African Journal of Biotechnology Vol. 8 (23), pp. 6645-6648, 1 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Antimicrobial and cytotoxic activity of *Streptomyces* sp. from Lonar Lake

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Accepted 23 September, 2008

Antibacterial substances from actinomycetes were isolated from marine environment of Lonar Lake and characterized. Out of the 24 isolates subjected to secondary screening, 12 isolates were active against *Bacillus subtilis,* 13 against *Staphylococcus aureus*, 7 against *Escherichia coli*, 3 against *Proteus vulgaris* and 4 against *Salmonella typhi*. Metabolites in the extract of broth of 48 hrgrown *Streptomyces* spp. culture no.2 proved to have antimicrobial and cytotoxic against human lung carcinoma cell A549.

Key words: Actinomycetes, antimicrobial activity, cytotoxic activity, human lung carcinoma cells -A549.

INTRODUCTION

Actinomycetes are the most economical and biotechnologically valuable class of prokaryotes producing bioactive secondary metabolites notably antibiotics (Blunt and Prinsep, 2006) anti-tumor agents, immunosuppressive agents (Mann, 2001) and enzymes (Berdy, 2005; Cragg and Newman, 2005; Strohl, 2004). Actinomycetes are the main source of clinically important antibiotics, most of which are too complex to be synthesized by combinatorial chemistry, making three quarters of all known products; the *Streptomyces* are especially prolific, producing around 80% of total antibiotic products (Bull and Stach, 2005; Imada and Okami, 1998; Kim and Garson, 2005). *Micromospora* is the runner up with less than one-tenth as many as *Streptomyces* (Lam, 2006). In addition to antibacterial components they also produce secondary metabolites with biological activities of which the *Streptomyces* spp. amounts for 80% of the total production by actinomycetes (Cragg and Newman, 2005).The aim of this study was to investigate the antimicrobial and cytotoxic activity of actinomycete isolates from Lonar Lake (Buldhana *Dist*, India).

MATERIALS AND METHODS

Isolation of actinomycetes and identification

Humic acid-vitamin agar (Hayakawa and Nonomura, 1993; Masayuki and Hideo, 1987) was used for the isolation of actinomycetes. The media components included cycloheximide (20 mg l^{-1}), pravastatin (10 mg l^{-1}), trimethoprim (2 mg l^{-1}) and nalidixic acid (10 mg l^{-1}) to prevent other non-actinomycete bacteria and fungal growth (Baltz, 2006). The samples were diluted with sterilized water and an aliquot of 0.1 ml from these samples was spread on the media. After incubation of 1 – 3 days at 28°C, the actinomycete colonies that developed on the plates were enumerated and were expressed in colony forming units.

In preliminary screening, determination of the antimicrobial activity of pure isolates was done by zone of inhibition method on Nutrient agar (NA) using *Salmonella typhi* NCIM 2501 as pathogen. Further screening was performed by zone of inhibition method against the standard test organisms. Actinomycetes were cultured for the screening of antibiotic substances in nutrient agar at 28°C for 3 days. The test organisms used were: *Bacillus subtilis* NCIM2722*, Staphylococcus aureus* NCIM 2127*, Escherichia coli* NCIM 2995*, Proteus vulgaris* NCIM2857*, Pseudomonas aeruginosa* NCIM 2074*, S. typh*i NCIM 2501 and *Enterococcus faecalis* NCIM 2080.

Identification of actinomycetes

The characterization of screened culture was conducted using biochemical assays including NaCl resistance, casein hydrolysis, starch hydrolysis, Tween 20 hydrolysis, urea hydrolysis, esculin hydrolysis, acid production from sugar, temperature tolerance and morphological characterization methods included macroscopic and microscopic determination. The structures observed under oil immersion (100X) were compared with Bergey's manual of Determinative Bacteriology, Ninth edition (2000).

Isolation and purification of antibacterial metabolites

Samples from *Streptomyces* Culture No.2 grown in 1 L nutrient

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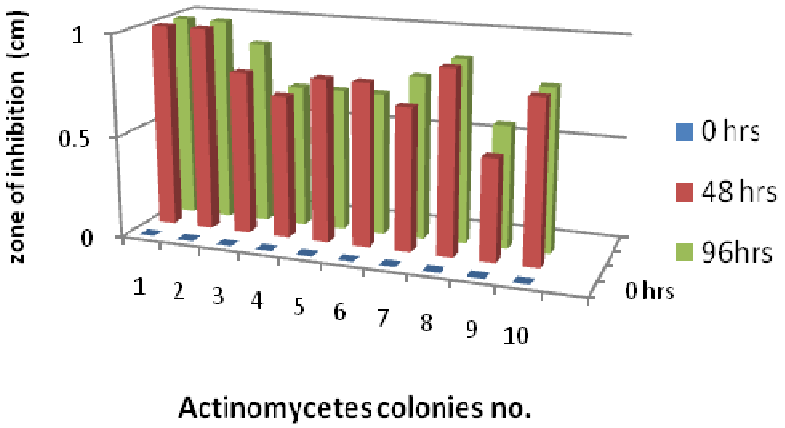

Figure 1. Screening of actinomycetes on *Salmonella typh*i NCIM 2501.

broth at 28°C and 220 rpm were taken and extraction was carried out using ethyl acetate solvent extraction [ethyl acetate : filtrate 1:1 (v/v)] on shaker at 220 rpm for 1 h. The ethyl acetate phase that contains antibiotic was separated from the aqueous phase and by evaporation in water bath at 80-90°C the residue was obtained and weighed. The compound thus obtained was used to determine antimicrobial activity and minimum inhibitory concentration.

The antimicrobial activity was determined by spectrophotometric method. The residue obtained was dissolved in 1 ml 0.2 M phosphate buffer (pH 7.0). 100 µl of preparation was loaded into a well in the 24 well plates, containing pathogenic cultures and were incubated at 37°C for 18 - 48 h and optical densities measured at 540 nm using ELISA plate reader (Figure 3).

For thin layer chromatography (Becker and Lechavier, 1964), silica gel plates (Merck) 5 X 20 cm, 1 mm thick were used. 10 µl of the ethyl acetate fractions and reference antibiotics were spotted on the plates and the chromatogram was developed using ethylacetate : iso-propanol : acetonitrile (1:4:5) as solvent system.

Cytotoxicity assay

Human Lung carcinoma cells-A549 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS). For cytotoxicity assays, cells were seeded into 24-well plates at a density of 2.1×10^5 cells/well and the dilutions (1:500 and 1:1000) of the extract made in DMEM in the volumes of 10 µl and 25 µl were added. After incubation period (24, 48 h), the growth media was removed and the cells were trypsinized and counted in a Hemocytometer (Freshney, 2006).

RESULTS AND DISCUSSION

Identification of actinomycetes

Out of 32 actinomycetes subjected for primary screening process, only 24 isolates showed activity against test organisms. Of which, only 3 were active against gram negative organism, 12 against gram positive organisms and 12 against both gram positive and gram negative organisms. Among these isolates, 31 of them showed positive inhibitory effect against *B. subtilis*, 27 against *S. aureus*, 17 against *E. coli*, 15 against *S. typhi* and 14 against *Proteus* species.

Out of the 24 isolates those were subjected for the secondary screening, 12 isolates were active against *B. subtilis,* 13 against *S. aureus*, 7 against *E. coli*, 3 against *P. vulgaris* and 4 against *S. typhi*. From Figure 1, it can be seen that the actinomycete culture no 2 shows maximum zone of inhibition against pathogenic culture. Culture no 2 which is of *Streptomyces* showed activity against all pathogenic strains (Figure 2). Figure 3 extrapolates the effect of extract of 48 h grown culture of *Streptomyces* spp. colony 2 on various pathogenic cultures in the form of optical densities obtained.

Identification of the potent antibiotic producing strains reveals that most of the specimens belong to the genus *Streptomyces* followed by *Streptoverticillium*. The minimum inhibitory concentration of the extract from *Streptomyces* spp. no.2 was 0.73 $mg.mL^{-1}$. Also the results from thin layer chromatography showed the spot given by the extract of *Streptomyces* spp. was with R_f value 0.78.

Cytotoxic activity

We examined the effect of *Streptomyces* spp. and its metabolites on the growth of eukaryotic cells. For the cytotoxicity studies A549 cells were chosen. Monolayer cultures of A549 cells were exposed to various concentrations of the extracts and cell viability evaluated by counting live cells after 24 and 48 h of exposure (Figures 4 and 5). The extract caused cell death at dilutions of 1/500 (10 µl). At dilution of 1/1000 (25 µl) cell viability greatly reduced after 48 h of exposure.

Conclusion

Actinomycetes are producers of potent metabolic compounds used commercially as antibiotics and other novel drugs (Shiomi and Takeuchi, 1990). Our work justifies the

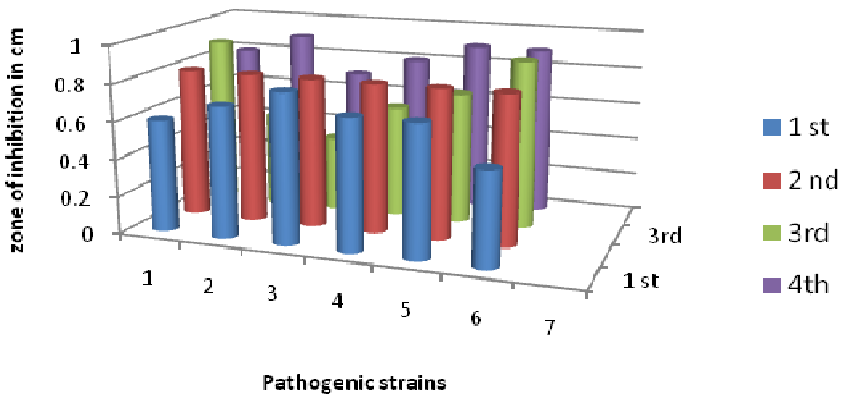

Figure 2. 25 µl of 48 h grown culture (1st, 2nd 3rd and 4th) of *Streptomyces* spp. *Streptomyces* spp. no.2 shows antimicrobial activity against all pathogenic strains. **1.** *Bacillus subtilis* NCIM2722, **2.** *Staphylococcus aureus* NCIM 212, **3.** *Escherichia coli* NCIM 2995 ,**4.** *Proteus vulgaris* NCIM2857, **5.** *Pseudomonas aeruginosa* NCIM 2074, **6.** *Salmonella typh*i NCIM 2501, and **7.** *Enterococcus faecalis* NCIM 2080.

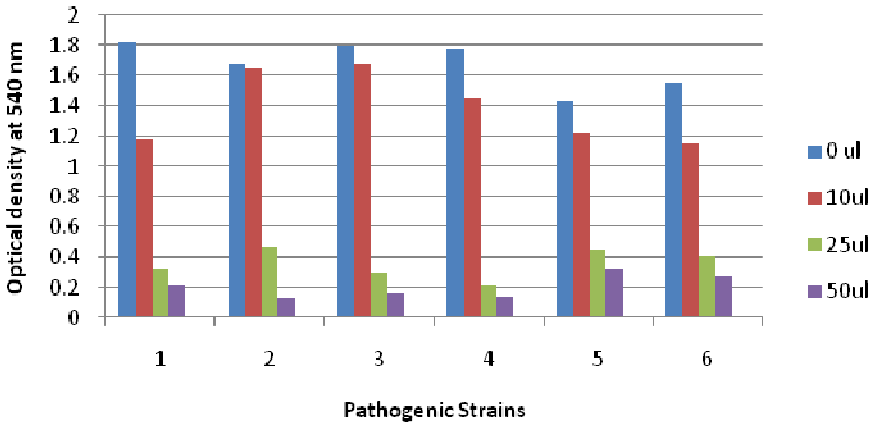

Figure 3. Effect of extract of 48 h grown culture of *Streptomyces* spp. no.2 of actinomycetes on various pathogenic cultures. **1.** *Bacillus subtilis* NCIM2722, **2.** *Staphylococcus aureus* NCIM 2127, **3.** *Escherichia coli* NCIM 2995, **4.** *Proteus vulgaris* NCIM2857, **5.** *Pseudomonas aeruginosa* NCIM 2074, **6.** *Salmonella typh*i NCIM 2501.

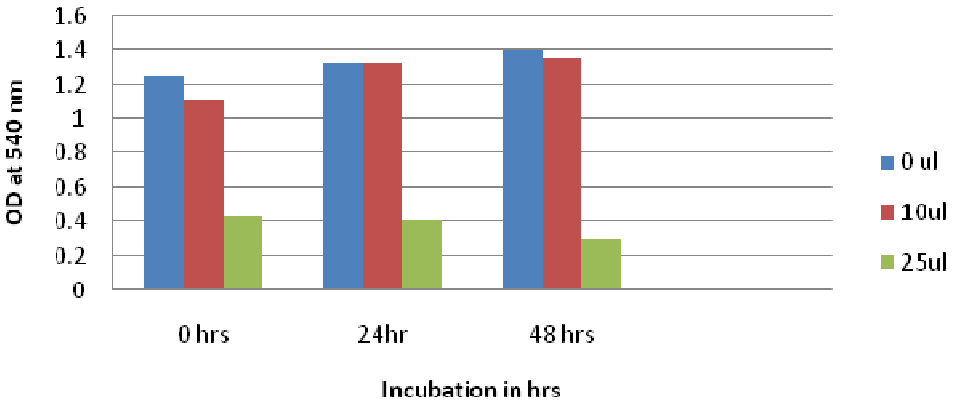

Figure 4. Cytotoxic effect of extract of 48 h grown culture of *Streptomyces* spp. no. 2 on Lung carcinoma cells A549.

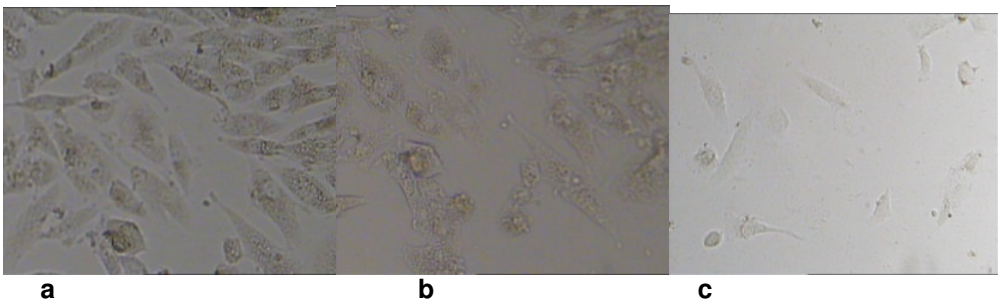

Figure 5. Lung carcinoma cells A549. **a)** Cells with 0 µl of extract of 48 h grown colony no . 2. **b)** Cells with 10 µl of extract of 48 h grown colony no . 2. **c)** Cells with 25 µl of extract of 48 h grown colony no. 2.

broad host range exhibited by the metabolites of the actinomycetes isolated using the above described methods and sources, which came to include even eukaryotes along with previously established action against prokaryotes. We also found that the metabolite from *Streptomyces* spp. colony 2 had inhibitory action against the growth of human lung carcinoma A549 cell line. Hence anti-cancerous property shown by this metabolite needs to be exploited further.

ACKNOWLEDGEMENTS

We wish to thank Dr. M. S. Andhale, for guidance on marine microbes. We are grateful to Dr S.G. Gupta for helpful discussion.

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