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Original Research Article

Molecular Profiling of *Glutamicibacter Mysorens* Strain YKIKM.MU and Bioactive Peptides Characterization for Antibacterial Activity

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Abstract

The increasing resistance of microbe to the existing synthetic drugs is being substituted with the alternative protein biomolecule as an importance source of new agents for treating various ailments. *Actinomycetes* are prevalent in diverse habitat across world. The coastal Mangrove vegetation is unique habitat for *Actinomycetes* isolation, high salinity and deprived oxygen levels are challenging conditions results a rare biologically important species. In present studies was carried to explore diversity of *actinomycetes* as a result *Glutamicibacter mysorens* isolated from Mangroves of Mangalore. This culture used for protein production using starch casein nitrate broth and yielded proteins of 1mg/ml and shown efficient antibacterial activity against *Bacillus cereus, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeroginosa* pathogenic bacteria. These proteins are characterized using 10% discontinuous anionic sodium dodecyl sulfate poly acryl amide gel electrophoresis and liquid chromatography-mass spectrophotometer analysis shown elution profile maximum at 3.11min with molecular weight of 265D, 266D, 267D. *Actinomycete* culture identification was carried through both polyphasic and molecular studies, biochemical tests, 16s rRNA gene sequence submitted to Genbank, accession number MW647910 under *Arthrobacter* genera species named as *Glutamicibacter mysorens* strain YKIKM.MU and Phylogenetic tree was plotted shown next maximum resemblance with *Arthrobacter mysorens* with 98.28%.

Key Words: Mangrove habitat, Proteins, SDS-PAGE, LC-MS, Antimicrobial activity, 16s rRNA sequencing, Phylogenetic studies.

Introduction

Actinomycetes are Gram-positive, spore-forming organism which shares characters of both bacteria and fungi. The "*Actinomycetes*" word is derived from Greek "atkis" (a ray) and "mykes" (fungus)[1-3]. These *actinomycetes* are the prominent source for the production of antibiotics are used in various biological applications[4-7]. The genus *Glutamicibacter* members are Gram-positive, catalase-positive, aerobic and sporogenous, and phenotypically heterogenous and grouped in the family *Micrococaceea*[8-10]

Mangrove habitats found in coastal tropical and subtropical areas harbor

unique flora which is specially adapted to grow on nearly stagnant water, river banks, and estuaries brackish water. Mangrove floras have adapted to grow in oxygen-deficient soil can be able to extract the nutrients needed for their survival. Additionally, these floras can survive in water with high salt content and in constantly changing water levels[1,11]. The unique ecological characteristic of mangrove's habitat to grow a variety of microbial flora with unique properties serves as a prominent source for various applications. As a result of convergent evolution through external stress and other environmental factors offers a wide variety of novel microbial species. The presence of environmental stress will also enhance the capability of an organism to withstand and survive in limited nutrients. These organisms by adapting themselves produce diverse biologically active compounds that were used to treat various diseases for chronic to human survival[12-14].

Mangroves cover a combined area of 52,300 square miles in America, Africa, Australia, and Asia. 118 countries around the world hold a mangrove forest with the largest 42% located in Asia. The next highest 21% has Africa, followed by North and Central America 15%, Oceania 12%, and South America 11%. Approximately three-fourths of all mangroves are located within only 15 countries[15]. India has a total mangrove cover of 4,482 square kilometers which is just 3% of the world's mangrove area. The coastline of Karnataka is over 320 kilometers cut across the join the Arabian Sea. In connection with the coast, the saltwater tides from the sea travel several kilometers interior through the river mouths providing compatible habitats for mangroves. Most mangroves are of the fringing type in linear formations along the river or estuarine banks[16].

Exploring antimicrobial peptides (AMP's) from *Glutamicibacter mysorens* strain YKIKM.MU under genus *Glutamicibacter* group of *Actinomycetes* offers many applications having biomedical importance[17-20]. Antimicrobial peptides are low molecular peptides with less than fifty amino acids capable of non-specific interaction with microbial membranes. Antimicrobial peptide affects the microbe's rapid death and decreases the probability of resistance development[21-24]. These molecules interact with the membrane molecules by ionic interactions leading to the formation of pores and membrane disruptions in the lipid membrane. Also, AMPs are capable of intracellular targeting of pathogens since AMPs can bind to nucleic acids and proteins[25,26].

Still the unexplored habitats of mangroves offer an excellent opportunity for finding novel *actinomycetes* with unique properties. The Karnataka state has a sizeable stretch of mangrove forests, a vibrant saline-water ecosystem associated with India's coastal region[16]. In the present study, we isolated and identified *Glutamicibacter mysorens* strain YKIKM.MU from the mangrove sediments of Mangalore, Karnataka. Identification is carried out through both polyphasic studies and Molecular techniques by 16s rRNA gene sequencing a Phylogenetic tree was plotted by program NEIGHBOR. This strain after mass culturing for protein extraction resulted in the identification of bioactive peptides. These microbial based proteins were partially characterized using 10% discontinuous anionic SDS-PAGE and LC-MS analysis. Also, when checked for antibacterial activity, these proteins also shown efficient antimicrobial activity against both Gram-negative and Gram-positive pathogenic bacteria.

Material and methods:

Soil sample collection:

Mangrove soil was collected from four different places in the region of Mangalore, Karnataka, India. The collected samples were transported aseptically in sterile plastic bags to the

Molecular Research Laboratory, Department of Microbiology, Jnana Kaveri, Mangalore University. The samples were subjected to preheat treatment at 60°C for 2 hours to avoid bacterial and fungal growth before serial dilution and plating technique. The texture of soil samples varied from sandy to loamy and parameters such as temperature showed 21°C at the site of collection and pH 7.2 was recorded[1].

Isolation of *actinomycetes:*

One gram of soil sample was serially diluted up to 10^{-7} dilution. Aliquots of 0.1ml of each dilution was spread plated on Starch Casein Nitrate Agar (SCN) plates in triplicates and incubated at 30°C for 7 days. After incubation time, the plates were examined for the presence of *actinomycetes* colonies. The prominent colonies were selected, and pure cultures were maintained on SCN media[14,27].

Culture Media:

Actinomycete culture was grown efficiently using Starch *Casein Nitrate Agar (SCN).* The composition of SCN media is as followed (Starch: 1%, Casein: 0.03%, KNO3: 0.2%, MgSO4.7H2O: 0.005%, K2HPO4: 0.2%, NaCl: 0.2%, CaCO3: 0.02%, FeSO4: 0.001%, Agar: 2%). All chemicals were procured from HiMedia, India[1].

Screening for hydrolytic enzyme production:

The mangrove *actinomycete* isolate (S6) was screened for the production of various hydrolytic enzymes, viz., amylase, lipase, protease, pectinase, and cellulase by following standard enzyme production methodology.

Amylase, Protease, and Lipase activity:

Nutrient agar medium (peptone 0.5g; beef extract 0.3g; agar 2g; NaCl 0.5g; pH 7.0) supplemented with starch (1%), gelatin (2%) and tributyrin (1%) were prepared separately for the respective enzyme assays. Plates were spot inoculated and incubated at room temperature (28 ± 2°C) for 3 to 5 days. Starch agar plates were flooded with grams iodine solution (Iodine 1g; potassium iodide 2g; distilled water 300ml) and gelatin agar plates with mercuric chloride solution (15%). The appearance of the clearance zone around the colonies was noted as positive and the diameter of the zone was recorded. Tributyrin agar plates were noted for a clear zone around the colonies for lipase production[28]

Pectinase:

Pectin Agar (pectin 0.5g; $CaCl_2.2H_2O$ 0.02g; NaCl 2g; $FeCl_3.6H_2O$ 0.001g; yeast extract 0.1g; agar 2g; NaCl 0.5g; pH 7.0) was used for testing the production of pectinase. The plates were spot inoculated and incubated at room temperature (28 ± 2°C) for 5 to 7 days. After incubation, the plates were flooded with 1% cetavlon (cetyl trimethyl ammonium bromide), allowed to stand for 20-30 minutes and the zone of clearance was noted as positive[27].

Cellulase:

Cellulose agar (casein hydrolysate 0.05g; yeast extract 0.05g; $NaNO_3$ 0.01g; cellulose powder 0.5g; agar 2g; NaCl 0.5g; pH 7.0) was used for testing cellulase production. The plates were spot inoculated and incubated at room temperature (28 ± 2°C) for 7 to 10 days. The zone of clearance around the colonies was noted as positive[29,30].

DNA isolation by CTAB Method:

The *actinomycetes* culture was grown on SCN broth and after maximum growth; culture biomass was collected by centrifugation. The culture biomass grounded using mortar and pestle after chilling in liquid nitrogen at room temperature. A fine powder obtained was used for extracting DNA. In brief, to 100mg homogenized tissue 500µl of CTAB Extraction Buffer was mixed and thoroughly vortexed. Homogenate was kept at 60°C bath for 30 minutes.

Following the incubation period, homogenate centrifuged for 5 minutes at 14,000 rpm. An equal volume of chloroform/isoamyl alcohol (24:1) and vortex it for 5 seconds then centrifuged the sample for 5 minutes at 14,000rpm to separate the phases. The DNA was precipitated by adding 0.7ml volume ice-cold isopropanol from the upper aqueous phase and incubated at -20°C for 30 minutes. Centrifuged sample at 14,000rpm for 10 minutes. The supernatant decanted without disturbing the pellet and subsequently washed with 500µl ice-cold 70% ethanol. The ethanol was removed by drying in Speed Vac. The final DNA obtained was dissolved in 30µl TE buffer with further treatment with 1µl of RNase solution by incubation at 37°C for 30 minutes. The purity and quantification was checked and recorded using Thermo scientific µ drop plate reader[32].

DNA purification using Column:

DNA purification method was carried out according to the manufacturer's instructions (Himedia) In brief, added 200µl of binding buffer to obtained DNA and mixed well and transferred to the column. Further spun at 12000 rpm for 1 minute and washed with wash buffer (750µl) and spun for 1 minute and repeated twice. Subsequently dried for 2 minutes and eluted with 20µl of elution buffer. With a final spinning for 1minute, the DNA obtained was analyzed using Agarose gel electrophoresis[33].

Agarose Gel electrophoresis:

0.8% Agarose gel was performed using 80ml of 1x TAE buffer and added 4µl ethidium bromide solution during solidification. The gel was run at 100V until the dye line has traveled approximately 75-80% down the gel and visualized using Gel Documentation[34].

Preparation of PCR reaction mixture:

DNA barcode primers contained 1µl DNA template (25ng), 20µl Master Mix, 1µl forward primer (10pM), 1µl reverse primer (10pM), and the final volume 25µl adjusted with molecular grade nuclease-free water. Standard primers forward primer (27F) 5'-AGAGTTTGATCCTGGCTCAG-3' Reverse primer (1492R) 5'-GGTTACCTTGTTACGACTT-3' for 16s rRNA gene amplification for actinobacteria was performed[35].

PCR conditions for 16s rRNA gene Amplification:

PCR reaction was set for Initial denaturation was 95°C for 2 minutes. Final denaturation 95°C for 30 seconds, Annealing 50°C for 30 seconds. Elongation 72 °C for 1 minute, Denaturation and was run for 35 cycles. Final Elongation 72 °C for 10 minutes holds at 4°C forever. The PCR amplified DNA was separated on 0.8% agarose gel electrophoresis and the gel was solubilized using gel solubilization buffer at 55ºC. The solubilized DNA was extracted by adding 200µl of isopropanol and further purified using column DNA purification Kit[35].

PCR Purification and 16s rRNA gene sequencing:

125mM EDTA (2.5µl) was added to each well-containing amplicons and centrifuged. 35µl of Ethanol was added and vortexed. Centrifuged at 3510rpm for 30 minutes. Ethanol was decanted using a tissue bed at 300 rpm. 40µl of 80% Ethanol was added to the wells and Centrifuged for 12 minutes. The above steps were repeated twice. Further,air-dried for 30-45 minutes by covering the plate with lint-free tissue. A short spin was given after adding, 13µl of Hi Di Formamide for denaturation at 95°C for 5 minutes, and the plate was placed in sequencer[12].

Data analysis:

Using software BioEdit the sequences were selected based on the quality of obtained sequence observed through Electropherogram peaks. The sequence file of ab1 format was viewed using software

BioEdit. Analyzed the sequencing data using the NCBI BLAST server.

Protein Extraction:

Strain of *Actinomycetes* were grown with agitation of 100rpm for 7-10days at 30°C in Starch Casein Nitrate broth (SCN) media composition (Starch: 1%, Casein: 0.03%, KNO_3: 0.2%, $MgSO_4.7H_2O$: 0.005%, K_2HPO_4: 0.2%, NaCl: 0.2%, $CaCO_3$: 0.02%, $FeSO_4$: 0.001%) with supplement of 1% soya tryptone. Cells of culture were harvested by centrifugation (7,000g), washed twice with phosphate-buffered saline without Mg2+ and Ca2+ and re-centrifuged. The cells were then suspended in 10ml of ice-cold acetone (analytical grade), allowed to stand on ice for 5 minutes, and collected by centrifugation (7,000g). Residual acetone was removed under a stream of nitrogen, and the proteins were then extracted by incubating with 1.0ml of 1% sodium dodecyl sulfate (SDS) for 2minutes. The acetone pretreatment was found necessary since the direct utilization of detergents did not extract cellular proteins from gram-positive microorganisms[31]. The resulted amount of protein produced was estimated by Biuret's method in comparison with standard BSA.

Electrophoretic analysis:

A discontinuous anionic gel electrophoresis was carried out composed of 10% separating and 5% stacking gel system. The gel was removed carefully and kept for protein staining (0.16% of $AgNO_3$) and stopped with1% acetic acid solution.

Antimicrobial activity:

The extracted crude protein was used with a concentration of 50µg/50µl loaded each well for antimicrobial activity by well diffusion method. Pathogenic test organisms selected for the study were *Escherichia coli* (ATCC8739), *Pseudomonas aeruginosa* (ATCC9027), *Klebsiella pneumoniae* (ATCC9621), *Staphylococcus aureus* (ATCC6538P) *Bacillus cereus (ATCC10876) Proteus vulgaris (ATCC13315) Salmonella typhimurium (ATCC23564) Enterobacter aerogenes (13048).* Nutrient agar plates were prepared, and swab inoculation of the 24hours pathogens cultures was made on the surface to produce a lawn culture.

Liquid Chromatography-Mass Spectrophotometer:

Chromatography was performed using an Agilent 1100 LC system including a vacuum degasser, binary pump, and well-plate autosampler. Chromatographic separation was achieved using a Supelco Discovery® RP-Amide C16 column (50mm × 4.6mm, 5m) with guard Discovery® RPAmide C16 column (20mm × 4.0mm, 5m) (Sigma-Aldrich, Oakville, ON, Canada). Mobile phases were 0.1% aqueous formic acid and methanol, with a linear gradient starting at 50% methanol, increased to 70% at 5 minutes, held at 70% for 2 minutes, then re-equilibrated at 50%. The flow rate through the chromatographic system was 1.0ml/minute, with a post-column tee to divert 0.6ml/minute to waste. Injection volume was 10L; a 5s flush port needle rinse before injection was incorporated into the procedure to prevent cross-contamination.

Results and Discussion

Description of *Actinomycete* culture *Glutamicibacter mysorens* strain YKIKM.MU

The *Actinomycete* isolate *Glutamicibacter mysorens* was shown efficient growth in starch casein nitrate media. Culture characteristics studied in polyphasic method showed Gram **+ve,** brown pigmentation, raised elevation, regular margin, powdery texture, grey-colored spores with both substratum and aerial mycelium appears brown colored as shown in Figure 1. The culture had shown positive for amylase and protease and negative for cellulase, lipase, and pectinase hydrolyzing enzyme activity.

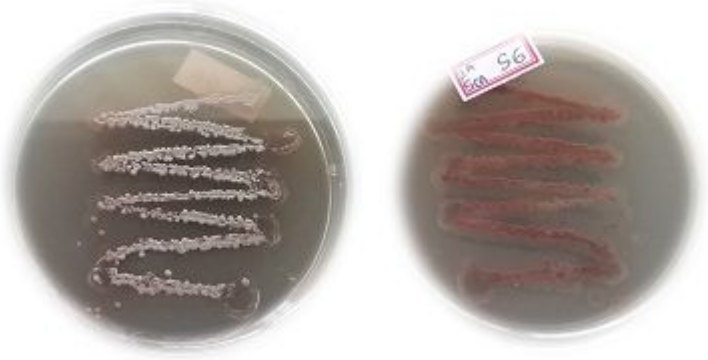


Figure 1: The culture had shown positive for amylase and protease and negative for cellulase, lipase, and pectinase hydrolyzing enzyme activity.

***Glutamicibacter mysorens* strain YKIKM.MU was grown on starch casein nitrate agar medium.**

The *Actinomycete* culture was subject to DNA isolation using the CTAB method yielded good quality of DNA and purified with a DNA purification kit. The obtained DNA is quantified and visualized using agarose gel electrophoresis as shown in **Figure 2.**

The purified DNA template is used for 16s rRNA gene amplification using Polymerase Chain Reaction by forward (27F) 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer (1492R) 5'-GGTTACCTTGTTACGACTT-3' and visualized in agarose gel electrophoresis as shown in **Figure 3.** Molecular weight was compared with a 1kb ladder.

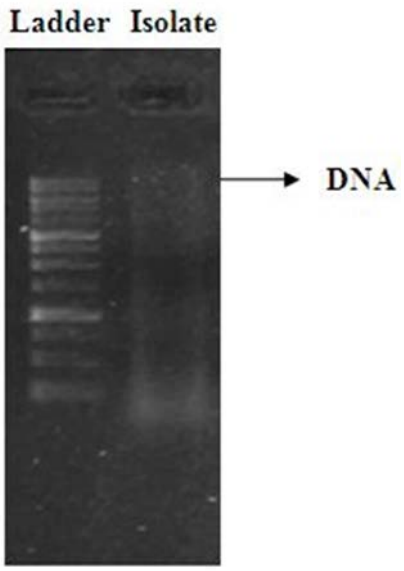


Figure 2: Agarose Gel Electrophoresis for isolated DNA from *Actinomycete* isolate using CTAB method compared with 1kb ladder.

The purified PCR amplified 16s rRNA gene was sequenced as shown in **Table 1**. The obtained sequence was shown highest similarity with *Glutamicibacter mysorens* strain YKIKM.MU with Genbank accession number MW647910.

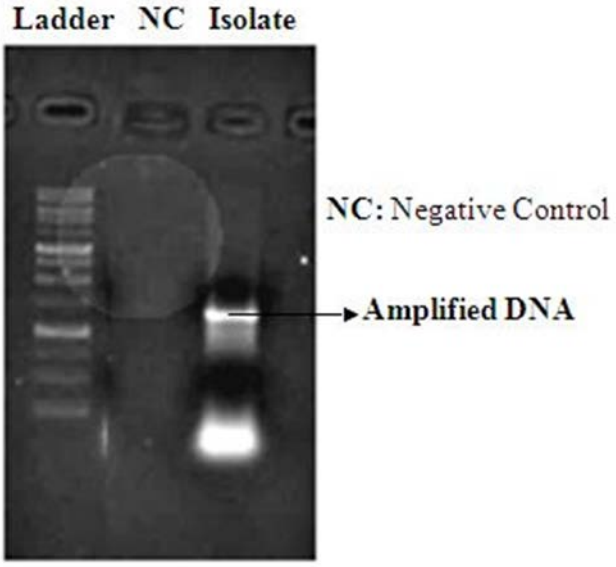


Figure 3: Agarose Gel Electrophoresis for PCR Amplified Genomic DNA isolated from *Actinomycete* isolate compared with 1kb ladder.

Table 1: Sequencing Results; Sequence data of Actinomyces isolate.

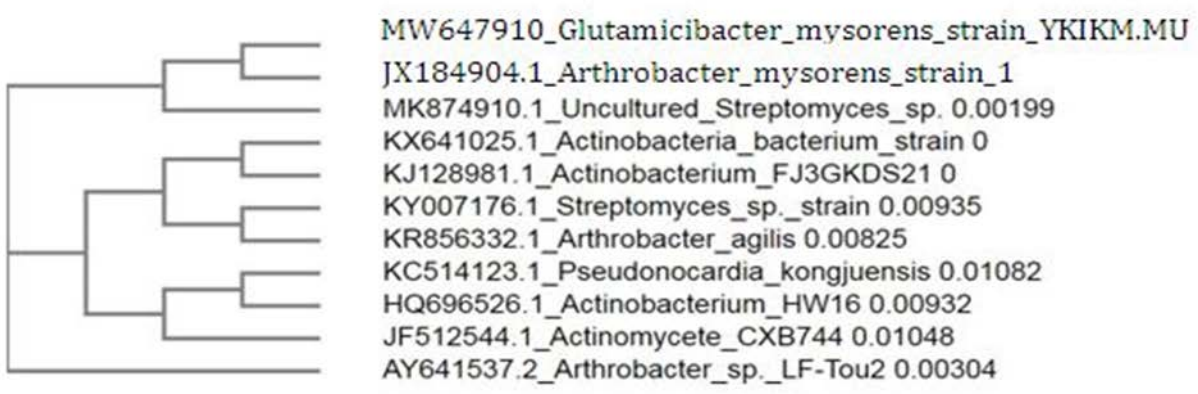
S6 Contig sequence:

>S6_query

TTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGG
GTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTGGAGGCAGCAGTG
GGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATG
ACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTA
CCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GCGCAAGCGTTATCCGGATTTATTGGGCGTAAATCGTAGGCGGTTTGTCGCGTCT
GCCGTGAAAGTCCGACTCAACCTCGGATCTGCGGTGGGTACGGGCAGACTAGAG
TGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG
GAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCATTTACTGACGCTGAGGAGC
GAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACG
TTGGGCACTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATT
AAGTGCCCGCCTGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCT
TACCAAGGCTTCATGTGCCAGACCGCTCCAGAGATGGGGTTTCCCTTCGGGGCTG
GTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG
TCCCGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTAGTGGTGGGGACT
CATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCAT
CATGCCTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGCAATGGGTTGCGAT
ACTGTGAGGTGGAGCTAATCCCTAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGC
AACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGCAGCAACGCTGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAGTTGGTAACA

The next highest resemblance is Arthrobacter mysorens with 98.28%, Glutamicibacter sp. 98.11%, Glutamicibacter mishrai 98.11%, Arthrobacter sp. strain 98.11%, Glutamicibacter arilaitensis 98.11%,

Glutamicibacter halophytocola 98.11% and few other species are under the genus of Arthrobacter as depicted in Table 2.



RNA gene, partial sequence

Table 2: 16s rRNA gene sequence similarity values for against all current recognized *Actinomycetes* species and phylogenetically related species.

***Actinomycetes* species**	**Query Cover**	**Percentage Identity**	**Strain Number**
***Arthrobacter mysorens* strain 1**	**100%**	**98.28%**	**JX184904.1**
Glutamicibacter sp. strain S_C_o-B10	100%	98.11%	MT533960.1
Glutamicibacter mishrai strain S5-52	100%	98.11%	CP032549.1
Arthrobacter sp. strain EB53	100%	98.11%	MH130339.1
Glutamicibacter arilaitensis strain EB36	100%	98.11%	MH130322.1
Glutamicibacter halophytocola strain ST67	100%	98.11%	MT186239.1
Glutamicibacter sp. strain Agri-11	100%	98.11%	MT102719.1
Glutamicibacter halophytocola strain WT14	100%	98.11%	MN733231.1
Glutamicibacter halophytocola strain DR408	100%	98.11%	CP042260.1
Glutamicibacter sp. strain SMB32	100%	98.11%	MH327514.1
Arthrobacter sp. strain IAE139	100%	98.11%	MK414833.1
Arthrobacter sp. strain L10	100%	98.11%	MG646026.1
Glutamicibacter sp. strain L6	100%	98.11%	MG646025.1
Glutamicibacter halophytocola strain KLBMP 5180	100%	98.11%	MK424284.1
Glutamicibacter arilaitensis strain ebst40	100%	98.11%	MG788347.1
Arthrobacter sp. djl-7H	100%	98.11%	KT230832.1
Arthrobacter sp. strain Art3	100%	98.11%	MG708154.1
Glutamicibacter nicotianae strain AKPEW109	100%	98.11%	KX698104.1
Glutamicibacter nicotianae strain LCX26	100%	98.11%	KY646077.1
Glutamicibacter nicotianae strain LCX24	100%	98.11%	KY646076.1

The PCR amplified DNA subjected for Sanger sequencing the obtained BLAST results showed *Glutamicibacter mysorens* strain YKIKM.MU and Phylogenetic tree was drawn using neighbour-joining method with the program NEIGHBOR as depicted in **Figure 4.** This is first report of organism isolated from mangrove habitat source of Mangalore.

In addition to the above characterizations, the culture was inoculated in 100ml of SCN broth 30ºC for 7d at 100rpm after incubation, the biomass was subjected for extraction of proteins using phosphate buffer was resulted 1mg/ml in Biuret assay. The protein concentration of 30µg was loaded onto 10% discontinuous anionic SDS-PAGE. As shown in **Figure 5.** Separated bands were compared with the protein molecular weights ranging from 11-53KDa.

The microbial protein was subjected to purification using size exclusion chromatography using Sephadex-G10. The eluted protein fraction number 72, 91, 126 was pooled. This pooled protein was tested for antibacterial activity against all the test organisms as assayed by well diffusion method. The partially purified protein from *Glutamicibacter mysorens* strain YKIKM.MU isolate showed optimum antibacterial activity against all test pathogens as shown in **Figure 6.** The protein had shown significant activity against *Bacillus cereus, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeroginosa* as shown in **Figure 7.**

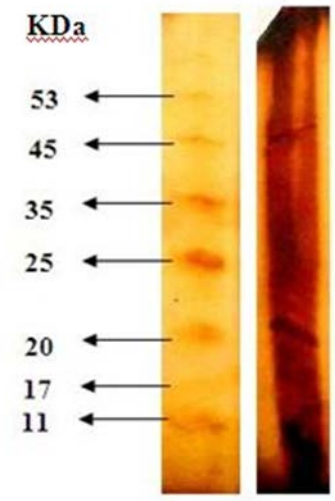


Figure 5: Electropherogram of anionic discontinues SDS-PAGE electrophoresis

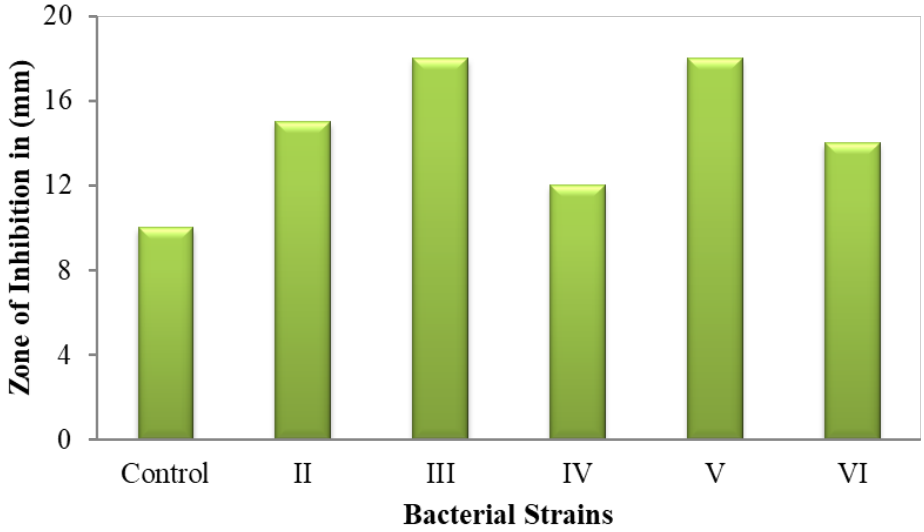


Figure 6: Antibacterial activity of protein extract. Control- Streptomycin, II-*Bacillus cereus*, III-*Proteus vulgaris*, IV-*Salmonella typhimurium*, V-*Staphylococcus aureus*, VI-*Pseudomonas aeroginosa*

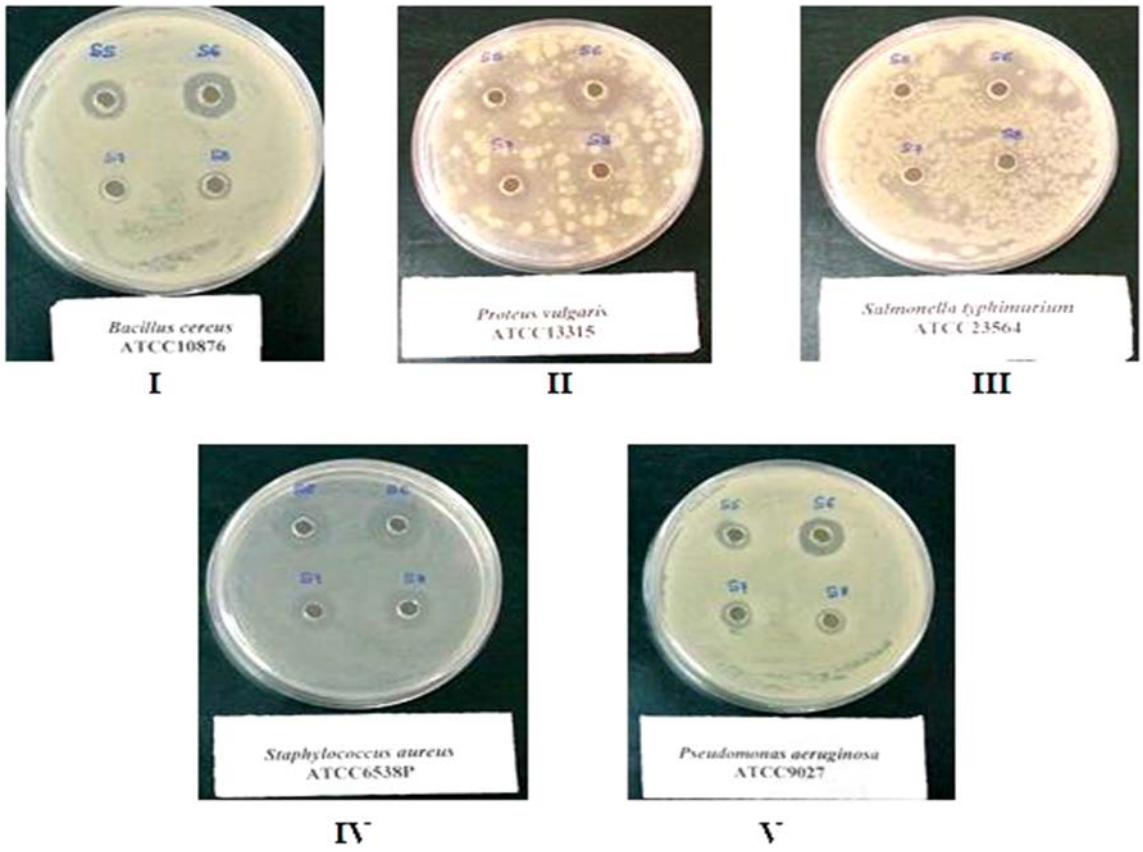


Figure 7: Antibacterial activity of protein extract by S6. I-*Bacillus cereus* (ATCC10876), II-*Proteus vulgaris* (ATCC13315), III-*Salmonella typhimurium* (ATCC23564), IV-*Staphylococcus aureus* (ATCC6538P), V-*Pseudomonas aeroginosa* (ATCC9027) cultures.

Thus protein sample is effective against Gram-positive pathogenic bacteria *Bacillus cereus* producing enterotoxins are responsible for causing diarrheal, gastrointestinal pain, and emetic syndrome[36]. The protein had shown efficiency against Gram-negative pathogenic bacteria *Proteus vulgaris* an opportunistic pathogen of humans. It is known to cause enteritis, colitis in infants and regulates an increase in mortality rate[37]. The protein is effective against Gram-negative bacteria *Salmonella typhimurium* is a predominantly infectious agent for causing typhoid fever in humans and salmonellosis[38,39].

The S6 peptides were also effective against Gram-positive pathogenic bacteria *Staphylococcus aureus* infectious agent for causing endocarditis, metastatic infection, or sepsis syndrome. It contributes for activation to the progression of endovascular disease[40]. This protein is effective against Gram-negative pathogenic bacteria *Pseudomonas aeroginosa* is causative agent for chronic obstructive pulmonary disease (COPD) and cystic fibrosis[41].

The zone of inhibition was recorded concerning to standard antibiotic streptomycin (10µg) that showed 10mm inhibition activity against test pathogens. This result indicates that protein obtained from the cell biomass contained anti-microbial peptides (AMPs) that could be novel concerning to the habitat and mechanism of antimicrobial action.

In comparison with other actinomycete isolate S5, S7 and S8. The S6 had shown efficient antibacterial activity. These antibacterial activities of partially purified proteins from S6 prompted further studies on protein characterization using liquid chromatography-mass spectrophotometer the elution profile is shown maximum

elution at 3.11min followed by 3.81min, 4.83min, and 8.69min depicted in Figure 8. The maximum protein elution time 3.11min showed a molecular weight of 265D, 266D, and 267D depicted in Figure 9.

The elution time of protein at 3.82min LC-MS had shown molecular weight of 133D, 211D, 264D, 265D, 293D, and 294D as depicted in **Figure 10.** Elution time at 4.84min had shown molecular weight of 147D, 199D, 211D, 281D, 282D, and 283D as depicted in **Figure 11**

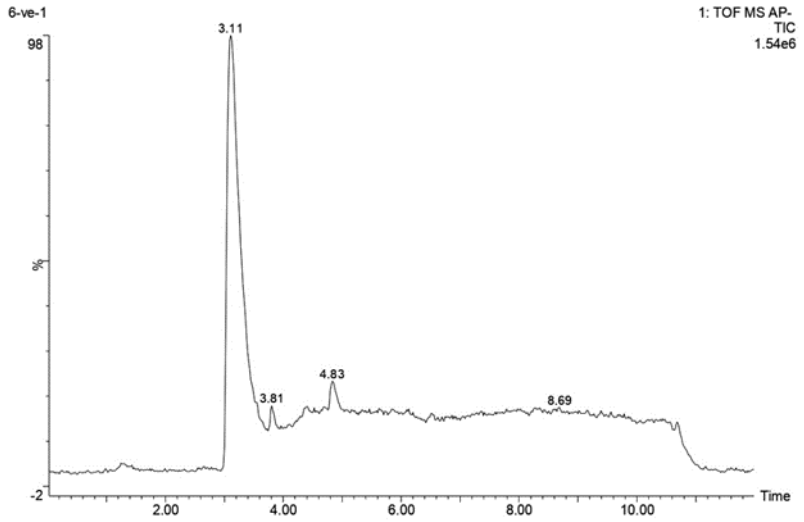


Figure 8: Elution profile of Liquid Chromatography-Mass Spectrophotometer.

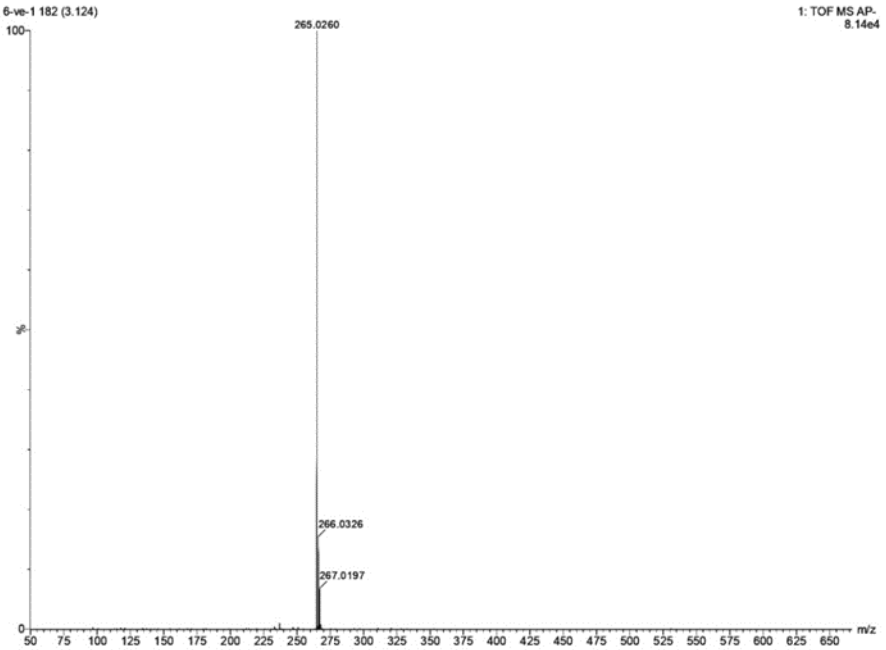


Figure 9. Maximum elution in Liquid Chromatography-Mass Spectrophotometer at 3.11min.

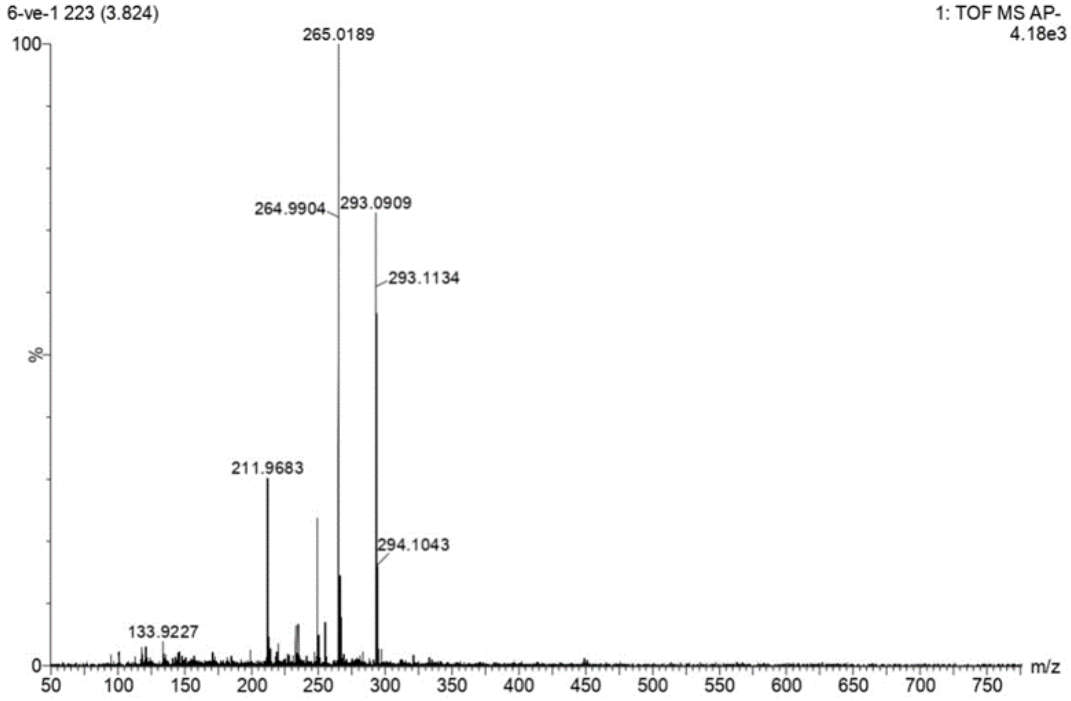


Figure 10. Elution profile in Liquid Chromatography-Mass Spectrophotometer at 3.8min.

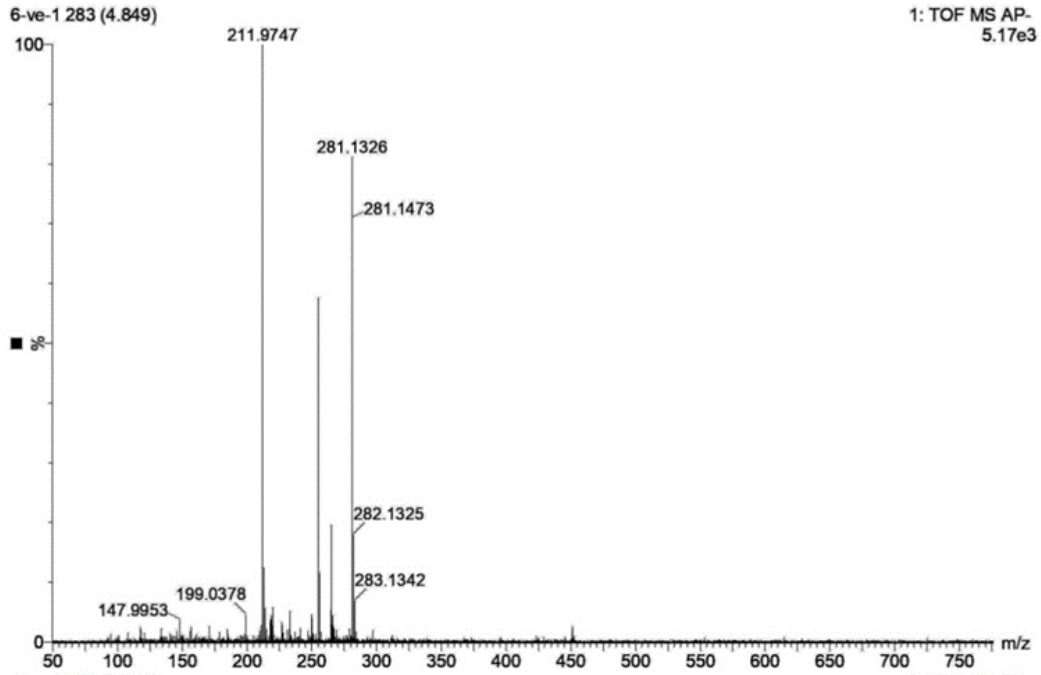


Figure 11. Elution profile in Liquid Chromatography-Mass Spectrophotometer at 4.8min.

Conclusion

The *Glutamicibacter mysorens* strain YKIKM.MU is an *actinomycete* culture isolated from unexplored Mangroves source of Mangalore coast. This culture producing proteins had shown effective antibacterial activity against *Bacillus cereus, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeroginosa* cultures. This offers a best prominent source isolating biologically important molecules for treating various ailments as evident by present study.

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References

1. Karthik, Y., Kalyani, M. I., S, S. K., D, R. & B, B. K. Cytotoxic and antimicrobial activities of microbial proteins from mangrove soil actinomycetes of Mangalore, Dakshina Kannada. Biomedicine 40, 59–67 (2020).
2. Kalyani, M. I. & Rajina, B. R. Peptide Analysis from Soil Actinomycetes Exhibiting Antimicrobial and Antiproliferative Activities. (2010).
3. Ashwini, B. & Manjula, I. K. Protein fraction from actinomycetes isolates inhibits protease activity secreted by staphylococcus aureus. European journal of pharmaceutical and medical sciences 2, 186–193 (2015).
4. Busse, H.-J. Review of the taxonomy of the genus Arthrobacter, emendation of the genus Arthrobacter sensu lato, proposal to reclassify selected species of the genus Arthrobacter in the novel genera Glutamicibacter gen. nov., Paeniglutamicibacter gen. nov., Pseudoglutamicibacter gen. nov., Paenarthrobacter gen. nov. and Pseudarthrobacter gen. nov., and emended description of Arthrobacter roseus. Int J Syst Evol Microbiol 66, 9–37 (2016).
5. Busse, H.-J. & Wieser, M. The Genus Arthrobacter. in The Prokaryotes: Actinobacteria (eds. Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E. & Thompson, F.) 105–132 (Springer, 2014).
6. Jones, D. & Keddie, R. M. The Genus Arthrobacter. in The Prokaryotes: Volume 3: Archaea. Bacteria: Firmicutes, Actinomycetes (eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. & Stackebrandt, E.) 945–960 (Springer, 2006).
7. Busse, H.-J., Wieser, M. & Buczolits, S. Arthrobacter. in Bergey's Manual of Systematics of Archaea and Bacteria 1–70 (American Cancer Society, 2015).
8. Groth, I. et al. Demetria terragena gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. Int J Syst Bacteriol 47, 1129–1133 (1997).
9. Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. Proposal for a new hierarchic classification system, Actinobacteria classis nov. International Journal of Systematic and Evolutionary Microbiology 47, 479–491 (1997).
10. Koch, C., Schumann, P. & Stackebrandt, E. Reclassification of Micrococcus agilis (Ali-Cohen 1889) to the Genus Arthrobacter as Arthrobacter agilis comb. nov. and Emendation of the Genus Arthrobacter. International Journal of Systematic and Evolutionary Microbiology, 45, 837–839 (1995).

11. Attenbrow, V. What's Changing: Population Size Or Land-use Patterns?: the Archaeology of Upper Mangrove Creek, Sydney Basin. vol. 21 (ANU E Press, 2007).
12. Hoyles, L. et al. Actinomyces marimammalium sp. nov., from marine mammals. International journal of systematic and evolutionary microbiology 51, 151–156 (2001).
13. Steinert, G., Taylor, M. W. & Schupp, P. J. Diversity of actinobacteria associated with the marine ascidian Eudistoma toealensis. Marine Biotechnology 17, 377–385 (2015).
14. Sridevi, V., Priyadarshini, P. S. & Gautam, Y. A. Selection of marine actinomycetes with bioactive potential isolated from sediments of Bay of Bengal and characterization of promising isolate, ABT-103. Microbiology Research Journal International 1–9 (2015).
15. Attenbrow, V. Introduction. in What's Changing: Population Size or Land-Use Patterns? vol. 21 1–10 (ANU Press, 2006).
16. Kumar, R. Conservation and management of mangroves in India, with special reference to the State of Goa and the Middle Andaman Islands. 51, 7 (2000).
17. Brandenburg, L.-O., Merres, J., Albrecht, L.-J., Varoga, D. & Pufe, T. Antimicrobial Peptides: Multifunctional Drugs for Different Applications. Polymers 4, 539–560 (2012).
18. Arouri, A., Dathe, M. & Blume, A. Peptide induced demixing in PG/PE lipid mixtures: a mechanism for the specificity of antimicrobial peptides towards bacterial membranes? Biochim Biophys Acta 1788, 650–659 (2009).
19. Seo, M.-D., Won, H.-S., Kim, J.-H., Mishig-Ochir, T. & Lee, B.-J. Antimicrobial peptides for therapeutic applications: a review. Molecules 17, 12276–12286 (2012).
20. Fernebro, J. Fighting bacterial infections-future treatment options. Drug Resist Updat 14, 125–139 (2011).
21. Pieters, R. J., Arnusch, C. J. & Breukink, E. Membrane permeabilization by multivalent anti-microbial peptides. Protein Pept Lett 16, 736–742 (2009).
22. Nicolas, P. Multifunctional host defense peptides: intracellular-targeting antimicrobial peptides. The FEBS journal 276, 6483–6496 (2009).
23. Brogden, K. A. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nature Reviews Microbiology 3, 238–250 (2005).
24. Bechinger, B. & Lohner, K. Detergent-like actions of linear amphipathic cationic antimicrobial peptides. Biochimica et Biophysica Acta (BBA) - Biomembranes 1758, 1529–1539 (2006).
25. Hancock, R. E. W. & Sahl, H.-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 24, 1551–1557 (2006).
26. Hoskin, D. W. & Ramamoorthy, A. Studies on anticancer activities of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes 1778, 357–375 (2008).
27. A. et al. Isolation, Characterization, and Screening of Antimicrobial-Producing Actinomycetes from Soil Samples. International Journal of Microbiology 2020, (2020).
28. Lakshmipathy, D. T. & Kannabiran, K. A morphological, biochemical and biological studies

of halophilic Streptomyces sp. isolated from saltpan environment. American Journal of Infectious Diseases 5, 200–6 (2009).
29. Das, S., Dash, H. R., Mangwani, N., Chakraborty, J. & Kumari, S. Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. Journal of microbiological methods 103, 80–100 (2014).
30. Mayfield, C. I., Williams, S. T., Ruddick, S. M. & Hatfield, H. L. Studies on the ecology of actinomycetes in soil IV. Observations on the form and growth of streptomycetes in soil. Soil Biology and Biochemistry 4, 79–91 (1972).
31. Bhaduri, S. & Demchick, P. H. Simple and rapid method for disruption of bacteria for protein studies. Appl Environ Microbiol 46, 941–943 (1983).
32. Pitcher, D. G., Saunders, N. A. & Owen, R. J. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 8, 151–156 (1989).
33. Cook, A. E. & Meyers, P. R. Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S rRNA gene restriction fragment patterns. International Journal of Systematic and Evolutionary Microbiology 53, 1907–1915 (2003).
34. Kalyani, M. I., Lingaraju, S. M. & Salimath, B. P. A pro-apoptotic 15-kDa protein from Bacopa monnieri activates caspase-3 and downregulates Bcl-2 gene expression in mouse mammary carcinoma cells. J Nat Med 67, 123–136 (2013).
35. Monciardini, P., Sosio, M., Cavaletti, L., Chiocchini, C. & Donadio, S. New PCR primers for the selective amplification of 16S rDNA from different groups of actinomycetes. FEMS Microbiology Ecology 42, 419–429 (2002).
36. Kotiranta, A., Lounatmaa, K. & Haapasalo, M. Epidemiology and pathogenesis of Bacillus cereus infections. Microbes and Infection 2, 189–198 (2000).
37. Neter, E. R. & Farrar, R. H. Proteus vulgaris and proteus morganii in diarrheal disease of infants. Am. J. Dig. Dis. 10, 344–347 (1943).
38. Moffatt, C. R. M. et al. Salmonella typhimurium and Outbreaks of Egg-Associated Disease in Australia, 2001 to 2011. Foodborne Pathogens and Disease 13, 379–385 (2016).
39. Gilman, R. H. & Hornick, R. B. Duodenal isolation of Salmonella typhi by string capsule in acute typhoid fever. Journal of Clinical Microbiology 3, 456–457 (1976).
40. Lowy, F. D. Staphylococcus aureus Infections. N Engl J Med 339, 520–532 (1998).
41. Murphy, T. F. et al. Pseudomonas aeruginosa in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 177, 853–860 (2008).