International Journal of Recent Technology and Engineering (IJRTE)
ISSN: 2277-3878 (Online), Volume-9 Issue-3 September 2020

Evaluation of a Potential Antibacterial, Produced by Streptomyces Cinereoruber Sp. Isolated from Chlika lake.

Anjani K. Upadhyay, Debasmita Chatterjee, Madhuri Swain, Lopamudra Ray

Abstract: Streptomyces, isolated from marine and estuarine habitat have been widely recognized as a potential source of antifungal, anti-tumour, anti-bacterial compounds. In the present study, the antimicrobial agent production potential of a Streptomyces cinereoruber sp was evaluated. The selective isolation of the strain was carried out on starch casein agar. The primary screening of the Streptomyces isolate was done by cross streak method against pathogenic test strains Escherichia.coli MTCC 82, Staphylococcus aureus MTCC 96, Bacillus cereus IP406 and Salmonella typhi MTCC 734 and Micrococcus leuteus and the antimicrobial property against Micrococcus leuteus was confirmed. The secondary screening was carried out by using the culture supernatant against the test strain by agar well diffusion method. The growth and antimicrobial production ability of the strain against Micrococcus leuteus was studied. The antimicrobial agent production was also observed till pH 11 and NaCl concentration 3% (w/v). The partially purified compound showed a peak similar to streptomycin in HPLC. The culture condition for the production of the compound was optimised.

Keywords: Streptomyces, Antibacterial, Optimization

I. INTRODUCTION

Streptomyces are well-known as the largest antibiotic-producing genus in the microbial world discovered so far (Taddi *et al.*, 2006; Jayapal *et al.*, 2007). Most *Streptomyces* and other Actinomycetes produce a diverse array of anti-biotics including aminoglycosides, anthracyclines, glycol-peptides, β-lactams, macrolides, nucleosides, peptides.

Streptomyces belong to a gorup of gram-positive bacteria that grow in marine environments, with a filamentous form similar to fungi. The morphological differentiation of *Streptomyces* involves the development of a layer of hyphae that can differentiate into a chain of spores. This process is exclusive among the Streptomyces, requiring a specialized and synchronised metabolism (de lima Procopio *et al.*, 2012).

The most attention-grabbing property of Streptomyces is the ability to produce bioactive secondary metabolites with antitumoral, antivirals, antifungals, anti-hypertensives, and mainly antibiotics and immunosuppressive activities. Estuarine habitats are among the most bio diverse and productive natural habitats in the world (Costanza *et al.*, 1993).

Chilika Lake (19^{o} 28' and 19 ^{o} 54' N and 85^{o} 05' and 85^{o} 38' E), a brackish water lagoon is situated on the east coast of India. Estuarine environments are transition zones between freshwater and marine or ocean environments.

Due to marine tidal influences like waves, influx of saline water and river influences like freshwater input and sediment, estuarine environment is subjected to mix of nutrients from both freshwater and marine water. This results in high productivity in ecotone regions and is responsible for increased biodiversity. Estuarine habitats are good sources for bio-prospecting of micro-organisms with halophilic and alkaline microbes with industrial applications. Microbes which belong to genus *Streptomyces*, isolated from marine and estuarine habitat have been widely recognized as a potential source of antifungal, anti-tumour, anti-bacterial compounds. Despite having tremendous biodiversity, the microbial diversity of Chilika Lake has been inadequately explored. Limited reports of phototrophic bacteria like *Shewanella chilikensis* (Sucharita *et al.*, 2009) and *Allochromatium phaeobacter Im* (Srinivas *et al.*, 2009) have been isolated and taxonomically identified from this habitat. The habitat is presumed to be a repository of industrially important microorganisms with biotechnological potential. Very few reports on isolation of *Streptomyces* strains from the lake are known. Thus, Chilika Lake could be a favourable environment for bio-prospecting of novel microbial isolates producing antimicrobial compound.

II. MATERIAL AND METHOD:

2.1. Sampling

Sediment samples were collected from the 21 different sampling stations of Chilika lake aseptically in zip lock packets and stored at 4^{o}C until further analysis. Temperature and pH of the water and soil samples were measured at the time of collection.

2.2. Enrichment and isolation of Streptomyces

The enrichment of the sample was carried out on starch casein media at 30^{o}C at 120 rpm.

Revised Manuscript Received on August 10, 2020.

Anjani K. Upadhyay, School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India.
Debasmita Chatterjee, School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India.
Madhuri Swain, School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India.
Lopamudra Ray, (PhD) Assistant Professor, School of Law, Adjunct faculty, Biotech, Campus Kalinga Institute of Industrial Technology (KIIT) Bhubaneswar, Odisha, India.

Five grams of sediment were added to 250 ml conical flasks containing 50 ml of sterile starch caesin medium. The flasks were then incubated in a shaker incubator (Innova New Brunswick, Germany) at 30^0C for 7 days. 1ml of the enrichment culture was then transferred to fresh broth and the process was repeated 4 times for 1.5 months. Isolates were obtained by plating 100µl of serially diluted enrichment culture, after 7 days on starch casein agar medium supplemented with nystatin.

2.3. Primary Screening of isolates for antimicrobial activities

The typical *Streptomyces* colony obtained on Starch casein media at the end of incubation period were then re-streaked to obtain pure culture. The pure colonies were maintained on Starch casein agar plate and slants. For long term storage the strains were grown in starch casein broth. The spore suspension of the grown culture along with a cryopreserver glycerol (50% spore suspension+50% sterile glycerol*) were then stored at -80°C. Antimicrobial activity of isolates were analysed on seawater (50 % v/v) LB for 7 days against indicator strains *Escherichia.coli* MTCC 82, *Staphylococcus aureus* MTCC 96, *Bacillus cereus* IP406 and *Salmonella typhi* MTCC 734 and *Micrococcus leuteus* procured from Microbial Type Culture Collection (MTCC, Chandigarh) by cross streak method.

2.4. Secondary screening of isolates for antimicrobial activities:

The secondary screening of the isolate was carried out by determining the antimicrobial activity of the culture supernatant of the *Streptomyces* strain against test bacterial strain *Micrococcus leuteus* by agar well diffusion assay (Holder and Boyce, 1994). In brief, the indicator strains were streaked on LB agar plate. 50 microlitres of the *Streptomyces* culture supernatant was then incorporated into the wells prepared in the same agar plate. The plates were incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition formed around the well.

2.5. Morphological characterization

Morphological and culture characteristics of spore chain morphology, spore surface, and spore chain ornamentation were observed by light and phase contrast (Leica DM300, Leica microsystem, Germany) and after incubation on ISP 3 agar medium for 3-4 weeks at 30^oC. Culture traits of strain MM1AG7 were recorded after 21 days incubation at 30^0C on several ISP (International Streptomyces Project; (Shirling and Gottlieb, 1996) media: Glucose yeast extract malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts starch agar (ISP 4), glycerol asparagine agar (ISP 5), peptone yeast extract iron agar (ISP 6) and tyrosine agar (ISP 7). The growth was also recorded on modified ISP fortified with sea water (50%, v/v). Colour evaluations for diffusible pigments and substrate mycelia were also observed on ISP media by comparing the cultures with colour chips from ISCC-NBS COLOR CHARTS standards no.2 2106 (Kelly, 1964). Biochemical and physiological tests were conducted at 30^oC according to the reported protocol **(**Shirling and Gottlieb, 1996**;** Gordan *et al.,* 1974*;* Williams *et al.,* 1983; Goodfellow *et al.,* 2007; Kampfer and Labeda, 2006). Production of hydrolysing enzymes for hydrolysis of starch, cellulose, gelatin liquefaction, casein, Tween 80, lecithin, tributyrin, chitin and utilization of urea and citrate were performed as described by (Goodfellow, 1989; Taddi *et al.,* 2006; Tindall *et al.,* 2007). Utilization of D-glucose, D-fructose, maltose, D-xylose, D-cellobiose, L-arabinose, L-rhamnose, D-raffinose, sucrose, D-galactose, D-mannose and inositol as sole carbon source (1% w/v) were tested on basal salt media fortified with sea water (50% v/v). Methods for estimation of melanin production and nitrate reduction were performed on modified ISP media as described by Shirling and Gottleib (Williams *et al.,* 1989; *Gordan et al., 1974).* Catalase activity was determined by oxygen bubble formation on addition of 4% H_2O_2 to well grown colonies and oxidase activity was determined by change in colour of the oxidase disk (Hi Media, Pvt. Ltd. Mumbai, India). The growth and pH tolerance of MM1AG7 was studied over a temperature range of 20-50ºC and pH 6-10 on LB and salt tolerance of the strain was observed on various NaCl concentrations (0-18 %, w/v) in LB media. Antibiotic susceptibility was investigated using antibiotic discs (Hi Media Pvt. Ltd. Mumbai) as described by Williams *et al*. (1983) on LB media. Antimicrobial activity of strain MM1AG7 was also examined on LB for 7 days against indicator strains Staphylococcus *aureus* MTCC 96, *Salmonella typhi* MTCC 734, procured from Microbial Type Culture Collection (MTCC, Chandigarh), *Micrococcus luteus* LBG B4291 and Bacillus *cereus* IP406 (a kind gift from Dr. Peter Luethy, Institute of Microbiology, ETH, Zurich), by observing formation of zone of inhibition.

2.6. Optimization of culture conditions for antimicrobial compound production by Streptomyces cinereoruber sp. MM1AG7

For large scale production of antimicrobial compound by the Streptomyces strain, the experiments were conducted to determine the optimum culture condition for the same including the effect of incubation period, culture media, temperature, pH, carbon source, nitrogen source and NaCl concentration. Cell growth was observed by calculation of cfu/ml and antimicrobial activity was tested by the well diffusion assay (Holder and Boyce, 1994) .

2.6.1. Effect of various carbon source on growth and antibacterial agent production by Streptomyces cinereoruber sp. MM1AG7

Preculture of strain ***Streptomyces cinereoruber* sp. MM1AG7** was grown in Starch casein media. 1% (w/v) of preculture was inoculated to 50 ml of mineral salt media (MSM) (Ref) supplemented with appropriate carbon source 1% (w/v) of (glycerol, starch, sucrose, glucose, and fructose) individually. Aliquots of 1ml were taken at every 24h interval for 5 days for measurement of growth and antimicrobial activity according to the methods described earlier.

All the experiments were carried out in triplicates**.** Cell growth was evaluated by plating 100 microlitres of the aliquot on starch casein media and incubated. The developed colonies were counted to determine cfu/ml and antimicrobial activity was tested by the classical well diffusion assay (Holder and Boyce, 1994) .

2.6.2. Effect of different Nitrogen source on growth and antibacterial agent production by Streptomyces cinereoruber sp. MM1AG7

Preculture of strain ***Streptomyces cinereoruber*** **sp. MM1AG7** was grown in Starch casein media. 1% (w/v) of preculture was inoculated to 50 ml of MSM supplemented with 1% (w/v) of different nitrogen sources (ammonium sulphate, potassium nitrate, peptone, yeast extract) separately.

Aliquots of 1ml were taken at every 24h interval for 5 days for measurement of antimicrobial activity and methods described earlier. All the experiments were carried out in triplicates and comparative study was done. Cell growth was observed by calculation of cfu/ml and antimicrobial activity was tested by the classical well diffusion assay (Holder and Boyce, 1994) .

2.6.3. Effect of different pH on growth and antibacterial agent production by Streptomyces cinereoruber sp. MM1AG7

The experiment was carried out by analysing activity and growth of the strain MM1AG7 in media with a pH range of 4-11. The preculture of isolates MM1AG7 was grown in Starch casein media. 1% (v/v) of the grown culture (containing approx.1.2-1.8 $x10^6$ cells/ml) was inoculated to 50 ml of MSM supplemented with appropriate carbon source having different pH (5, 7, 8, 9, and 11) separately in flasks. Aliquots of 1ml were taken at every 24h interval for 5 days for measurement of antimicrobial activity and methods described earlier. All the experiments were carried out in triplicates**.** Cell growth was observed by calculation of cfu/ml and antimicrobial activity was tested by the classical well diffusion assay (Holder and Boyce, 1994) .

2.6.4. Effect of different NaCl concentration on growth and antibacterial agent production by Streptomyces cinereoruber sp. MM1AG7

Preculture of strain ***Streptomyces cinereoruber*** **sp. MM1AG7** was grown in Starch casein media. 1% (w/v) of precultures were inoculated to 50 ml of MSM supplemented with appropriate carbon source with different NaCl conc. (0.5%, 1%, 2%, 4% 65 and 8% w/v) separately in flasks. Aliquots of 1ml were taken at every 24h interval for 5 days for measurement of antimicrobial activity and methods described earlier. All the experiments were carried out in triplicates and comparative study was done. Cell growth was observed by calculation of cfu/ml and antimicrobial activity was tested by the classical well diffusion assay (Betina, 1983).

2.6.5. Determination of the Minimum Inhibitory Concentration (MIC) of the antimicrobial compound against Micrococcus leuteus

Minimum inhibitory Concentration is the lowest concentration of antibiotic that completely inhibits bacterial growth (Andrews, 2001) . Quantitative assay was carried out to determine the MIC of the antibacterial agent present in the culture supernatant of isolated *Streptomyces* strain. The experiment was carried out by adding and serially diluting the antibacterial extract of the *Streptomyces* strains to tubes containing 5ml of Luria Bertanii medium followed by addition of 5 µl of overgrown test strains such as *Micrococcus luteus*. The tubes were incubated at 37^0C for 24h. The absorbance was then measured at 600nm. The activity of the purified extract of MM1AG7 culture was considered as 100 %. Different concentration of the extract was prepared by adding different volumes i.e. 500µl, 400µl, 300µl, 200µl, 100µl, 50µl, 40µl, 30µl, 20µl and 10µl of the same to tubes containing 5ml of LB, followed by addition to 5µl of *M. leuteus* culture in each. Culture tubes were incubated at 37^OC for 24hrs and then OD was measured for each.

2.7. Purification and characterisation of the antimicrobial compound

2.7.1. Purification of the antimicrobial compound by column chromatography:

MM1AG7 strain was inoculated in 500ml starch casein media and incubated at 37^OC for 5 days. Fully grown culture was centrifuged at 6500 rpm for 10 minutes. The culture supernatant obtained was extracted twice with ethyl acetate, concentrated in rota-vapour and again dissolved in 5ml of ethyl acetate. 5ml of this sample is loaded onto a silica gel column and eluted successively with 50ml of 100% hexane, 50ml of linear gradient hexane: ethyl acetate (v/v, 75:25, 50:50, 25:75), 50ml of 100 % ethyl acetate, 50ml of 100 % methanol; resulting in 31 fractions of 10ml each. All the fractions were tested for antimicrobial activity by well diffusion assay against *M.leuteus*.

2.7.2. Qualitative Determination of the antimicrobial agent by Thin Layer Chromatography (TLC)

For TLC analysis was carried according to the method of (Kagan and Flythe, 2014) 300µl of reaction mixture was spotted on TLC plate (Silica gel 60, F_{254} (20 x20cm); E. Merck, Darmstadt, Germany) using micro-capillary (Drummond, Scientific company, USA), along with respective chito-oligosaccharide standards. The plates were air dried. Mobile phase (butanol: acetic acid: water (v/v 4:4:1)) was allowed to run along the TLC plate till solvent front reached more than $3/4^{th}$ of its length after which the plate was marked and dried. The plates were then developed by exposing the plate under UV (354nm).

2.7.3. Confirmation of the purified antimicrobial compound by HPLC

A modified method was developed from the studies so far was used to determine the presence of secondary metabolite in terms of antibiotic. In brief, sample MM1AG7 was extracted twice using ethyl acetate in an equal volume ratio (1:1, v/v).

after concentrating the sample by using rotary evaporator (Eyela CCA-1141, Tokyo) residues dissolved in methanol (HPLC grade) were determined by HPLC (Agilent Technologies 1260 Infinity) equipped with an Ascentis@ C18 reversed phase column (4.6 nm × 5 nm) with detection at 230nm with a manual injector. A mixture of methanol and water (85:15) was used as the mobile phase at a flow rate of 1.2ml/min. the injection volume was 20µl.

2.8. Characterisation of the purified antimicrobial compound produced by Streptomyces cinereoruber sp. MM1AG7

The solvent extracted and partially purified antimicrobial compound produced by *Streptomyces cinereoruber* sp. MM1AG7 was analysed for various parameters as following;

2.8.1. Effect of temperature on activity of the antimicrobial agent:

Effect of temperature on stability of the antibacterial agent was determined. 1ml of supernatant was harvested from broth culture and treated at 4^{O}C, 25^{O}C, 37^{O}C, 56^{O}C, 70^{O}C, 90^{O}C for 1hr. The residual antibacterial activity of heat-treated samples was determined by measurement of zone of inhibition against *M.leuteus.*

2.8.2. Effect of pH on activity of the antimicrobial agent:

For determining the effect of pH on stability of the antibiotic, 1ml of supernatant was harvested from broth culture and treated with pH 5, 6, 7, 8, 10 for 1 hour. The residual antibacterial activity of pH-treated samples was determined by measurement of zone of inhibition against *M.leuteus.*

2.8.3. Effect of mutation on activity of the antimicrobial agent

The Streptomyces strain was inoculated in 100 ml (4 Nos) conical flasks containing 50 ml SC broth in each. Ethidium-bromide was added in 3 of them in different concentrations (5µg/ml, 10µg/ml, and 15µg/ml) and one was without ethidium-bromide was considered as control. All were incubated till 120 hours. cfu/ml count and antimicrobial activity was observed in every 24 hours to check the effect of the mutagen (EtBr).

III. RESULTS AND DISCUSSION

A total of 161 Actinomycetes strains were obtained from 21 sampling stations of Chilika lake. The strains were checked for their antimicrobial production ability by cross streak method against the test strains. Antimicrobial activity of isolates were analysed on seawater (50 % v/v) LB for 7 days against indicator strains *Escherichia.coli* MTCC 82, *Staphylococcus aureus* MTCC 96, *Bacillus cereus* IP406 and *Salmonella typhi* MTCC 734 and *Micrococcus leuteus* procured from Microbial Type Culture Collection (MTCC, Chandigarh) . A total of 67 *Streptomyces* strains were then selected for secondary screening. The Actinobacterial strains were grown in Starch casein broth for 5-7 days. The culture supernatant was then checked for their potential by agar well diffusion method against the test strains (Fig 1). Eventually the strain MM1AG7 was selected for further studies out of 15 selected Streptomyces strains.

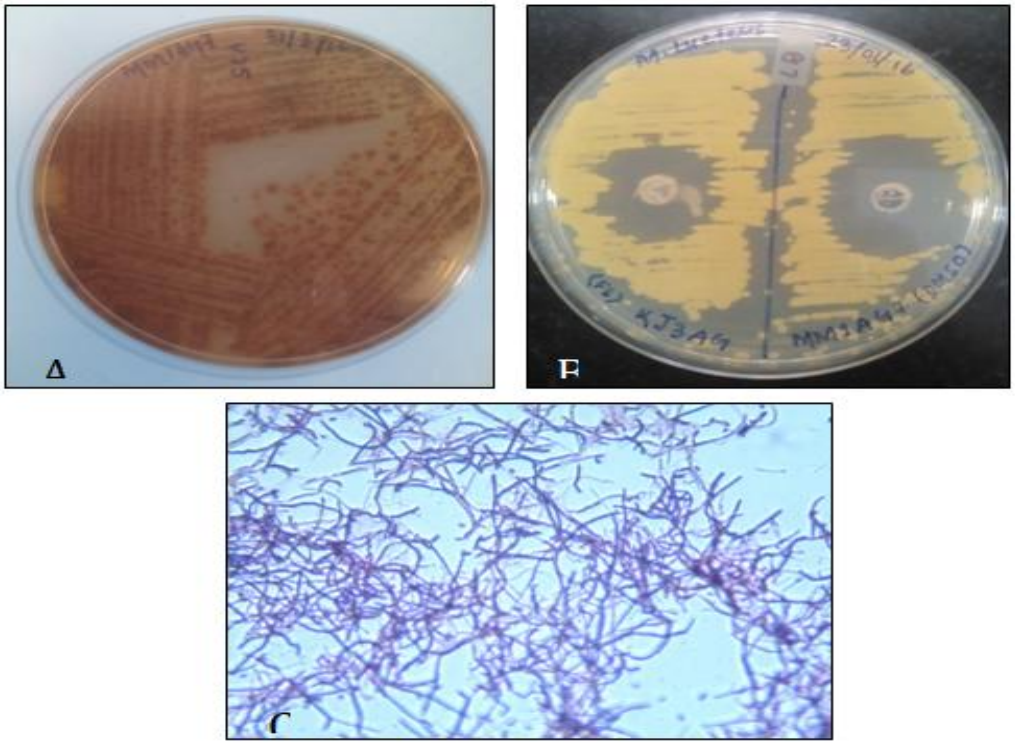

Fig 1 (A): Colony morphology of the isolate MM1AG7 on starch casein plates. (B): Agar well diffusion assay for the antimicrobial agent produced by MM1AG7. (C): 100X oil immersion microscopic view of MM1AG7

The 16srRNA sequencing and phylogenetic analysis showed that the strain MM1AG7 may belong to a novel species of Streptomyces i. e *Streptomyces cinereoruber subsp. cinereoruber* NBRC 12756^{T} (Fig 2).

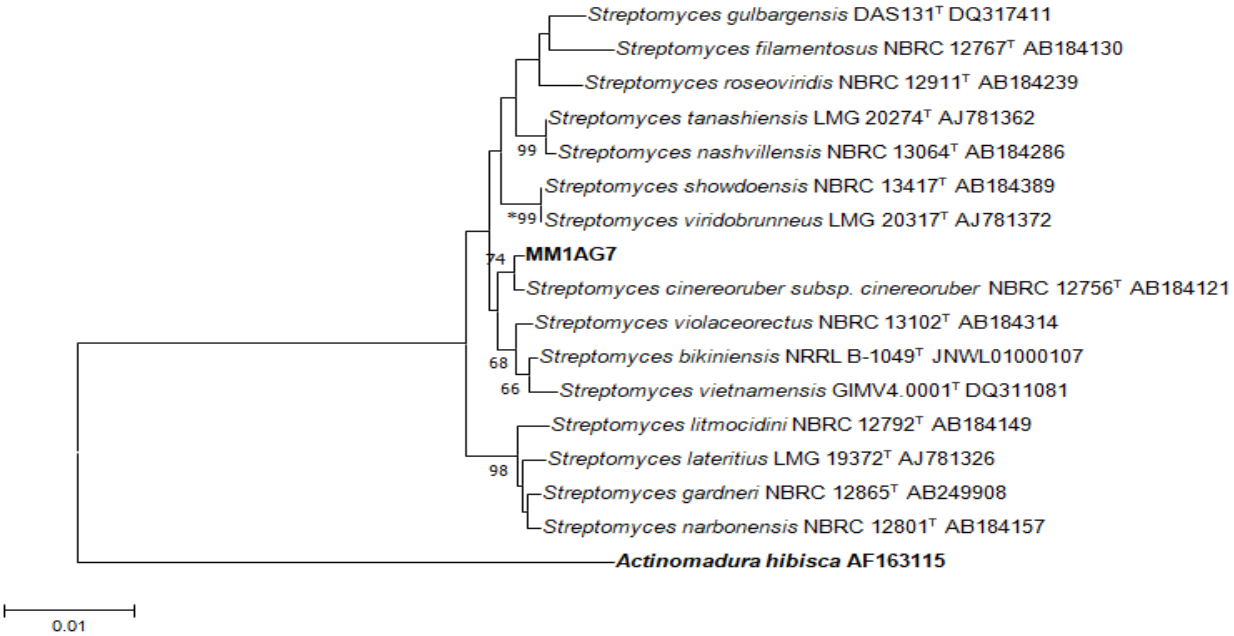

Fig 2. Evolutionary relationships of taxa for MM1AG7 inferred by Neighbor Joining method.

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.14154585 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions per site. The analysis involved 22 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1321 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [4]. The novelty of the strain can be confirmed by polyphasic taxonomy (Result not shown for all). The specific growth rate, generation time and the respective antimicrobial potential of the isolate was determined by growing it in starch casein broth (Fig3).

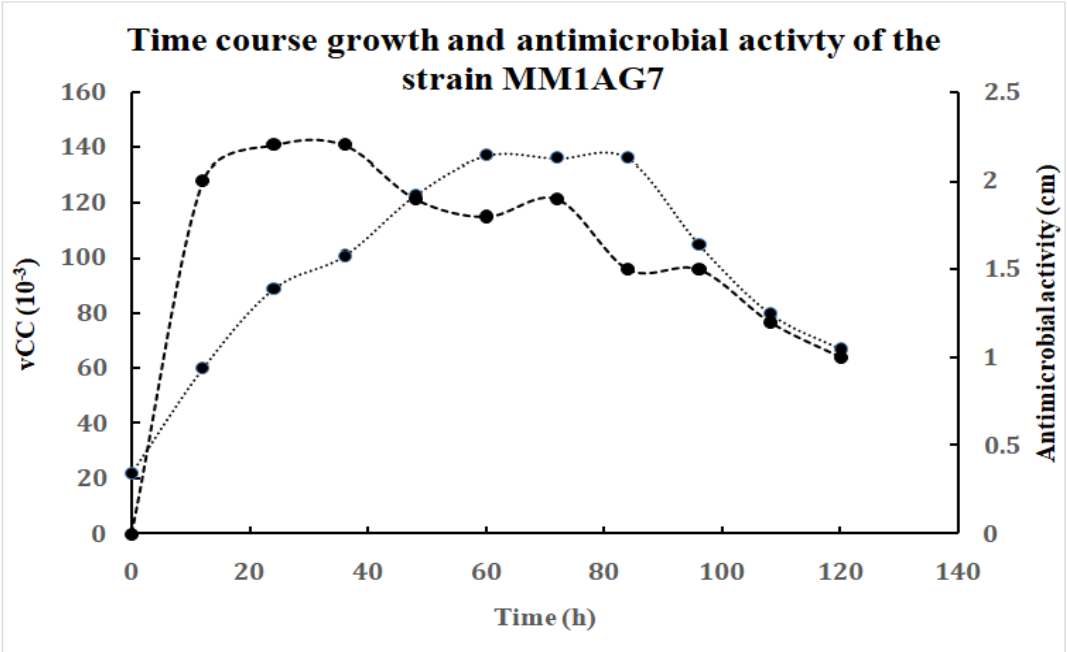

Fig3. Time course growth curve and antimicrobial activity of the strain MM1AG7

Effects of supplying different carbon sources, on cell growth and antimicrobial activity were studied. Among the different carbon sources used, glycerol was the best for the production of the antimicrobial agent by MM1AG7against *M.luteus* followed by starch (Fig 4).

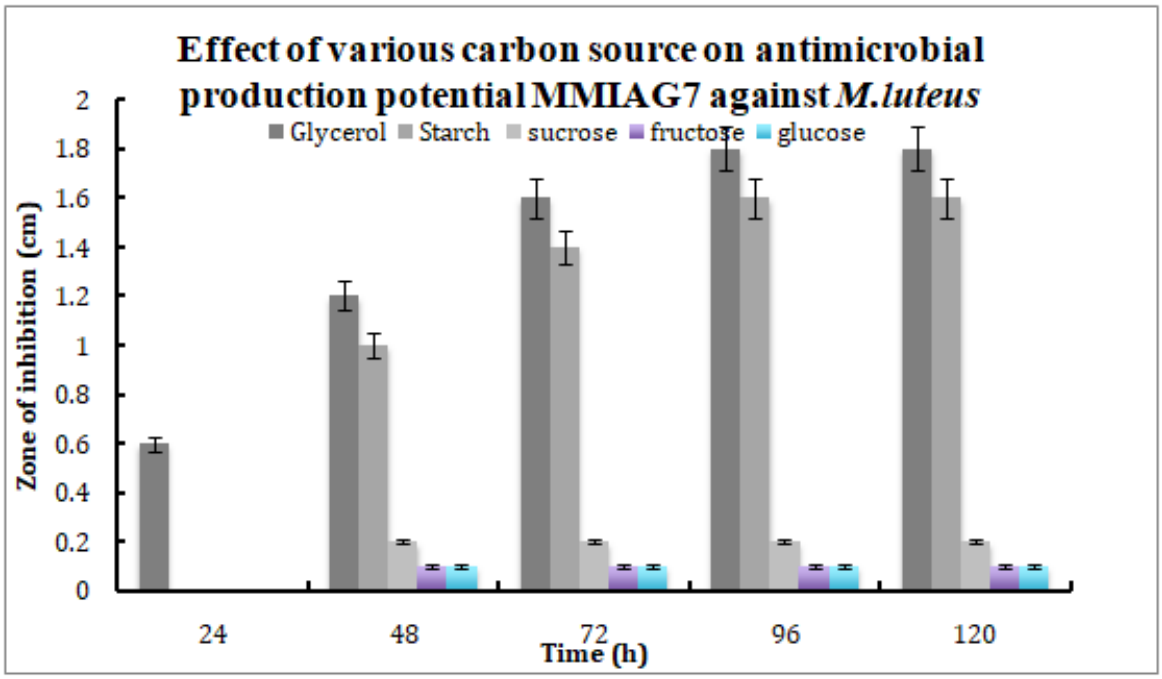

Fig 4. Effect of various carbon source on antimicrobial production potential MMIAG7 against *M.luteus.*

Effects of different nitrogen sources on cell growth and antimicrobial activity were studied. Among the nitrogen sources tested, the potassium nitrate was the best for the productivity of antimicrobial agent by MM1AG7 against *M.luteus* followed by peptone, whereas the other nitrogen source yeast extract used in this study reduced both cell growth and antimicrobial activity. Ammonium sulphate completely prevented the cell growth and antimicrobial activity (Fig 5).

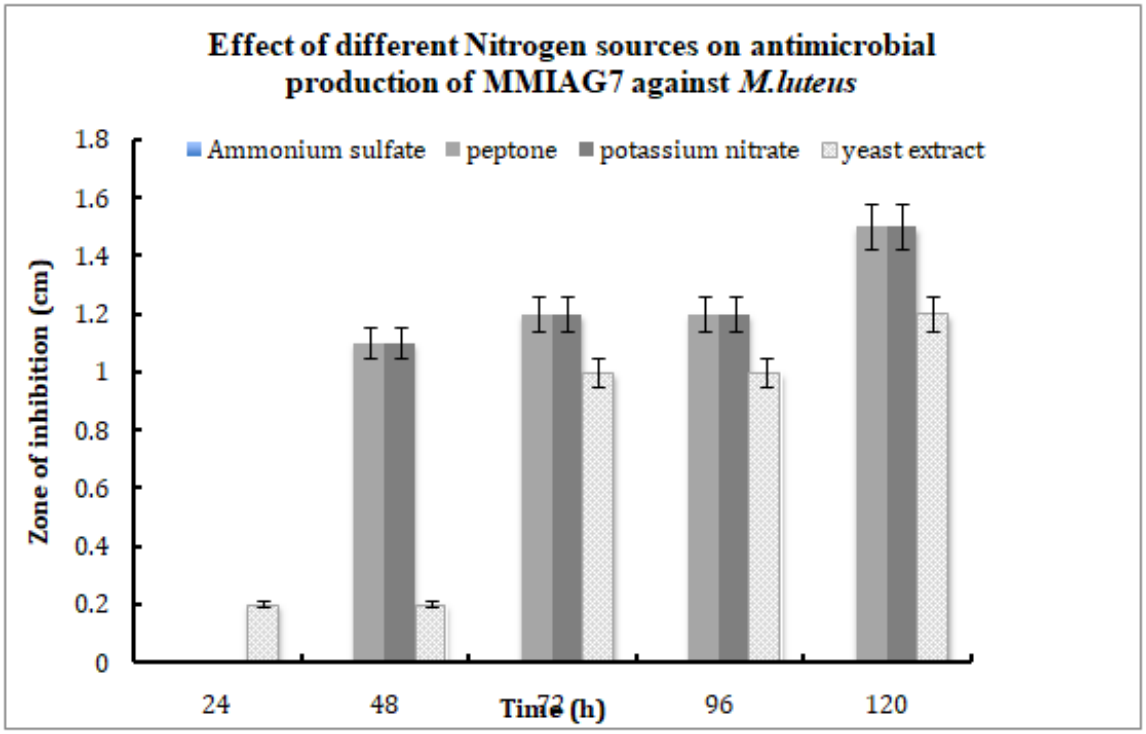

Fig 5. Effect of different Nitrogen sources on antimicrobial production of MMIAG7 against *M.luteus*

Effects of different pH on the cell growth and antimicrobial activity were studied. pH 11 was found to be optimum for cell growth and antimicrobial agent production by the strain against *M.luteus.* (Fig 6).

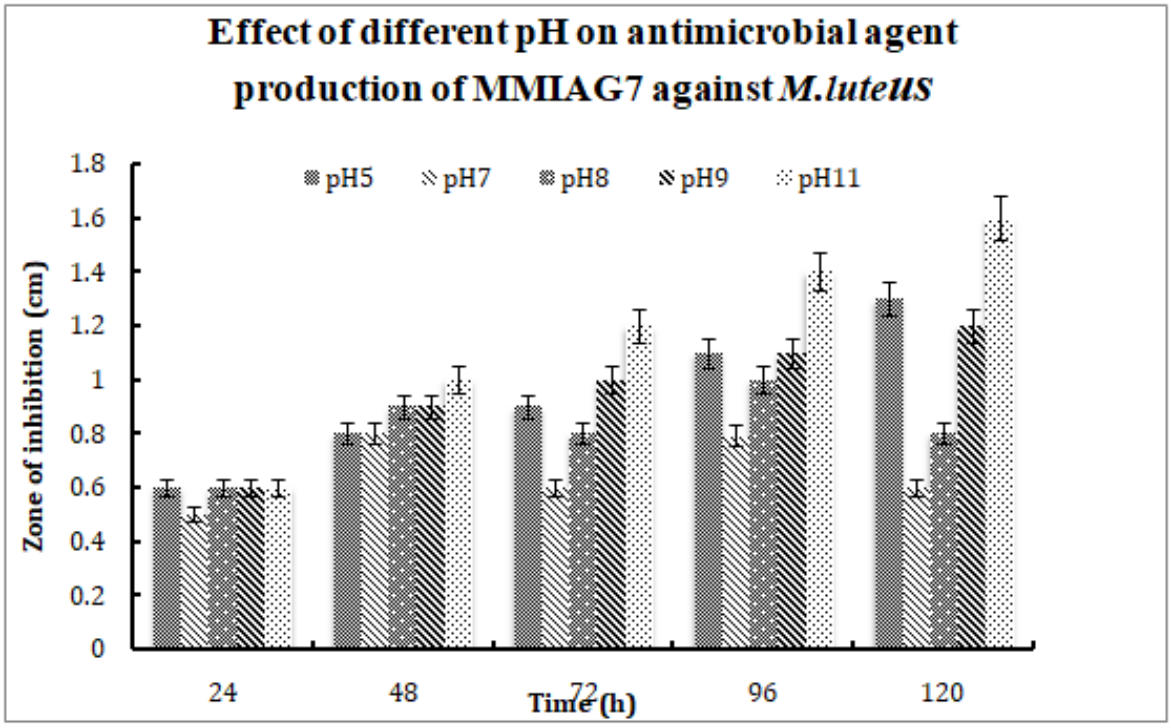

Fig 6. Effect of different pH on antimicrobial agent production of MMIAG7 against *M.luteus.*

Effects of different NaCl conc. to the media, on cell growth and antimicrobial activity were studied. 0.5% (w/v) NaCl conc. was found to be optimum, followed by 1% and 2% for the production of antimicrobial agent by MM1AG7 against *M. luteus* followed by 1% and 2% NaCl conc. (Fig 7).

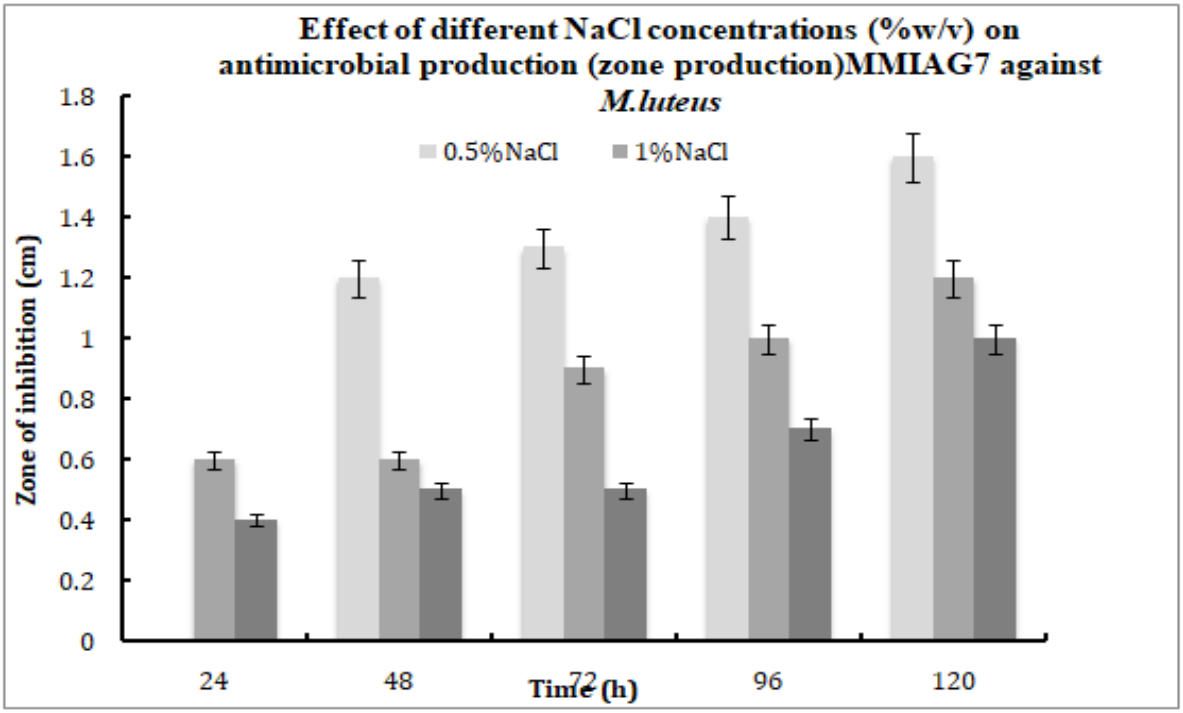

Fig 7. Effect of different NaCl concentrations (%w/v) on antimicrobial production of MMIAG7 against *M.luteus.*

Effects of mutation by different concentration of ethidium bromide on cell growth and antimicrobial activity was studied. Among them only the MM1AG7 culture mutated with 5µg/ml conc. of EtBr showed cell growth and productivity of antimicrobial agent against *M. luteus*; whereas with the increasing conc. of the mutagenic agent cell growth and antimicrobial activity both were prevented (Fig 8).

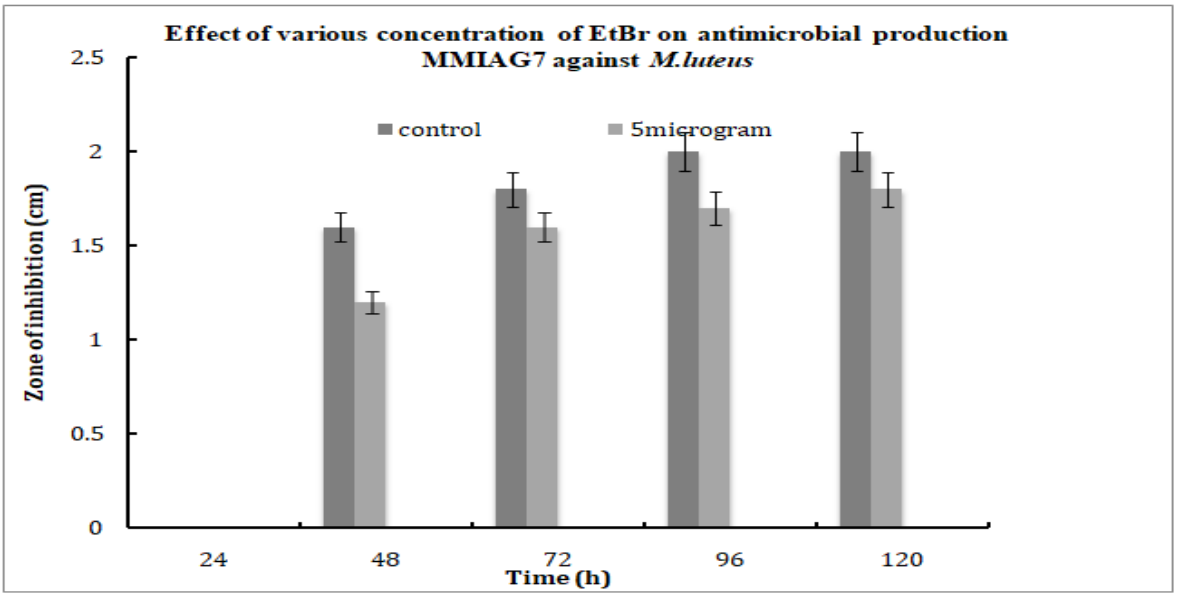

Fig 8. Effect of various concentration of EtBr on antimicrobial production MMIAG7 against *M.luteus.*

The antimicrobial compound was found to be a highly polar compound as it was eluted by methanol in column purification process. It also efficiently showed activity in pH ranging from 5-9; but there was no activity starting from pH 11 (Fig 9).

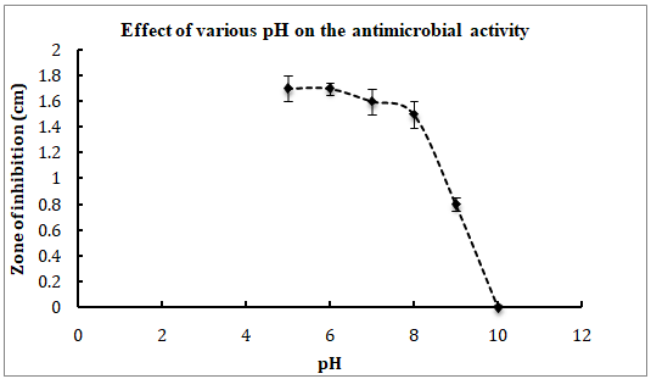

Fig 9. Effect of different pH on the activity of the antimicrobial agent partially purified from MM1AG7

Purified antimicrobial compound was found to be heat, enzyme (lysozyme, proteinase k) and pH tolerant. It showed its activity efficiently in all the temperature ranges; but maximum activity was found in 90ºC (Fig 10) and highest activity at NaCl concentration 0.5%(w/v) (Fig 11).

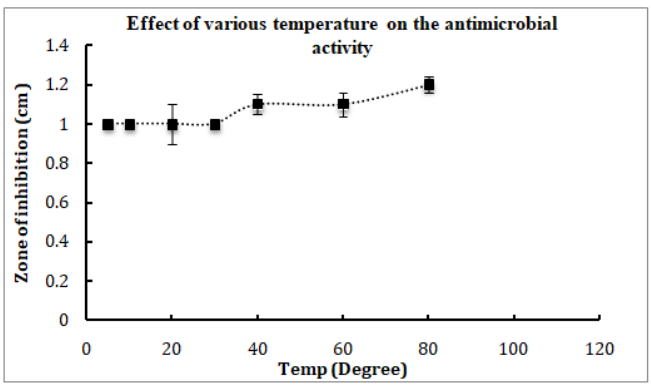

Fig 10. Effect of various temperature on the activity of the partially purified antimicrobial agent from MM1AG7.

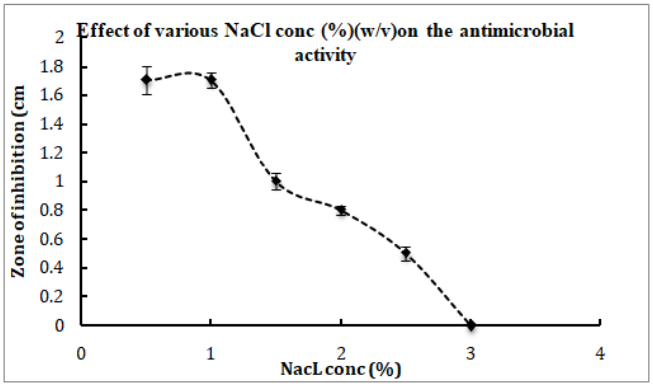

Fig 11. Effect of varying NaCl concentrations on the activity of the antimicrobial agent.

The compound when analysed in TLC under UV showed an evident spot for the antimicrobial agent (Fig 12).

HPLC analysis showed a significant peak for the antimicrobial compound (Fig 13).

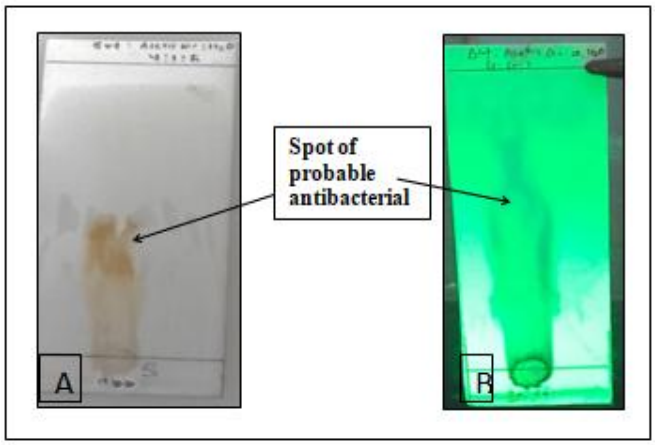

Fig 12. Thin layer chromatography of the antibacterial agent.

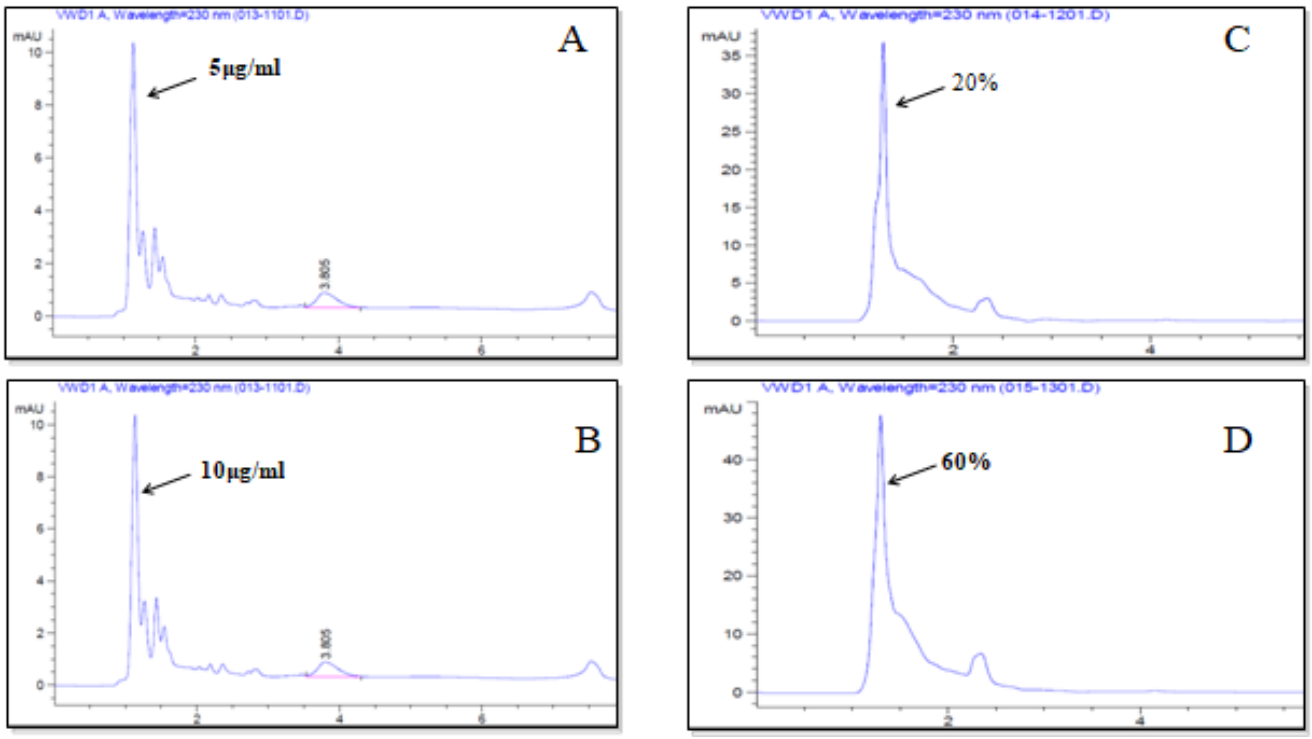

Fig 13. HPLC analysis shows the peak of the antimicrobial agent similar to Streptomycin

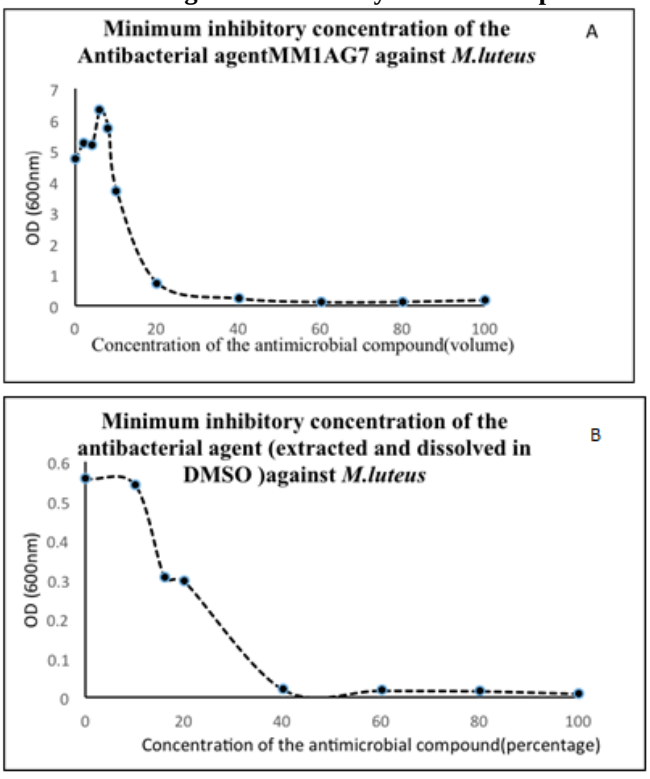

Fig 14. Determination of Minimum Inhibitory Concentration of the antibacterial agent.

The antimicrobial agent produced to the supernatant was extracted with ethyl acetate and the minimum inhibitory concentration was determined to be 40%. (Fig 14).

IV. CONCLUSION

Actinomycetes particularly genus *Streptomyces* are well known for their ability to produce various antimicrobial compound and other secondary metabolites. Chilika with its unique habitat harbours many such strains. The isolate MM1AG7 which showed similarity with *Streptomyces cinereoruber subsp. cinereoruber* NBRC 12756^{T} was found to produce a potent antimicrobial compound against *Micrococcus leuteus.* The antimicrobial agent produced to the supernatant was extracted with ethyl acetate and the minimum inhibitory concentration was determined to be 40%. Since, the extract was partially pure and cannot be represented as gram or milligram as far as efficiency is concerned thus, the extract was considered as 100% efficient and further dilutions were made to determine the MIC. The preliminary HPLC analysis showed that the antimicrobial agent may have similarity with Streptomycin. The antimicrobial agent was effective at pH 5 to pH 11 which shows that its acid tolerant and alkali tolerant as well.

The NaCl tolerance was found to be 0.5% (w/v). The temperature tolerance was from 10°C-80° C which indicates the efficiency of the antimicrobial agent at a higher temperature. Glycerol was the most effective carbon source for the production of the antimicrobial agent, which shows the possibility of using glycerol waste for the production of the antimicrobial agent, that is a cost-effective process.

REFERENCES

1. Andrews, J. M. 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48(Suppl. 1):5–16
2. Betina V.1983. The chemistry and biology of antibiotics. Amsterdam: Elsevier Scientific Pub. Co; p. 190.
3. Costanza R, Kemp WM, Boynton WR.1993. Predictability, scale, and biodiversity in coastal and estuarine ecosystems: implications for management. Ambio.1:88-96.
4. de lima procopio, R. E; da silva, I. R; Martins, M.K; de azevedo, J.L & de Araujo, J. M. (2012) Antibitics produced by Streptomyces. *Braz J Infect Dis*. 16: 466-471.
5. Goodfellow, M. (1989). The Actinomycetes I. Supragenericclassifcation of actinomycetes. In Bergey's Manual of SystematicBacteriology, vol. 4, pp. 2333±2339. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams &Wilkins.

6. Goodfellow, M., Kumar, Y., Labeda, D. P. & Sembiring, L. 2007. The *Streptomyces violaceusniger* clade: a home for streptomycetes with rugose ornamented spores. *Antonie Van Leeuwenhoek* 92,173-99.
7. Gordon, R. E., Barnett, D. A., Handerhan, J. E. and Pang, C. H.N. 1974. *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Bacteriol* 24, 54-63.
8. Holder, I.A., and Boyce, S. T. 1994. Agar well diffusion assay testing of bacteriak susceptibilitu to various antimicrobials in concentration non-toxic for human cell in culture. Burns, 20 426-429.
9. Jayapal KP, Lian W, Glod F, Sherman DH, Hu WS. 2007. Comparative genomic hybridizations reveal absence of large Streptomyces coelicolor genomic islands in Streptomyces lividans. BMC Genomics, 8:229.
10. Kagan IA, Flythe MD. 2014. Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. JoVE. (85).
11. Kampfer, P. & Labeda, D. P. 2006. International Committee on Systematics of Prokaryotes; Subcommittee on the taxonomy of the *Streptomycetaceae*: Minutes of the meeting, 25 July 2005, San Francisco, CA, USA. *Int J Syst Evol Microbiol* 56, 495.
12. Kelly, K.I. 1964. Inter-society color council-national bureau of standard color-name charts illustrated with centroid colors. US Government Printing Office, Washington.
13. Shirling, E. B. & Gottlieb, D. 1966. Methods for characterization of Str8eptomyces species. Int J Syst Bacteriol 16, 313–340.
14. Srinivas TN, Kumar PA, Sucharitha K, Sasikala C, Ramana CV.2009. Allochromatium phaeobacterium sp. nov. *Int J Syst Evol Microbiol*. 59:750-3.
15. Sucharita K, Sasikala C, Park SC, Baik KS, Seong CN, Ramana CV.2009. Shewanella chilikensis sp. nov., a moderately alkaliphilic gammaproteobacterium isolated from a lagoon. International journal of systematic and evolutionary microbiology.59:3111-5.
16. Taddi, A; Rodriguez, H. J.; Marquez-Vilchez; Castelli, C. 2006. Isolation and identification of streptomyces sp. From venezuelan site: Morphological and biochemical studies. Microbiological Research 161:222-231.
17. Tindall, B. J., Sikorski, J., Smibert, R. M. & Kreig, N. R. 2007. Phenotypic characterization and the principles of comparative systematics. In Methods for General and Molecular Microbiology, 3rd edn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.
18. Williams, S.T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A., Sackin, M. J. 1983. Numerical classification of Streptomyces and related genera. *J. Gen Microbiol* 129, 1743-1813.
19. Williams, S.T., Goodfellow, M., & Alderson, G. 1989. Genus *Streptomyces* Waksman and Henrici 1943. 339 AL. *In Bergey's manual of Sytematic Bacteriology*, vol. 4, pp. 2452-2492. Edited by S.T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williama &Wikins.

AUTHORS PROFILE

Anjani Kumar Upadhaya, Obtained his MTech (Biotechnology) from School of Biotechnology, KIIT University. He and team members carried out this work during their 10th semester project work..

Debasmita Chatterjee, obtained her MTech (Biotechnology) from School of Biotechnology, KIIT University. She and team members carried out this work during their 10th semester project work.Currently she is doing Phd in IIT, Kharagpur.

Ms madhuri Swain obtained her MSc Biotechnology from KIIT university. She did this work during her MSc project work.

Dr Lopamudra Ray, the corresponding author of the paper has obtained dher PhD in Biotechnology in 2012 from KIIT university. Currenly she is working as an Assistant Professor in KIIT University. She has 3 registered Phd students. Her basic resaerch area is enzyme purification from microorganisms, and waste degradation. She works on Streptomyces. Her current project is on chitin waste degaradation using novel microroganisms from Chilika Lake.