Mycobiology 37(3) : 211-214 (2009)

Optimal Conditions for Antimicrobial Metabolites Production from a New *Streptomyces* sp. RUPA-08PR Isolated from Bangladeshi Soil

F. A. Ripa[1], F. Nikkon[2], S. Zaman[3] and P. Khondkar[1]*

[1]*Pharmaceutical Microbiology and Biotechnology Laboratory, Department of Pharmacy, Rajshahi University, Rajshahi- 6205, Bangladesh*
[2]*Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi- 6205, Bangladesh*
[3]*Department of chemistry, Rajshahi University, Rajshahi- 6205, Bangladesh*

(Received August 31, 2009. Accepted September 21, 2009)

An actinomycete strain was isolated from northern part of Bangladesh and identified as a new *Streptomyces* species on the basis of its morphological, biochemical, cultural characteristics and 16S rRNA data. Attempts were made to optimize the culture conditions for the production of antimicrobial metabolites by this strain. Antimicrobial metabolites production was started after 7 days of incubation of culture broth and reached its maximum levels after 10 days and thereafter gradually decreased. The maximum production of antimicrobial metabolites was obtained when the culture medium pH was adjusted to 8. The optimum temperature for antimicrobial metabolites production was 39°C, indicated the new strain as mesophilic organism. Basel medium supplemented with glucose and yeast extract as carbon and nitrogen sources, respectively, was proved to be the best for the production of bioactive metabolites. Maximum production of bioactive metabolites was when NaCl concentration was 1% and among different minerals tested, K_2HPO_4 and NaCl showed positive influence on antibiotic production by the strain.

KEYWORDS : Antibacterial activity, Optimization of condition, 16S rRNA, *Streptomyces* sp.

The genus *Streptomyces* is an aerobic, spore-forming actinomycetes and is classified in the family *Streptomycetaceae* on the basis of morphological and cell-wall chemotaxonomic characters (Waksman and Henrici, 1943). The taxon currently accommodates aerobic, Gram-positive bacteria that have high DNA G-C% content (69~78 mol%) and produce extensively branched substrate mycelium and aerial hyphae (Williams *et al.*, 1983; Embley and Stackebrandt, 1994). With more than 500 validly described species and subspecies, the taxon currently contains the largest number of species in the domain *Bacteria* (Hain *et al.*, 1997). Molecular-systematic methods, notably 16S rDNA phylogenetic analysis, are having an increasing impact on *Streptomyces* systematics (Kim *et al.*, 1998). Several thousand (about 4000) of antibiotics have been isolated and identified. Some of them are useful and some of them are useless. Most antibiotics have been discovered during soil screening program. It is reported that soil microorganisms would provide a rich source of antibiotics (Bibb, 2005). Antibiotics discovered in the US and Japan between 1953 and 1970 reveals approximately 85% of the antibiotics were produced by Actinomnycetes, 11% by fungi and 4% by bacteria (Reiner, 1982). Among the Actinomycetes, more than 70% of known antibiotics are produced by *Streptomycetes* including, streptomycin, neomycin, tetracycline and chloramphenicol (Tanaka and Mura, 1993; Harvey, 1993). As part of our ongoing research of microbial metabolites (Khondkar, 1999; Hossain *et al.*, 2004), we isolated an actinomycetes, *Streptomyces* sp. RUPA-08PR, from a soil sample collected in the region of Rajshahi. The nutritional source like carbon, nitrogen and minerals as well as the environmental factors such as incubation period, pH and temperature are known to have profound effect on antibiotic production by actinomycetes (Himabindu and Jetty, 2006). Optimization of culture conditions is essential to get high yields of the antimicrobial metabolites. Hence, the present study described the optimization of culture conditions for the isolation of antimicrobial metabolites from this species.

Materials and Methods

Collection and identification of organism. The organism was isolated from a soil sample collected from Rajshahi, Bangladesh at the depth of 0.75 m using crowded plate technique (Hammond and Lambert, 1978). The organism was identified as a novel, *Streptomyces* species on the basis of morphological, physiological, biochemical (Shirling and Gottlieb, 1969) and 16S rDNA studies (GenBank accession number BankIt1256035 GQ500975) and designated as *Streptomyces* sp. RUPA-08PR (Ripa, 2008). Pure culture of the strain was maintained on Czapek Dox (alkaline) agar slant.

*Corresponding author <E-mail : proma_khondbar@yahoo.co.uk>

Effect of incubation period. Shake flask fermentations were run in 500 *ml* flasks containing 100 *ml* Czapek-Dox (alkaline) broth. The flasks were plugged with cotton and covered with parafilm and sterilized. The flasks were allowed to cool and the liquid media inoculated with spores of the organisms from previously prepared agar slant were poured into it. Then they were incubated at 37°C for optimum yields on a rotary shaker at 250 rpm. At every 24 h interval, the flasks were harvested and antimicrobial metabolites production determined in terms of their antimicrobial spectrum. The culture filtrates were extracted with ethyl acetate by using separating funnel. The concentrates were tested for antibacterial activity by disc diffusion method (Bauer *et al.*, 1966) against *B. subtilis* for 15 consecutive days. All results presented in the paper are mean ± SD of triplicate analysis.

Effect of pH and temperature on the production of bioactive metabolites. The effect of pH and temperature on the antimicrobial production by the strain was studied by inoculating 24 h old culture in Czapek-Dox (alkaline) broth. The pH of the media was adjusted using hydrochloric acid (1 M) and sodium hydroxide (1 M). For temperature study, after sterilizing and inoculating with spores the flask were incubated at different temperatures (25~45°C). The production of antimicrobial metabolites was tested for antimicrobial activity after 120 h of incubation by disc diffusion method against *B. subtilis*.

Effect of carbon and nitrogen sources on the production of bioactive metabolites. To determine the effect of carbon sources on antimicrobial metabolites production, different carbon sources such as sucrose, D-glucose, D-fructose, mannitol, D-(+) galactose, xylosa, lactose, D (+) mannose, rhamnose and maltose were added to the basal medium containing KCl (0.05%), KH_2PO_4 (0.1%), $MgSO_4$, $7H_2O$ (0.05%) and $FeSO_4$, $7H_2O$ (0.001%). Carbon sources were added in 3% concentration to the basal medium supplemented with $NaNO_3$ (0.2%) as nitrogen source. The effect of various nitrogen sources such as $NaNO_3$, yeast extract, KNO_3, ammonium sulphate, ammonium chloride, peptone, casein, L-asparagine and beef extract was studied by adding nitrogen source (0.2%) to the basal medium containing glucose (3%). Final pH of the medium adjusted to 8 and antibacterial activity was checked by disc diffusion method against *B. subtilis* for 15 consecutive days.

Effect of salt (NaCl) concentration on antibiotic production. Salt concentration has a profound effect on the production of antibiotic from microorganism due to its effect on the osmotic pressure to the medium (Pelczer *et al.*, 1993). To observe this effect NaCl was added to culture media at different concentrations such as 0%, 0.5%, 1%, 2%, 3%, 4% and 5% respectively. Glucose (3%) and yeast extract (0.2%) was used as carbon and nitrogen source respectively.

Results and Discussion

The production of antimicrobial metabolites at different days was determined by disc diffusion assay method measuring the zone of inhibition against *B. subtilis*. Antimicrobial metabolites production by the strain was started after 7 days of incubation. The highest level was obtained after 10 days of incubation and then production was declined gradually (Fig. 1). Thus the organism was allowed to incubate for 10 days for the production of antimicrobial metabolites. The effect of pH and temperature on antimicrobial metabolites production by the strain is presented in Fig. 2 and 3. The optimum pH for antibiotic production was 8.0. The organism produced high levels of antibiotic production when culture medium incubated at 39°C. Therefore the strain was strictly mesophilic for secondary metabolites production. Extreme pH and temperature were unfavorable for antibiotic production (Fig. 2 and 3).

The effect of different carbon sources on antibiotic pro-

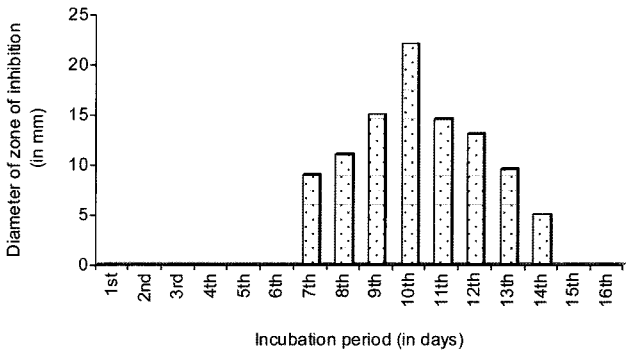

Fig. 1. Effect of incubation period on antimicrobial metabolites production.

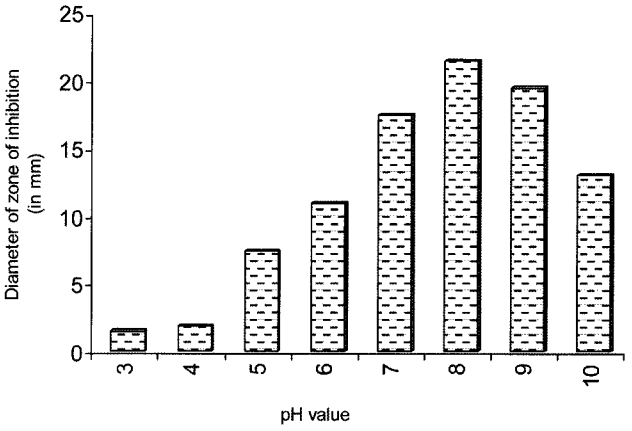

Fig. 2. Antimicrobial metabolites production at different pH.

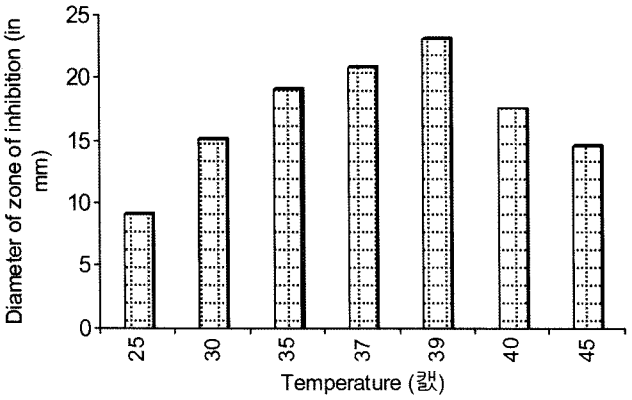

Fig. 3. Effect of temperature on antimicrobial metabolites production.

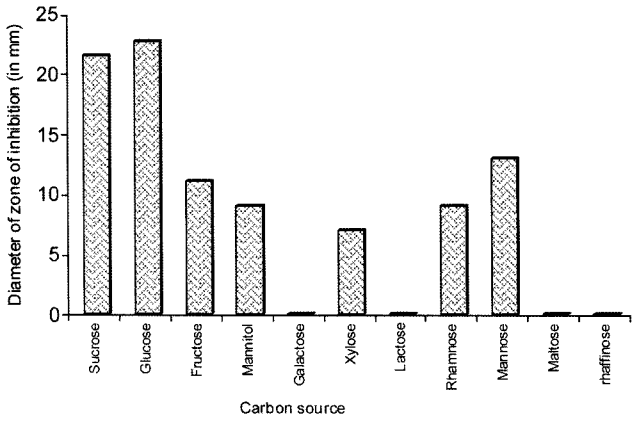

Fig. 4. Effect of different carbon sources on the production of antimicrobial metabolites.

duction by the strain is presented in Fig. 4. Among the carbon sources, glucose proved to be the best carbon source for both cell growth as well as antimicrobial metabolites production by the strain. Sucrose also gave a similar pattern result followed by mannose, fructose, mannitol, rhamnose and xylose respectively. No antibiotic was produced when the medium was supplemented with galactose, lactose, raffinose and maltose as a sole carbon source. Carbohydrates such as glycerol, maltose, lactose and some others are known to have interference with the production of secondary metabolites (Demain and Fang, 1995). In the present study, the strain was found to produce high levels of antimicrobial metabolites in the medium supplemented with glucose (2%) as sole carbon source. In case of *Streptomyces* species, with regards to carbon sources species specific variation may occur for cell growth and secondary metabolites production (Jonsbu *et al.*, 2002).

The effect of nitrogen sources on antimicrobial metabolites production is given in Fig. 5. Both inorganic and organic nitrogen sources produced reasonable amount of antimicrobial metabolites. The highest activity was obtained

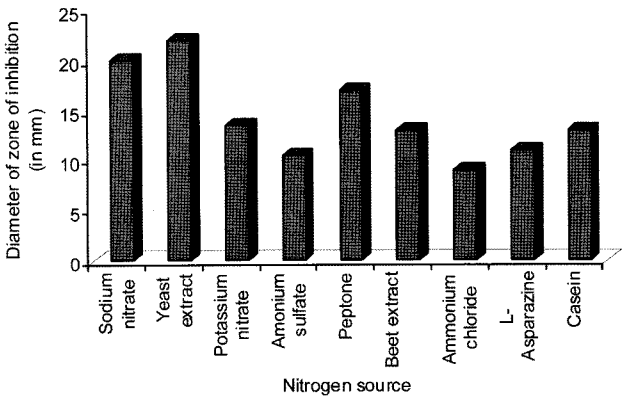

Fig. 5. Effect of different nitrogen sources on the production of antimicrobial metabolites.

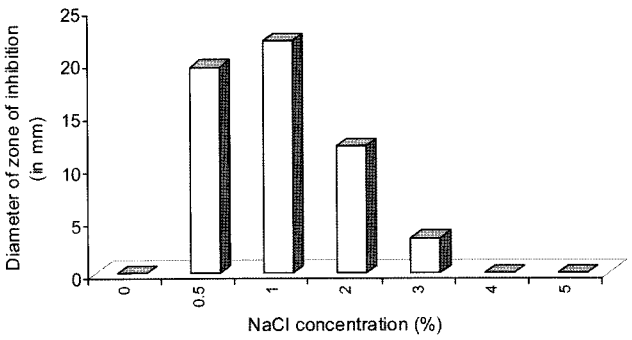

Fig. 6. Effect of NaCl concentration (%) on antimicrobial metabolites production.

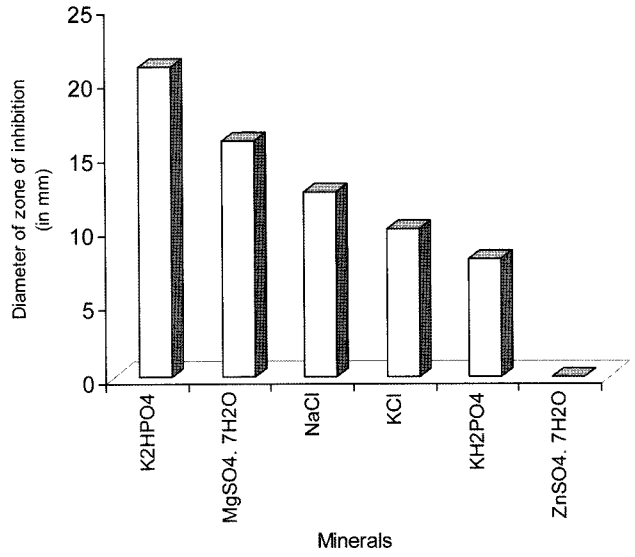

Fig. 7. Effect of minerals on antimicrobial metabolites production.

with yeast extract. Medium supplemented with $NaNO_3$ and peptone gave similar results followed by KNO_3, beef extract, casine, L-asparazine, ammonium sulphate and ammonium chloride.

It was observed that the production of antimicrobial

metabolites was maximum in presence of 1% NaCl. The growth of the organism gradually decreased with the increase of NaCl concentration. The results are given in the Fig. 6. Among different minerals tested only K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ had positive effects on antibiotic production followed by NaCl, KCl and KH_2PO_4. However, $ZnSO_4 \cdot 7H_2O$ exerted negative effect on secondary metabolites production (Fig. 7). In the present study optimum levels of culture conditions were determined for antimicrobial production by *Streptomyces* sp. RUPA-08PR.

References

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardised single disk method. *Am. J. Clin. Pathol.* 44:493-496.

Bibb, M. J. 2005. Regulation of secondary metabolism in Streptomycetes. *Curr. Opin. Microbiol.* 8:208-215.

Demain, A. L. and Fang, A. 1995. Emerging concepts of secondary metabolism in actinomycetes. *Actinomycetology* 9:98-117.

Embley, T. M. and Stackebrandt, E. 1994. The molecular phylogeny and systematics of the actinomycetes. *Annu. Rev. Microbiol.* 48:257-289.

Hain, T., Ward-Rainey, N., Kroppenstedt, R. M., Stackebrandt, E. and Rainey, F. A. 1997. Discrimination of *Streptomyces albido avus* strains based on the size and number of 16S-23S ribosomal DNA intergenic spacers. *Int. J. Syst. Bacteriol.* 47:202-206.

Hammond, S. M. and Lambert, P. A. 1978. Antimicrobial Action, pp. 4-9. Edward Arnold Ltd., London.

Harvey, A. L. 1993. Drugs From Natural Products - Pharmaceuticals and Agrochemicals. Ellis Horwood, New York.

Himabindu, M. and Jetty, A. 2006. Optimization of nutritional requirements for gentamicin production by *Micromonospora echinospora*. *Indian J. Exp. Biol.* 44:842-848.

Hossain, M. S., Hossain, M. A., Rahman, M. M., Mondol, M. A., Bhuiyan, S. A., Gray, A. I., Flores, M. I. and Rashid, M. A. 2004. Amides from the fungus *Streptomyces hygroscopicus* and their antimicrobial activity. *Phytochemistry* 65:2147-2151.

Jonsbu, E. M., Intyre, M. and Nielsen, J. 2002. The influence of carbon source and morphology on nystatin production by *Streptomyces noursei*. *J. Biotechnol.* 95:133-144.

Khondkar, P., Islam, M. A. and Rahman, M. M. 1999. Isolation and *in vitro* antimicrobial screening of 5-hydroxymethylfurfural from a *Monocillium* species. *Raj. Uni. Studies part-2* 27: 83-87.

Kim, S. B., Falconer, C., Williams, E. and Goodfellow, M. 1998. *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. *Int. J. Syst. Bacteriol.* 48:59-68.

Pelczar, M. J., Chan, E. C. S. and Krieg, N. R. 1993. Microbiology: Concepts and Applications. 5th ed. McGraw-Hill, USA.

Reiner, R. 1982. Antibiotics: An Introduction. Roche Scientific Service, Switzerland.

Ripa, F. A. 2008. Isolation and characterization of a novel *Streptomyces* species: *Streptomyces profariensis*, M. Pharm Thesis, Rajshahi University, Bangladesh.

Shirling, E. B. and Gottlieb, D. 1969. Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. *Int. J. Syst. Bacteriol.* 19:391-512.

Tanaka, Y. T. and Mura, S. O. 1993. Agroactive compounds of microbial origin. *Annu. Rev. Microbiol.* 47:57-87.

Waksman, S. A. and Henrici, A. T. 1943. The nomenclature and classification of the actinomycetes. *J. Bacteriol.* 46:337-341.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. and Sackin, M. J. 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* 129:1743-1813.