Caerulomycin D [19]. Additional natural derivatives of Caerulomycin F-K were isolated from marine derived actinomycetes (*Actinoalloteichus cyanogriseus*) that showed anticancer and antibacterial activity [29]. Lin *et al.* identified the biosynthetic gene cluster for Caerulomycin A from marine actinomycetes *Actinoalloteichus cyanogriseus*. It mainly consists of unusual hybrid polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) [30]. The biosynthesis of Caerulomycin A based on enzymatic modifications leading to structural changes, have been studied [31]. Chemical synthesis of Caerulomycin A-C has also been reported [32] [33]. The immunosuppressive activity of Caerulomycin A and its natural analogues has been reported [34].

Although Caerulomycin A has not been entered in clinic so far, may be due to its limitations, it could be the ideal molecule for generating semi synthetic druggable derivatives having potential application as an anticancer, antifungal or immunosuppressive drug. The strain and the optimized fermentation process described here could be useful for large scale production of this molecule for further drug development studies.

5. Conclusion

In our in-house screening program in the quest of novel and superior antifungal compounds, an actinomycetes strain PM0525875 was isolated from a marine invertebrate. The active principle was characterized to be Caerulomycin A. Minimum inhibitory concentration (MIC) of the compound was found in the range of 0.39 - 1.56 µg/ml against pathogenic fungal test strains. The phylogenetic analysis of producer strain using 16S rRNA sequence showed closest match with *Actinoalloateichus cyanogriseus*. Overall, Caerulomycin A was isolated from marine invertebrate-associated *Actinoalloteichus* sp. using optimized medium and fermentation conditions.

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