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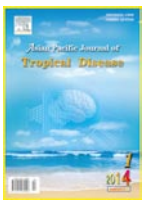

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Bioprospecting of marine *Streptomycetes* sp. for its antagonistic activity on MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates

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PEER REVIEW

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Comments

This is a good relevant study dealing with the current issues of multidrug resistance and the synergistic approach of antimicrobials which is an important dimension to marine natural product research using Actinobacteria. They have also insisted on the importance of the stress tolerance in Hsp70 under the influence of antibiotics implicating that optimal dosage is critical during treatment that curbs development of drug resistance.

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ABSTRACT

Objective: To assess the antimicrobial activity of the Actinobacteria bioactive secondary metabolite and characterize the drug resistance mechanisms of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*).

Methods: Potential marine Actinobacteria were isolated and the crude extract was purified using thin layer chromatography, the fractions were tested for antimicrobial activity and phylogeny of the selected strain was analyzed. Isolated pathogenic strains were screened for extended spectrum beta-lactamase, mannan-binding lectin, AmpC production, efflux mechanism and polymerase chain reaction. The cephalosporin and carbapenem antibiotics were synergistically tested along with *Streptomyces* sp. PM49 fraction by combination disc test and double-disc synergy test. Heterogeneous susceptibility assay, minimum inhibitory concentration and expression of DnaK (Hsp70) were determined.

Results: *Streptomyces* sp. PM49 active fraction of R_f value 0.69 showed antimicrobial activity and an inhibitory zone of 15 to 7 mm obtained. About 34.1% of *P. aeruginosa* and 4.8% of *A. baumannii* were multiple drug resistant. AmpC β-lactamase was found in 12% of *A. baumannii*, efflux mechanism was putatively positive in 8/23 of *P. aeruginosa* and 3/20 of *A. baumannii*. Combination disc test and double-disc synergy test with both PM49 compound and antibiotics showed an increase in the inhibitory zone of <3 mm to 4 mm, three *P. aeruginosa* isolates expressed bla_{IMP}. Heteroresistant subcolonies grew at a frequency of 3×10^{-5} to 1×10^{-5}. Stress induction analysis showed increase of DnaK during heat shock at 52 °C, the levels of protein doubled after exposure to the antibiotics.

Conclusions: Novel unexplored *Streptomyces* spp. antimicrobial constituents can be developed as an inhibitor and can be substituted along with the available antibiotics to combat the drug resistant pathogens.

KEYWORDS

Streptomyces sp., Multidrug resistance, Synergistic activity

1. Introduction

Infectious diseases are the major cause of morbidity and mortality caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*) and have successfully evolved numerous strategies for resisting the action of many antibacterial drugs. They harbor several drug resistant mechanisms such as target alteration, efflux pump, low intrinsic susceptibility and cross-resistance to chemically unrelated classes of antibacterial molecules challenging human health[1]. Extended spectrum beta-lactamase (ESBL) producing organisms are in the family Enterobacteriaceae commonly in *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumonia*), but infections,

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colonization and nosocomial spread involving other ESBL producing organisms are reported[2]. There is also increasing prevalence of metallo-β-lactamases reported in non lactose fermenting *P. aeruginosa* and *A. baumannii*. Organisms over expressing AmpC β-lactamases is another major clinical concern since these organisms are usually resistant to all the available β-lactam drugs of third generation cephalosporins and carbapenems[3]. There are reports of carbapenem heterogeneous phenotypes among Gram-negative isolates in which these small proportion of cells express a very high resistance to antimicrobials, which are a serious threat[4]. The scenario of emerging multidrug resistance and practice of monotherapy have led to therapeutic failure urging the need of new antimicrobial agents[5]. Multiple combinations of antibacterial agents are suggested as an alternative option synergistically enhancing the role with other antibiotics and natural compounds[6].

This century has paved foundation of marine derived bioactive compound primarily of microbial origin for modern pharmaceutical industries[7]. Emerging source of new compounds from recent studies has led to the isolation of novel potential strains of marine Actinobacteria, and a mathematical model has predicted that the number of undiscovered antibiotics on the order of 10^7 from these strains[8]. Synergistic effects of two or more compounds are essential for many reasons to prevent or suppress the emergence of resistant strains, to decrease dose-related toxicity and to attain a broad spectrum of activity; synergistic effects of phytochemicals with antibiotics have been reported[9]. There are various environmental stress factors that contribute for the expression of heat shock proteins in pathogenic microorganisms and the induction of the proteins varies with the duration of the prevailing factors[10]. Subinhibitory concentration of antibiotics is an important factor influencing the level of stress tolerance, acid and bile salt stress in enteropathogenic *E. coli* that has been demonstrated to enhance the adhesion to epithelial cells and also alter glycolipid receptor binding specificity[11].

In this study we attempted to evaluate the antagonistic effect of a novel antimicrobial compound of a marine *Streptomyces* sp. PM49, multidrug resistant non lactose fermenters were characterized and the synergy of the bioactive compound was determined along with ceftazidime and meropenem antibiotics.

2. Materials and methods

2.1. Isolation of potential Actinobacteria

A total of 100 Actinobacteria strains were isolated from Bay of Bengal, Parangipettai coastal area (11 29′N; 79 47′E) of Tamil Nadu. Preliminary screening for potential antimicrobial strain was done with cross-streak method against Gram-positive and Gram-negative multidrug resistant isolates[12]. The potential strains were tested for its biological activity against the test pathogens using 0.5 McFarland′s standard broth culture swabbed on Mueller-Hinton agar (MHA) plates agar plug method. A 5 mm agar bored core containing diffused metabolite from ISP2 media was impregnated on the surface of the test plate and incubated at 37 °C. The ethyl acetate extracts of the potential Actinobacterial strains PM49, PM36 and PM52 were tested for antibacterial activities using Kirby-Bauer disc diffusion method. The selected strain *Streptomyces* sp. PM49 was morphologically characterized and phylogentically determined. The mycelium structure and arrangement of spores were examined under light and scanning electron microscope (JEOL model 6390). Various physiological characteristics and biochemical tests were performed for the isolate.

2.2. Production and characterization of crude extract

Solid state fermentation was adopted for the production of bioactive compound from PM49 strain which was streaked onto yeast extract malt extract agar plates (ISP2 medium) and incubated at 28 °C for 10 to 15 d. The mycelial growth was scrapped from the plate after 10 d and the compound was harvested with ethylacetate was concentrated under reduced pressure at 45 °C with a rotary evaporator and quantified. The extract was subjected to thin layer chromatography (TLC) on silica gel (60F 254 nm Merck) and the solvent system for separation was standardized with *n*-hexane, *n*-butanol, diethyl ether, chloroform, ethyl acetate, methanol and water in different proportions. To visualize the efficiency of separation, the air dried sheets were left in iodine chamber and R_f value was recorded[13]. The crude extract was further purified by preparative TLC and tested for antimicrobial activity by disk diffusion method. Chemical screening was done by spreading the TLC sheets with vanillin-sulphuric acid, naphthoresorcin-sulphuric acid and Ehrlich reagents, kept in hot air oven at 120 °C and observed[14]. For bioautography the chromatogram was inverted on top of culture plate seeded with test organism sprayed with triphenyl tetrazolium chloride and observed for zones of inhibition.

2.3. Phylogenetic analysis

Genomic DNA was extracted from *Streptomyces* sp. PM49 grown on ISP2 using the standard protocol and polymerase chain reaction (PCR) amplified using consensus 16S rRNA primers. Sequencing was performed using ABI 3500 XL Genetic Analyzer with Big Dye Terminator version 3.1 Cycle Sequencing Kit. The 10 μL reaction mixture included 4 μL Big Dye Terminator Ready Reaction Mix, 1 μL template (100 ng/μL), 2 μL primers (10 pmol/λ) and 3 μL milli Q water. The PCR conditions were initial denaturation at 96 °C for 1 min, denaturation at 96 °C for 10 seconds, hybridization at 50 °C for 5 seconds and elongation at 60 °C for 4 min. Phylogenetic analysis was carried out with phylogeny.fr webserver[15]. Multiple sequence alignment was performed to identify closely related homologs with the help of BLASTN search tool available at NCBI. Sequences were aligned using the MUSCLE software (multiple sequence comparision by log-expectation) and phylogentic tree was constructed using the PhyML program[14].

2.4. Tests for ESBL and mannan-binding lectin (MBL) detection

A total of 87 samples from endotracheal secretions, cerebrospinal fluid, blood, catheter, peritoneal fluid, pus and surgical swabs were processed for this study collected during a period of 6 months at a tertiary care hospital Bangalore, India. The phenotypically positive meropenem and ceftazidime resistant *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* isolates were characterized. The test strains of 0.5 McFarland standards were inoculated on MHA plate, disk approximation method was adopted and an increase in the zone diameter of 5 mm or greater with ceftazidime plus amoxyclav disks in comparison with ceftazidime alone was interpreted as positive ESBL. A 5-mm Whatman filter paper disk with 0.5M ETDA of 5 μL (930 μg per disk) and a 10 mg meropenem disk constituted with 10 μL of 50 mmol/L zinc sulphate was dispensed at 15 mm distance. The presence of an enlarged zone of inhibition towards the EDTA disk was interpreted as double-disc synergy test (DDST) positive for an MBL producer[16].

2.5. Detection of efflux and AmpC β-lactamase production

By random selection about 23 isolates of highly carbapenem resistant *P. aeruginosa* and 20 of *A. baumannii* from above test were tested for efflux mechanism[17]. MHA plates with and without reserpine (25 and 50 μg/mL) along with meropenem by agar dilution method for minimum inhibitory concentration (MIC) assay was determined. Difference in the MICs of the strains (decrease in MIC in the control plates in comparison with test plates for reserpine) suggests a putative efflux mechanism. The AmpC β-lactamase activity of isolates was tested with cefoxitin (30 μg) by enzyme extraction method enhanced growth of the surface organism *E. coli* ATCC 25922 (sensitive to cefoxitin) at the point where the slit intersected the zone of inhibition was interpreted positive[18], the test was further confirmed using cloxacillin disks for the isolates that did not show complete inhibition.

2.6. PCR for metallo-β-lactamase

Template DNA was extracted from *P. aeruginosa* and *A. baumannii* by rapid alkali lysis method using primers specific for bla_{VIM} and bla_{IMP}[19]. PCR conditions were 3 min at 93 °C, 40 cycles of 1 min at 93 °C, 1 min at 55 °C and 1 min at 72 °C, and finally, 7 min at 72 °C. Amplification was carried out in a thermocycler (Eppendorf). The amplicon was purified and separated in 1% agarose gel visualized by staining with ethidium bromide (0.5 μg/mL) and compared with 100 bp ladder.

2.7 Heterogeneous phenotype susceptibility assay

The analysis was performed for 15 selected strains of *P. aeruginosa*, *A. baumannii* and *Staphylococcus aureus* (*S. aureus*) strains displaying heterogeneous phenotype with meropenem and methicillin. The heteroresistant subpopulation at the highest drug concentration of the above antibiotics were tested along with *Streptomyces* sp. PM49 compound and observed for complete zone of inhibition by agar plug and disk diffusion method.

2.8. Combination disc test (CDT) and DDST with antibiotics and bioactive compound

To evaluate the potential of secondary metabolite along with antibiotic disks, the ceftazidime and meropenem disks alone and with *Streptomyces* sp. PM49 compound of 50 μg/5μL by combined disk test on *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*. Methicillin was also included for *S. aureus* isolates. Screening of ESBLs and MBLs was performed as described above along with bioactive compound disks and the assays were triplicated.

2.9. Stress induction of Hsp70

The non lactose fermenters were induced to grow in the presence of ceftazidime and meropenem and protein profile of the strains were observed. The cultures were inoculated in Brain Heart Infusion containing the 20 μg/mL of antibiotics and cells were harvested between OD_{600} 0.6 after (0 to 60 min). For heat shock the 0.6 OD cultures were incubated in a water bath at 50 °C at the mentioned time intervals. Expression of Hsp70 was assessed before and after stress by SDS-PAGE immunoblotting using anti-Hsp70 diluted 5 000 times in phosphate buffer solution (PBS)-Tween 20. The secondary antibody (anti-mouse IgG HRP conjugate-Promega) diluted 5 000 times in PBS-Tween 20 was applied for 1.5 h and the blots were washed again with PBS-Tween 20.

2.10. Determination of MIC

MIC for the test isolates was performed with the doubling dilution of *Streptomyces* sp. PM49 extract ranging in concentration from 8 μg/mL to 128 μg/mL by agar plate method using MHA. *P. aeruginosa* ATCC 27853 was used as a standard strain for quality control. Plates were incubated at 37 °C for 18 h and the zones of inhibition were measured.

2.11. Statistical analysis

To analyze the data of antibiotics resistance pattern, one way ANOVA was performed to check the significant difference among the lactose and non lactose fermenter groups for the antibiotics tested. A difference was considered significant if the probability of the results was less than 0.05.

3. Result

From the sampling site, a total of 100 Actinobacteria isolates designated as PM1-PM100 based on the different colony morphology were selected and preserved. The isolates were characterized and micromorphology of the strain was examined by scanning electron microscope (Figure 1).

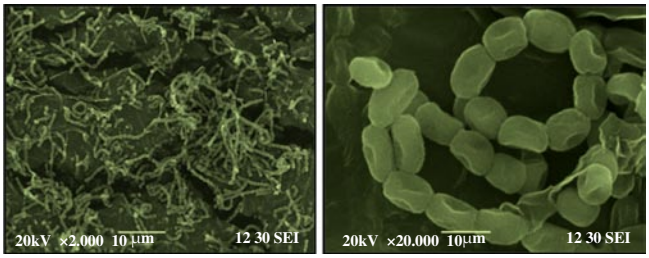

Figure 1. Scanning electron microscopic image of *Streptomyces* sp. PM49.
SEM of *Streptomyces* sp. PM49 showing colony morphology and chain of smooth spores 2 000×, single spore magnified 20 000× grown on yeast extract-malt extract agar (ISP2) for 7 d at 28 °C, bar=10 µm.

The resistance percentage of the pathogens are shown in Tables 1 and 2. The isolate PM49 was active against both Gram-positive and Gram-negative isolates with a zone diameter of 12 to 17mm. The crude compound was best resolved using chloroform and ethylacetate (1:1 v/v), the spots resolved into five bands of R_f value 0.53, 0.69, 0.72, 0.87, 0.90 and 0.92. Bioautography revealed that the second and third spot exhibited good antibacterial activity on spraying with 2% (w/v) aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride. Active spots on exposure to naphthoresorcin- sulphuric acid developed brown color. The antimicrobial activity for the separated spots by disk diffusion method on multiple drug resistant pathogens demonstrated zone of inhibition for *E. coli*, *K. pneumoniae* 15-17 mm and non-lactose fermenters *P. aeruginosa* and *A. baumannii* of 13-16 mm. The 16S rRNA sequences was gel eluted and sequenced (Figure 2). G block curation was done and 99% identity with 16S rRNA sequences of *Streptomyces djakartensis*, *Streptomyces misakiensis*, *Streptomyces rochei* and *Streptomyces yanii* were obtained comparative analysis that showed the new isolate to be *Streptomyces* sp. PM49 and submitted in Genbank with accession no. JX904061 (Figure 3).

Table 1
Resistance of lactose fermenters percentage of Gram-negative bacilli for various antibiotics.

Antibiotics	Resistance for individual antibiotics of lactose fermenters (%)		
	E. coli	*Klebsiella* spp.	*Enterobacter* spp.
Amikacin	100.0	100.0	100.0
Ciprofloxacin	90.7	100.0	84.6
Gentamicin	75.7	85.7	89.2
Nitrofurantion	100.0	100.0	26.9
Norfloxacin	77.2	80.7	80.7
Cotrimoxazole	68.1	88.4	100.0
Impenem	100.0	83.8	100.0
Colistin	22.0	34.0	58.0

Table 2
Resistance of non lactose fermenters percentage of Gram-negative bacilli for various antibiotics.

Antibiotics	Resistance for individual antibiotics of non lactose fermenters (%)	
	Pseudomonas spp.	*Acinetobacter* spp.
Amikacin	56.1	56.7
Ciprofloxacin	72.1	100.0
Gentamicin	77.0	100.0
Piperacillin	57.7	100.0
Impenem	77.5	98.5
Colistin	65.0	37.0
Tigecyclin	NA	0.0
Netilmicin	67.3	61.5

NA: not administered.

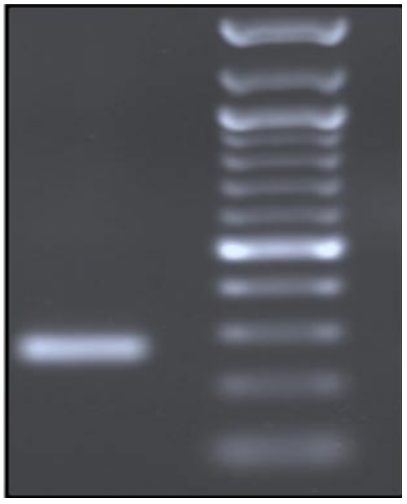

Figure 2. PCR amplified 16S rRNA fragment.
PCR amplification using consensus 16S rRNA gene specific primers of marine *Streptomyces* sp. PM49 strain, Lane-1 gene product, Lane-2 standard molecular 1 kb ladder.

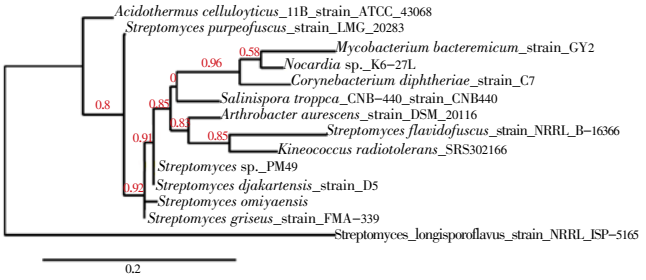

Figure 3. Phylogenetic tree of *Streptomyces* sp. PM49.
Phylogenetic analysis of the new strain marine *Streptomyces* sp. PM49 had 99% identity with 16S rRNA sequences of terrestrial *Streptomyces djakartensis* isolate.

The clinical isolates screened were commonly found multidrug resistant and exhibited resistance for 8 to 11 antibiotics, *E. coli* was predominant with 71.28% followed by *K. pneumoniae* 34.5%, *P. aeruginosa* 34.1% and *A. baumannii* 4.8%, but the frequency of multiple drug resistant strains prevailed in the non lactose fermenters. ESBL was high in number in *K. pneumoniae* by disk approximation method, metallo-β-lactamase producers were detected better in DDST. A distance of 15 mm centre to centre between meropenem with 0.5M EDTA disks of 930 µg showed that 20.4% of *P. aeruginosa* were MBL positive. About 12% of *A. baumannii* isolates were found to be AmpC β-lactamase producers with cefoxitin and further confirmed using cloxacillin disks for the isolates that did not show complete inhibition (Figure 4). On testing for efflux mechanism none of the isolates showed decrease in the MIC of meropenem with reserpine but 8/23 of *P. aeruginosa* and 3/20 of *A. baumannii* were putatively positive. Genes encoding MBLs gave positive results in 3 isolates for IMP in *P. aeruginosa* and none in *A. baumannii*. In case of CDT and DDST with both PM49 compound and ceftazidime, meropenem disks an increase in the inhibitory zone of <3 mm to 4 mm was observed.

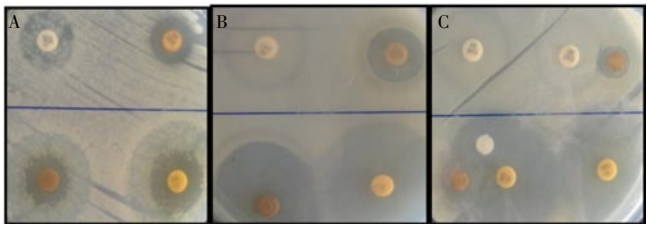

Figure 4. Combined disk test and double disk synergy test.
A & B: Dark disks substituted with compound are showing complete inhibition of *P. aeruginosa* and *A. baumannii* strains (Plates-Top slot ceftazidime, bottom meropenem). C: Positive bla_{IMP} metallo-β-lactamse *P. aeruginosa* strain also exhibited sensitivity to meropenem with EDTA disk+meroepenem disk+*Streptomyces* sp. PM49 fraction disk (triple disk) compared to meropenem alone.

Heteroresistant subcolonies grew within the zone till 32 μg/mL of meropenem at a frequency of 3×10^{-5} to 1×10^{-5} for both the strains, but complete inhibition was observed in *S. aureus*. To investigate if the colonies were gaining heterogenenous phenotype under selective pressure with high antibiotic concentration, the colonies from 32 μg/mL concentration were again subcultured on antibiotic free MHA plate. The culture remained heterogenenously resistant with a frequency of 4×10^{-5} in the non lactose fermenters. Appearance of subcolonies around ceftazidime disk within the apparent zone of inhibition of heterogenenous colonies was also observed with agar plug from novel *Streptomyces* sp. PM49 data not shown. Stress induction analysis by western blot assay of DnaK proteins showed a transient increase during heat shock at 50 °C. The levels of DnaK did not increase during the first 15 min of heat shock and no changes were observed in the expression for untreated control during the 60 min period. Drug susceptible strain did not survive in the presence of meropenem in contrast to the multidrug-resistant strain tested under the same conditions. The levels of DnaK protein more than doubled after 30 min exposure to the antibiotics and thereafter produced near-constitutively increased levels of the protein up to 60 min. Heat shock response was not prominently expressed as that of meropenem induced stress in both *P. aeruginosa* and *A. baumannii*. In *P. aeruginosa*, DnaK level was more than *A. baumannii*, but both the strains exhibited a steady state increase of the protein (Figures 5 and 6).

Figure 5. PCR amplification of bla_{IMP} gene.
Metallo-β-lactamase gene bla_{IMP} was positive in 3 *P. aeruginosa* isolates, Lane-1 DNA 100 bp ladder and Lane 2, 3 & 4 bla_{IMP} of 188 base pairs gene product.

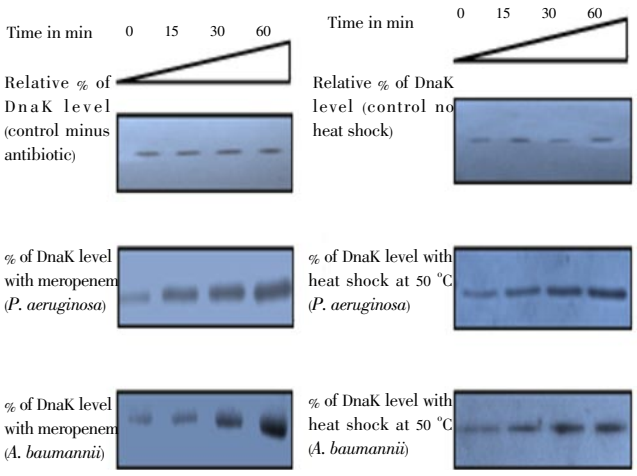

Figure 6. Stress induction analysis of Hsp70 (DnaK).
Stress tolerance under the influence of meropenem on the expression of DnaK in *P. aeruginosa* and *A. baumannii* strain grown at 37 °C demonstrated a significant increase in the level of the protein. Western blot analysis after a shift to a raise in temperature at 50 °C also shows the induction of DnaK increased till 60 min of time interval in both the isolates.

The statistical package using (SPSS17.0) shows a significant difference in P value existing among the organisms for the antibiotics tested ($P<0.01$).

4. Discussion

The potential *Streptomyces* strain PM49 from marine sediments had wide antimicrobial activity on both Gram-positive and Gram-negative organisms. The strain can be placed in rectus-flexibilis group of *Streptomyces* sp. since the sporophores were straight to flexuous in nature. The functional group of the active compound should be a sugar containing molecule since it developed brown color spot on exposure to naphthoresorcin-sulphuric acid. The R_f values of the compound with antimicrobial activity was at 0.53 to 0.69 consistent with previously reported values. The unmatched ends were deleted to obtain homogenous matrix of characters which will increase the reliability of the tree[20]. Taxonomic position of the strain PM49 using *in-silico* sequence analyzer Phylogeny.fr along with comparative biological analysis data of the closely related microbial strains confirms new isolates very efficiently[21]. ESBL production although frequently reported in *E. coli* is commonly found in *K. pneumonia*[22], *P. aeruginosa* clonally spread MBLs gene to Enterobacteriaceae members carrying intrinsic resistant to many antibiotics they are largely associated with nosocomial outbreaks[23]. *Acinetobacter* spp. acquire resistance to chemotherapeutic agents, 86% of the strains were found resistant to the tested antibiotics except tigecyclin unlike other reports[24]. A putative efflux mechanism may be responsible for the resistance to carbapenem in all the positive isolates[25]. Sequence comparison of specific genes for the metabolite produced, structure elucidation using physico-chemical characteristic and functional group analysis of the beta lactamase inhibitory compound revealing the novelty of this strain has to be executed. DDST is relatively reproducible compared to CDT[26], although synthetic activity of sulbactam

with imipenem and cefoperazone has been demonstrated natural compounds which are known for their less toxicity and efficiency[27]. Actinobacteria are known for their antimicrobial compounds especially *Streptomyces* are known for robust novel pharmacological compounds.

Streptomyces clavuligerus produces the β-lactamase inhibitor clavulinic acid which synergistically enhanced the activity of cephalosporins[28]. The synergy of natural compounds and antibiotics has been reported, but this is the first study to the best of our knowledge for synergistically testing the combination of Actinobacterial compound with antibiotics for heteroresistant strains. It is difficult to discriminate if the heteroresistant phenotype of our isolate resembles persisted cells or mutants since few *P. aeruginosa* strains that had colonies growing in the inhibitor zone for the novel *Streptomyces* sp. PM49 bioactive compound. *P. aeruginosa* and *A. baumannii* on being exposed to sub inhibitory concentration of meropenem expressed increased amount of DnaK. Growth in the presence of sub inhibitory concentration of amoxicillin, clindamycin and metronidazole is found generally upregulate both transcription and translation machinery in *Clostridium difficile*[10]. The sub optimal dose of antibiotic stimuli can provide the ability for the strains to cope up with the stress and could enable selection of resistant cells for the administrated antibiotics. Cell wall stimuli upregulated in the vancomycin intermediate resistant *S. aureus* strains have been studied[28]. Jenny *et al.* have demonstrated that genes encoding many ribosomal proteins, penicillin binding proteins and a putative β-lactamase enzyme have been induced in the presence of amoxicillin[10]. The incidence of ESBL and MBLs producing strains among these Gram-negative non lactose fermenters clinical isolates have increased over the past few years resulting in limited therapeutic options and outbreaks of multiple drug resistant isolates. Although 16S rRNA study reveals the persistence of new marine *Streptomyces* is yet to be explored from marine sediments[29], sequence comparison of specific genes for the metabolite produced, structure elucidation using physico-chemical characteristic and functional group analysis of the beta lactamase inhibitory compound that reveals the novelty of the strain has to be executed.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Non lactose fermenting Gram-negative *P. aeruginosa* and *A. baumannii* are becoming increasingly resistant to wide range of broad spectrum of antibiotics which are reported globally. The versatility to hydrolyze all types of β-lactamases necessitated intensifying treatment to these pathogens with new antimicrobials. This study aimed to screen for a novel unexplored marine *Streptomyces* spp. antimicrobial compound to effectively control the multiple drug resistant strains and characterize the various drug resistance mechanisms harbored by them.

Research frontiers

This study reveals the persistence of new marine *Streptomyces* yet to be explored from marine sediments and their antimicrobial bioactive compounds are found to inhibit multiple drug resistan strains. The sub-optimal dose of antibiotic stimuli provides ability for the strains to cope up with stress enabling selection of resistant cells.

Related reports

Fenical *et al.* have reported that marine Actinobacteria are resource of new drug leads and monotherapeutic practices leading to therapeutic failure. In this study, the authors extracted *Streptomyces* sp. PM49 bioactive compound tested for its potential and enhanced activity of ceftazidime, meropenem disks with an increase in the inhibitory zone of <3 mm to 4 mm was observed as demonstrated by Coutinho *et al.* using plant extract against MRSA. Stress induction analysis of DnaK proteins by western blot analysis showed an increase in the expression of the protein being upregulated both in the presence of heat and antibiotic stress which has been reported by Jenny et al. This study emphasis the importance of stress stimuli and prospects of Actinobacteria bioactive compounds for the control of multidrug resistant pathogens.

Innovations & breakthroughs

Stress tolerance under the influence of meropenem on the expression of DnaK in *P. aeruginosa* and *A. baumannii* strain grown at 37 °C demonstrated a significant increase in the level of the protein. Western blot analysis to a raise in temperature at 50 °C also showed the induction of DnaK in both the isolates.

Applications

It is becoming increasingly clear that less explored marine habitats are rich sources of novel Actinobacteria having capability to produce interesting new bioactive compounds, including antibiotics. Synergistic effects of two or more compounds are essential for many reasons to prevent or suppress the emergence of resistant strains, to decrease dose-related toxicity and to attain a broad spectrum of activity. This study emphasises existing antibiotics can be reused along with novel natural compound and the importance of optimal dosage of antibiotics for therapy.

Peer review

This is a good relevant study dealing with the current issues of multidrug resistance and the synergistic approach of antimicrobials which is an important dimension to marine natural product research using Actinobacteria.

They have also insisted on the importance of the stress tolerance in Hsp70 under the influence of antibiotics implicating that optimal dosage is critical during treatment that curbs development of drug resistance.

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