Current Trends in Biotechnology and Pharmacy
Vol. 6 (1) 99-111 January 2012, ISSN 0973-8916 (Print), 2230-7303 (Online)

Influence of Cultural Conditions for Improved Production of Bioactive Metabolites by *Streptomyces cheonanensis* VUK-A Isolated from Coringa Mangrove Ecosystem

Usha Kiranmayi Mangamuri[1], Sudhakar Poda[2], Krishna Naragani[1] and Vijayalakshmi Muvva[1]*

[1]Department of Botany and Microbiology and [2]Department of Biotechnology,
Acharya Nagarjuna University, Guntur-522510, Andhra Pradesh, India
*For Correspondence - profmvl@gmail.com

Abstract

The influence of culture conditions and the effect of environmental factors on the growth and production of bioactive metabolites by *Streptomyces cheonanensis* VUK-A was the focus of this study. The strain exhibited broad spectrum antimicrobial activity against Gram-positive, Gram-negative, unicellular and multicellular fungi. The optimum pH and temperature for bioactive metabolite production were 7 and 30°C respectively. Production of bioactive metabolites by the strain was high in asparagine glucose broth as compared to other media tested. Studies on nutritional factors revealed that highest antimicrobial metabolite production was obtained when lactose and peptone at 1% and 0.25% were used as carbon and nitrogen sources respectively. Ninty six hours of incubation was found to be the optimum for bioactive metabolite production by the strain. As the strain exhibited potent antimicrobial activity, it may be explored for biotechnological purposes.

Keywords: Optimization, Bioactive metabolites, Nutritional factors, Culture conditions, Environmental Parameters.

Introduction

There is an immediate need to discover and develop new antibiotics as spread and prevalence of infectious diseases resistant to chemotherapy is on the rise (1). Special attention was focused on the microbes that have been proved as the natural dumps for the bioactive metabolites since decades for resolving the problem of the antibiotic resistance (2). These microbes are capable of producing a wide array of the potent antimicrobial compounds that have broad application in the field of medicine (3). Many microbes that thrive in extreme conditions have the potentiality to produce unusual bioactive metabolites that acts as a chemical defense against the pathogenic microbes (4). The most promising source of the future antibiotics that the society expects is the natural microbial products (5). The exploration for the new antimicrobial compounds and the new strains continue to be the most important research programme around the world (6). The microbial natural products remain the most potent and important source for the novel antibiotics, although new methodologies are needed to improve the efficiency of the discovery of compounds. Numerous antibiotics have been

obtained from various microbes isolated from the marine environment played a significant role in the discovery of anti-metabolites (7). Production of the secondary metabolites by the microbes differs in quality and quantity based on the type of strains and also species used (8). It is also a known fact that appropriate fermentation medium is critical and crucial for the production of the secondary bioactive metabolites (9) and prior experience and knowledge is required in developing a suitable basal medium since it plays an important role in further media optimization (10). Additionally, biosynthesis of the secondary metabolites is influenced by numerous environmental factors including nutrients (nitrogen, phosphorous and carbon sources), growth rate, feedback control, and other physical conditions (oxygen supply, temperature and pH) (11, 12, 13, 14). Therefore, influence of the growth conditions and environmental conditions are important to improve the production of the secondary metabolites.

Streptomyces species has been widely reported for the production of a number of anti-microbial metabolites that have therapeutic applications (15). These species have gained a special prominence for their characteristic ability to produce potent antibiotics and other secondary metabolites including anti-tumor agents (7). Each of the strain has a genetic set up that influences the production of 10-20 different kinds of secondary compounds. The objective of the present study is to design an appropriate culture medium and also optimize the cultural conditions of the *Streptomyces cheonanensis* VUK-A strain in order to reduce the cost of fermentation process and improve the formation of antimicrobial compounds.

Materials and Methods

***Isolation*:** The *Streptomyces cheonanensis* VUK-A was used in the investigation. The strain was isolated from soils of "Coringa Mangrove Ecosystem" of south coastal Andhra Pradesh, India by using soil dilution plate technique on starch-casein agar medium (16) and further maintained on yeast extract malt extract dextrose (ISP-2) agar medium at 4°C (17). The 16s rRNA sequence of the strain *Streptomyces cheonanensis* VUK-A was submitted to the Genbank (accession number JN087502) (48).

***Incubation Period on Bioactive Metabolite Production*:** The growth pattern and bioactive metabolite production of the strain was studied at regular intervals up to 7 days. One week old culture of the strain was cultivated in seed medium (starch casein broth) at room temperature for 48 h. Seed culture at a rate of 10% was inoculated into the production medium of the same composition. The fermentation process was carried out for one week under agitation at 120 rpm. At every 24 h interval, the flasks were harvested and the biomass was separated from the culture filtrate. Biomass was determined in terms of total cell dry weight. Antimicrobial metabolite production determined in terms of their antimicrobial spectrum (18). The culture filtrates were extracted with ethyl acetate and evaporated to dryness in a water-bath at 80°C. The solvent extracts were concentrated and 50µl of crude extract was tested for antimicrobial activity by employing agar well–diffusion method (19) against test organisms like *Bacillus subtilis* (ATCC 6633), *Streptococcus mutans* (MTCC 497), *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (ATCC 10231).

***Culture Conditions for the Optimum Production of Bioactive Metabolites*:** Antimicrobial metabolite production of the strain was optimized by using different parameters such as pH,

temperature, culture media, carbon and nitrogen sources and minerals.

***pH and Incubation Temperature on Biomass and Bioactive Metabolite Production*:** To determine the influence of initial pH on growth and bioactive metabolite production, *Streptomyces cheonanensis* VUK-A was cultured in the medium with different initial pH, ranging from 4-10 and at different starting temperatures, from 20 - 60°C. The biomass and bioactive metabolite production were estimated to determine optimal pH and temperature conditions which were used in this study (20, 21).

***Culture Media on Biomass and Production of Bioactive Metabolite*:** In order to determine ideal conditions for the maximum production of antimicrobial metabolite from the *Streptomyces cheonanensis* VUK-A, the strain was cultivated in 10 different media such as asparagine-glucose broth, tyrosine broth (ISP-7), starch inorganic salts broth (ISP-4), glycerol-asparagine broth (ISP-5), yeast-starch broth, malt extract broth, tryptone yeast extract broth (ISP-1), czapek-dox broth, maltose-tryptone broth and soya-bean meal broth. The biomass accumulation and bioactive metabolite production in each medium was evaluated. The medium in which the strain exhibits optimum levels of bio-active metabolite production was used for subsequent study.

***Carbon and Nitrogen Sources on Biomass and Bioactive Metabolite Production*:** To determine the effect of carbon sources on biomass and bioactive metabolite production of the strain, different carbon sources like maltose, lactose, fructose, sucrose, dextrose, starch, mannitol, arabinose, xylose, glycerol and inositol (each at a concentration of 1%) were added separately to the optimized medium. Furthermore, the effect of varying concentrations of the best carbon source (0.5 - 5%) on bioactive metabolite production was also determined. Similarly, influence of various nitrogen sources on bioactive metabolite production was evaluated by supplementing different nitrogen sources like sodium nitrate, ammonium sulfate, ammonium oxalate, peptone, yeast-extract, tryptone, casein, tyrosine, phenyl alanine, glycine and glutamine (each at a concentration of 0.5%) to the optimized medium containing an optimum amount of the superior carbon source as determined above (22). Furthermore, the impact of varying concentrations of optimized nitrogen source (0.1 - 2%) was studied to standardize the maximum antimicrobial metabolite production.

***Minerals on Biomass and Bioactive Metabolite Production*:** Impact of minerals on the production of biomass and bioactive metabolites was studied by supplementing different minerals like K_2HPO_4, $MgSO_4$, $FeSO_4$, K_2HPO_4 and $ZnSO_4$ each at a concentration of 0.05% (w/v) to the optimized medium (23).

***Antimicrobial Activity Against Test Organisms*:** The antimicrobial metabolites of the strain produced under optimized conditions were tested against various strains of bacteria viz., *Streptococcus mutans* (MTCC 497), *Lactobacillus casei* (MTCC 1423), *Lactobacillus acidophilus* (MTCC 495), *Enterococcus faecalis* (MTCC 439), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (MTCC 7299), *Shigella flexneri* (MTCC 1457) and *Xanthomonas campestris* (MTCC 2286) and fungi such as *Candida albicans* (ATCC 10231), *Aspergillus niger*, *Aspergillus flavus, Fusarium oxysporum* (MTCC 3075) and *Penicillum citrinum* by agar-diffusion assay (19).

***Statistical Analysis*:** Data obtained on the bioactive metabolite production under different

microbial culture conditions were statistically analyzed and expressed as mean ± standard error with one-way analysis of variance (ANOVA).

Results

***Incubation Period on Biomass and Bioactive Metabolite Production*:** The growth pattern of *Streptomyces cheonanensis* VUK-A was studied on starch casein broth. The stationary phase of *Streptomyces cheonanensis* VUK-A extended from 72 h to 120 h of incubation (Fig.1). The secondary metabolites obtained from four day old culture exhibited high antimicrobial activity against the test microorganisms, which is in agreement with the earlier reports (24, 25, 26, 27, 28).

***pH and Incubation Temperature on Biomass and Bioactive Metabolite Production*:** The maximum growth and antimicrobial activity of the strain was obtained at pH 7 (Fig. 2) suggesting its inclusion in the neutrophilic actinomycetes group. Bhattacharya *et al.* (29) reported that 7 is the optimum pH for antibiotic production by *Streptomyces hygroscopicus* D1.5. Atta *et al.* (30) stated that the optimum initial pH value capable of promoting biosynthesis of antimicrobial agents by *Streptomyces torulosus* KH-4 was found to be 7. Similarly, bioactive metabolites obtained from the isolate *Streptomyces* sp. VITSVK 9 at pH 7 exhibited good antimicrobial activity (21). There was an increase in the growth of the cell as well as the production of bioactivie metabolic production with the increase of the incubation temperature from 20°C -30°C (Fig 3). However, further increase in temperature (above 30°C) resulted in the decreased growth rate and decline in the production of bioactive metabolite (Fig.3). In terms of its optimum temperature for growth, the organism appeared to be mesophilic in nature. This is in agreement with earlier reports for several of the *Streptomyces* species (31, 32, 33, 34, 35).

***Culture Media on Biomass and Bioactive Metabolite Production*:** Biomass and bioactive metabolite production of the strain was studied in different culture media (Fig. 4). Among the media tested, asparagine-glucose broth produced higher levels of bioactive metabolites followed by tryptone broth (ISP 1) and tyrosine broth (ISP 7). Similarly production of biomas was higher in czapek–dox broth followed by soya-bean meal

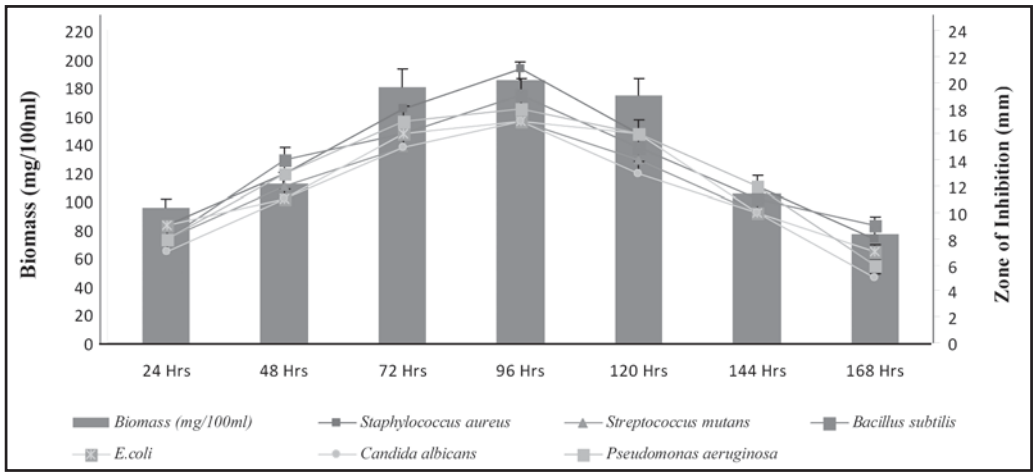

Fig 1. Growth pattern of *Streptomyces cheonanensis* VUK-A. Data are statistically analyzed and found to be significant at 5%.

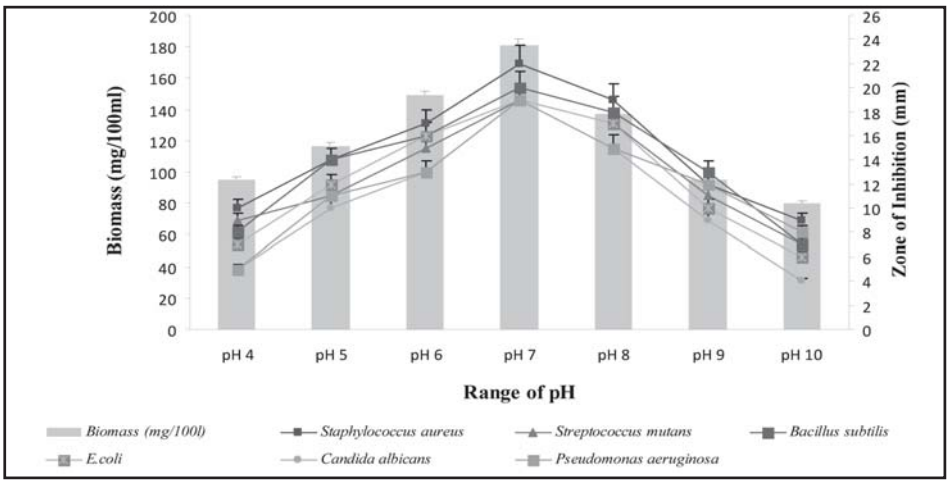

Fig. 2. Effect of pH on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK –A. Data are statistically analyzed and found to be significant at 5%.

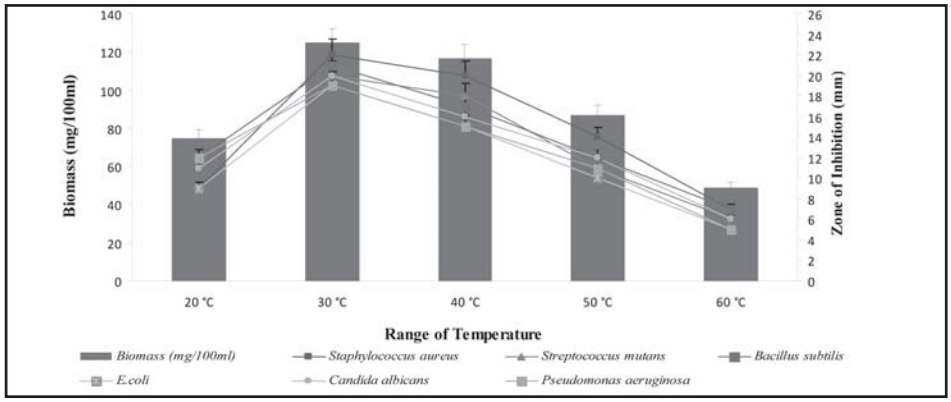

Fig. 3. Effect of temperature on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK-A. Data are statistically analyzed and found to be significant at 5%.

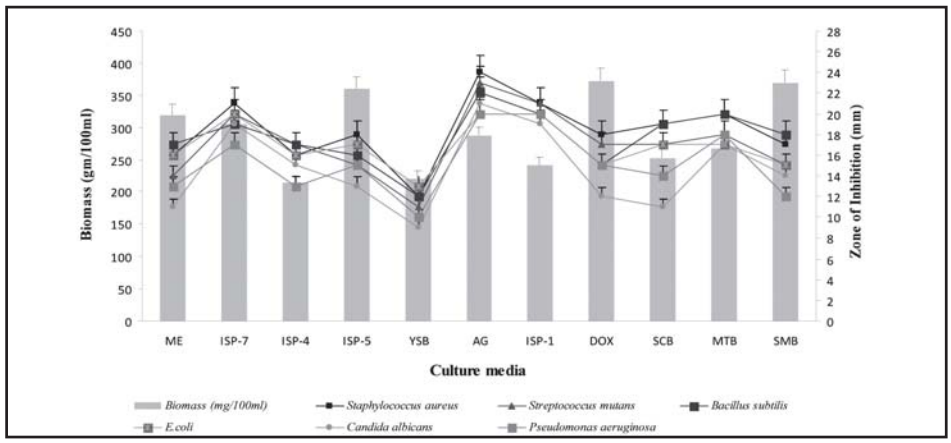

Fig. 4. Effect of different culture media on cell growth and bioactive metabolite production by *Streptomy ces cheonanensis* VUK-10. Data are statistically analyzed and found to be significant at 5%.

broth and asparagine-glycerol broth (ISP 5). There is a significant increase in the bioactive metabolite production cultured in asparagine-glucose broth. Saha *et al.* (7) reported that czapek- dox broth favored high rates of antibiotic production by *Streptomyces* sp. MNK-7 isolated from soil samples from Bangladesh. Kavitha and Vijayalakshmi (28) showed that maltose-tryptone broth favored maximum production of bioactive metabolite production by *Nocardia levis* MK-VL-113.

Carbon and Nitrogen Sources on Biomass and Bioactive Metabolite Production: The details of the effect of carbon and nitrogen sources on production of biomass and bioactive metabolites by *Streptomyces cheonanensis* VUK-A were showed in figs. 5 and 6. Significant production of bioactive metabolite was obtained in lactose amended media followed by fructose and sucrose. Similarly, the production of biomass was high with fructose followed by sucrose and lactose. These results are comparable with Streptomyces hygroscopicus strains AK-111-81, CH-7, which utilized lactose as carbon source for antibiotic production (36, 37). As lactose emerged as the most preferred carbon source for bioactive metabolite production by the strain, varying concentrations of lactose (0.5-5%) was tested to determine its optimal concentration. As shown in Fig 7, lactose at levels of 2% and 1% showed optimal yields of biomass and bioactive metabolites, respectively. A few reports suggested that maximum growth and bioactive metabolite production occurred with glucose (1%) as sole carbon source (32, 38, 39). In order to develop effective composition of growth medium, the roles of different nitrogen sources were evaluated for their influence on growth and antimicrobial agent production by the strain. Of all examined nitrogen sources, bacteriological peptone was found to be the best nitrogen source for growth as well as bioactive metabolite production. It should be noted that tryptone and tyrosine, as nitrogen sources, also favored good growth but the antimicrobial compound yield was less in comparison to peptone. Inorganic nitrogen sources like ammonium sulfate, ammonium oxalate, sodium nitrate and some organic nitrogen sources like yeast extract and casein did not show significant effect on antibiotic production by the strain. Peptone enhanced the biomass and

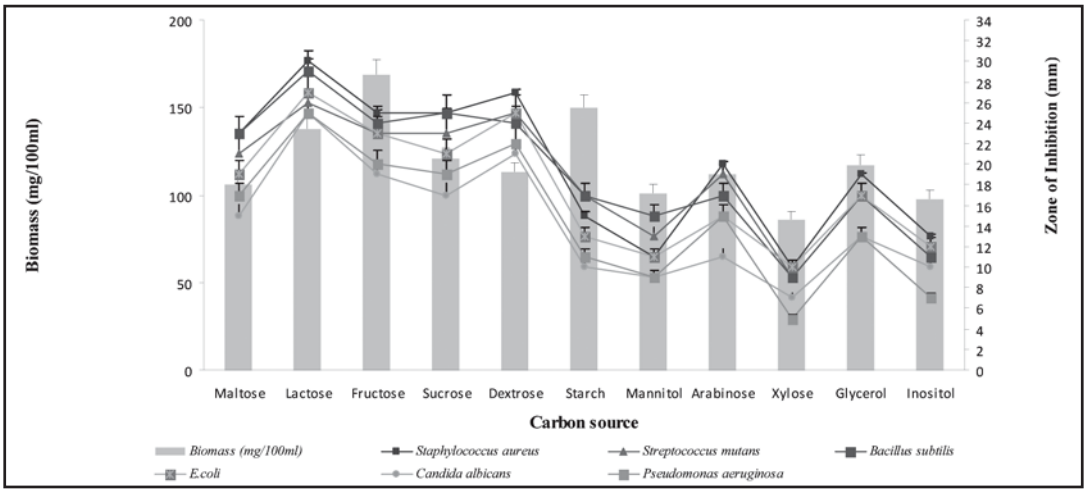

Fig. 5. Effect of different carbon sources on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK –A. Data are statistically analyzed and found to be significant at 5%

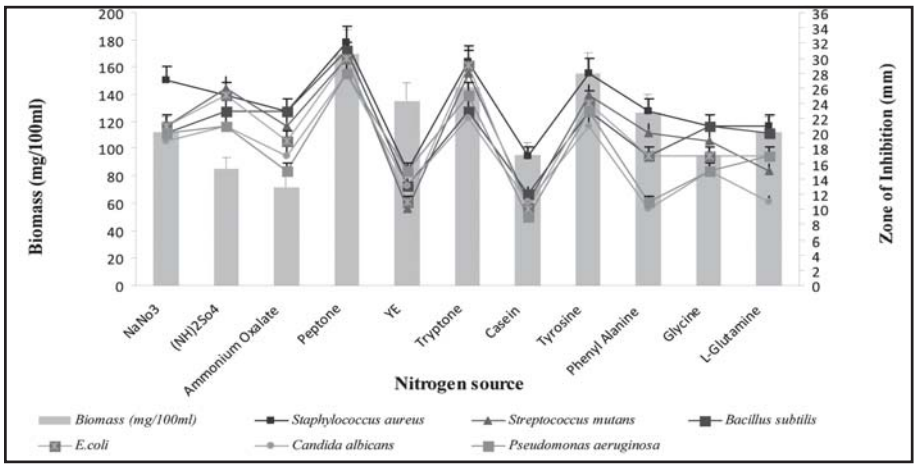

Fig. 6. Effect of different nitrogen sources on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK –A. Data are statistically analyzed and found to be significant with 5%.

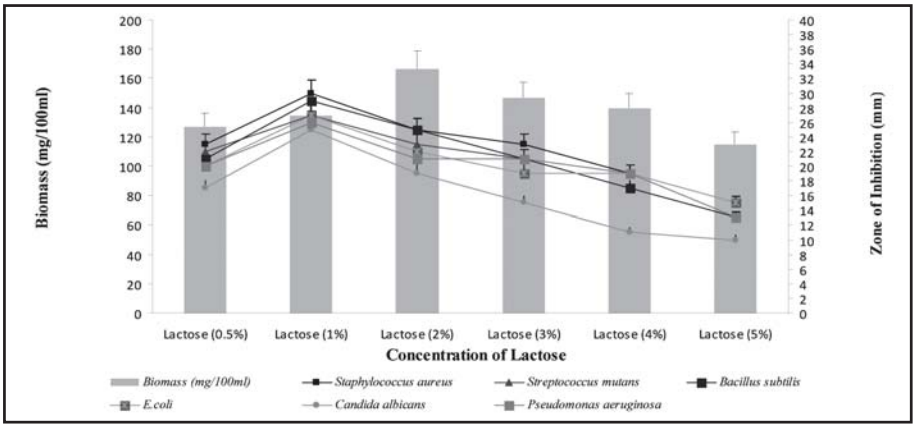

Fig. 7. Effect of different concentrations of Lactose on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK –A. Data are statistically analyzed and found to be significant at 5%.

bioactive metabolite production by *Streptomyces cheonanensis* VUK-A which is in conformity with earlier reports (21, 40, 41, 32). Effect of different concentrations of peptone on the production of bioactive metabolite production has been shown in Fig.8. It should be noted that peptone at a concentration of 1% and 0.25% exhibited optimal production of biomass and bioactive metabolite, respectively. Hassan *et al.* (42) recorded that $NaNO_3$ followed by peptone and alanine at a concentration of 0.25% increased antibiotic production by Streptomyces violates. Ismet *et al.* (43) also reported that corn steep powder at a concentration of 0.25% enhanced cell growth and bioactive metabolite production by *Micromonospora* sp. M 39.

Minerals on Biomass and Bioactive Metabolite Production: Effect of minerals on biomass and bioactive metabolite production by the strain has been shown in Fig 9. Among the minerals tested, K_2HPO_4 supported biomass and bioactive metabolite production whereas lower antimicrobial metabolite production was obtained with $FeSO_4$ and $ZnSO_4$. Ripa et al. (44) also reported that K_2HPO_4 showed positive influence on antibiotic production by the strain. Narayana

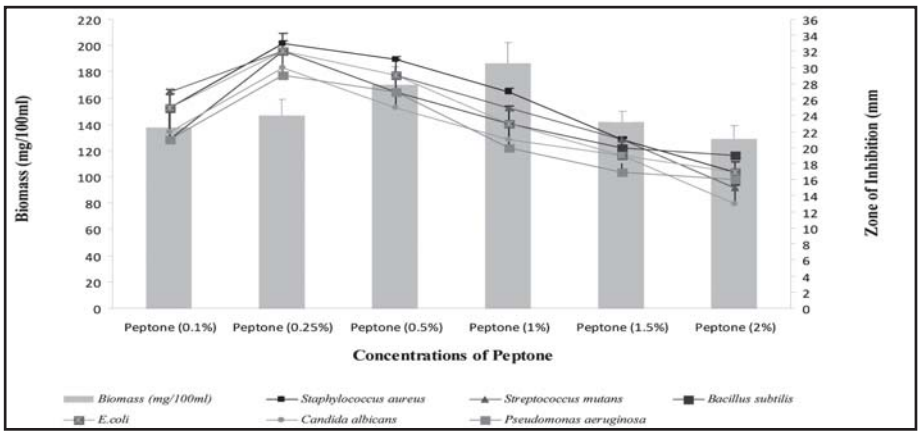

Fig. 8. Effect of different concentrations of peptone on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK –A. Data are statistically analyzed and found to be significant at 5%.

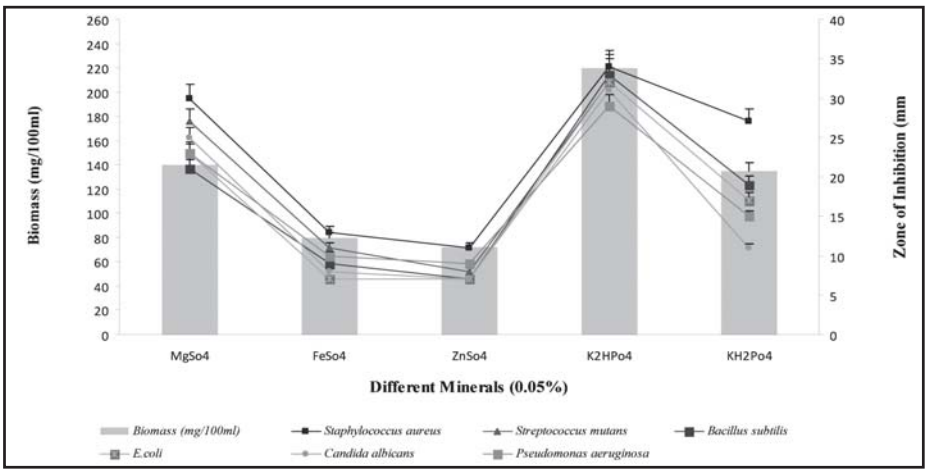

Fig. 9. Effect of different minerals on biomass and bioactive metabolite production by *Streptomyces cheonanensis* VUK –10. Data are statistically analyzed and found to be significant at 5%.

and Vijayalakshmi, (18) also noted that K_2HPO_4 slightly enhanced the production of cell mass and bioactive metabolites of *Streptomyces albidoflavus.*

Discussion

A broad spectrum bioactive metabolite producing actinomycetes isolate VUK-A from Coringa mangrove ecosystem, Andhra Pradesh, India has been identified as *Streptomyces cheonanensis* VUK-A. Ninety six hours of incubation at 30ºC and pH 7 was found to be optimum for bioactive metabolite production. It has been reported that the environmental factors like temperature, pH and incubation time have profound influence on antibiotic production (45). It was found that the bioactive metabolite production by *Streptomyces cheonanensis* VUK-A was positively influenced by the carbohydrates, nitrogen sources and minerals. The results suggest that antibiotic production was higher in medium having 1% lactose as carbon source. The

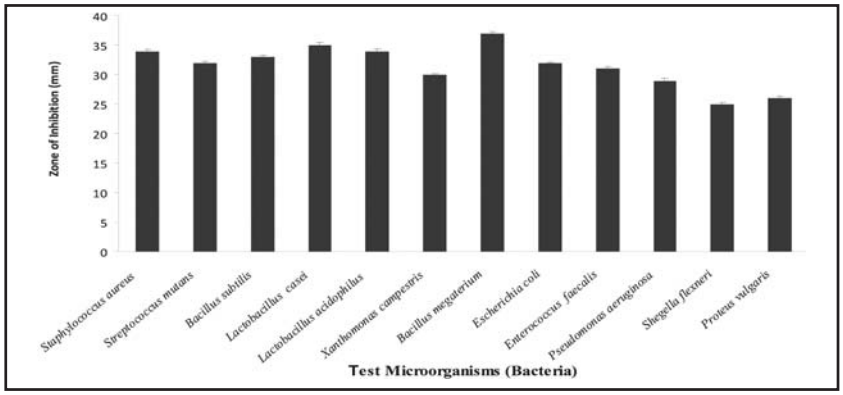

Fig. 10. Figure representing the anti-microbial metabolite produced by *Streptomyces cheonanensis* VUK-A (presented in terms of zone of inhibition) under optimized conditions tested against various bacteria. Data are statistically analyzed and found to be significant at 5%.

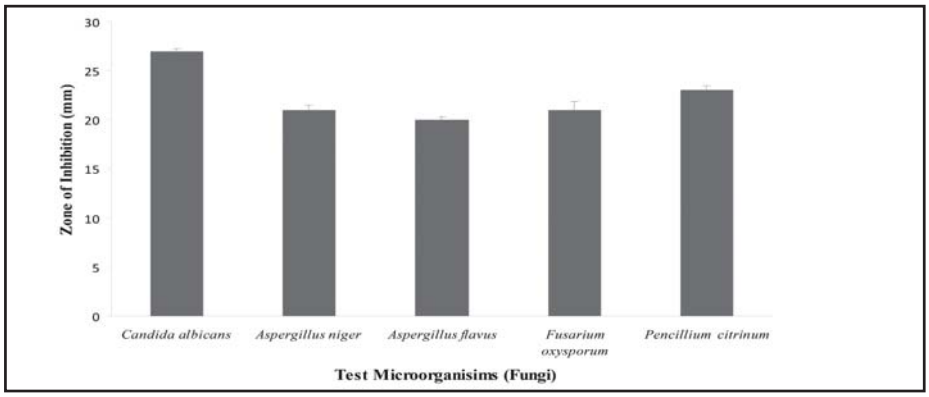

Fig. 11. Figure representing the anti-microbial metabolite produced by *Streptomyces cheonanensis* VUK-A (presented in zone of inhibition) under optimized conditions tested against various fungi. Data are statistically analyzed and found to be significant at 5%.

bioactive metabolite production got reduced with increase or decrease of lactose concentration. Among the nitrogen sources tested maximum antibiotic production was obtained with 0.25% peptone. In comparison with inorganic nitrogen sources, organic nitrogen sources gave relatively higher antimicrobial agent production by *Streptomyces cheonanensis* VUK-A. This is in conformity with the findings of Vahidi *et al.,* (46) which showed that the organic nitrogen sources are better for the production of antifungal agents. Among minerals, K_2HPO_4 favored slight enhancement of antibiotic production. The results indicated that antibiotic production is greatly influenced by medium constituents. Vilches *et al.*, (47) stated that the nature of carbon and nitrogen sources strongly effect antibiotic production in different organisms. In the present study, the metabolites produced by *Streptomyces cheonanensis* VUK-A grown under optimized conditions exhibited good antimicrobial activity against gram positive, gram negative bacteria and fungi (Figs. 10, 11 and 12). Hence, further studies regarding the purification, characterization and identification of bioactive compounds produced by *Streptomyces cheonanensis* VUK-A are in progress.

Acknowledgements:

The authors are thankful to CSIR, India for providing the financial support.

References

1. Xinxuan, W., Lili, H., Zhensheng, K., Heinrich, B. and Xiaoning, G. (2010). Optimization of the Fermentation Process of Actinomycete Strain Hhs.015T . Journal of Biomedicine and Biotechnology, Article ID 141876, 10 pages.

2. Berdy, J. (2005). Bioactive microbial metabolites. The Journal of Antibiotics , 58(1): 1-26.

3. Wise, R. (2008). The worldwide threat of antimicrobial resistance. Current Science, 95: 181-187.

4. Jain, P. and Pundir, R. K. (2011). Effect of fermentation medium, pH and temperature variations on antibacterial soil fungal metabolite production. Journal of Agricultural Technology, 7: 247-269.

5. Thumar, J.T., Dhulia K. and Singh, S.P. (2010). Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. World Journal of Microbiology and Biotechnology, 26: 2081-2087.

6. Mustafa, O. (2011). Effects of some environmental conditions on biomass and antimicrobial metabolite production by *Streptomyces* Sp., KGG32. International Journal of Agriculture and Biology, 3: 317-324.

7. Saha, M.R., Rifa, F.A., Islam, M.Z. and Khondkar, P.(2010). Optimization of conditions and *in vitro* antibacterial activity of secondary metabolite isolated from *Streptomyces* sp. MNK 7. Journal of Applied Science Research, 6:453-459.

8. Lam, K.S., Mattei, J. and Forenza, J (1989). Carbon catabolite regulation of rebeccamycin production in *Saccharothrix aerocolonigenes*. Journal of Industrial Microbiology, 4: 105-108.

9. Gao, H., Liu, M., Liu, J., Dai, H., Zhou, X., Liu, X., Zhuo, Y., Zhang, W. and Zhang, L. (2009). Medium optimization for the production of avermectin B1a by *Streptomyces avermitilis* 14-12A using response surface methodology. Bioresource Technology, 100: 4012-4016.

10. Jia, B., Jin. Z. H. and Mei, L.H. (2008). Medium Optimization based on Statistical Methodologies for Pristinamycins Production by *Streptomyces pristinaespiralis*. Applied Biochemistry and Biotechnology, 144: 133-143.

11. Lin, J., Bai, L., Deng. Z. and Zhong. J. J. (2010). Effect of ammonium in medium on Ansamitocin P-3 Production by *Actinosynnema pretiosum.* Biotechnology and Bioprocess Engineering, 15: 119-125.

12. Ruiz, B., Chávez, A., Forero, A., García-Huante, Y., Romero.A., Sánchez, M., Rocha, D., Sánchez, B., Rodríguez-Sanoja, R., Sánchez, S. and Langley, E (2010). Production of microbial secondary metabolites: Regulation by the carbon source. Critical Reviews in Microbiology, 36: 146-167.

13. Sánchez, S., Chavez, A., Forero, A., Garcia-Huante, Y., Romero, A., Sánchez ,M., Rocha., D, Sánchez.,B Avalos., M Guzman-Trampe, S., Rodriguez-Sanoja,R., Langley., E and Ruiz.,B (2010). Carbon source regulation of antibiotic production. The Journal of Antibiotics, 63: 442-459.

14. Atlas, R. M. and Bartha. R. (1998). Fundamentals and applications in Microbial Ecology. (4th edition) Benjamin/ Cummings, Science Publishing, New York, pp. 174-217.

15. Argoudelis, A., Baczyneky, D. L., Duo, M.T., Laborde, A. L., Sebek, O. K., Truesdel. S. K. and Shilliday. F.B. (1987). *In-vitro* studies of arginomycin and its biodegradation products. The Journal of Antibiotics, 40: 750-760.

16. Wellington, E. M. H. and Cross, T. "Progress in industrial microbiology?" (1983). In: Bushell, M.E editor. Taxonomy of antibiotic producing actinomycetes and new approaches to their selective isolation. Elsevier, Amsterdam. pp.36.

17. Williams, S. T. and Cross, T. (1971). Isolation, Purification, Cultivation and Preservation of Actinomycetes. Methods in Microbiology, 4: 295-334.

18. Narayana, K. J. P. and Vijayalakshmi, M. (2008). Optimization of antimicrobial metabolites production by *Streptomyces albidoflavus*. Research Journal of Pharmacology, 2: 4-7.

19. Cappuccino, J. G. and Sherman, N. (2004). Microbiology, Laboratory manual. Person education, INC, New Delhi. pp. 282-283.

20. Srinivasan, M. C., Laxman. R. S. and Deshpande. M.V. (1991). Physiology and nutritional aspects of actinomycetes: An overview. World Journal of Microbiology and Biotechnology, 7: 171-184.

21. Saurav, K. and Kannabiran. K. (2010). Diversity and Optimization of process parameters for the growth of *Streptomyces* VITSVK 9 sp. Isolation from Bay of Bengal, India. Journal of Natural and Environmental Sciences , 1: 56-65.

22. Elliah, P., Srinivasulu, B. and Adinarayana, K. (2000). Optimization studies on Neomycin production by a mutant strain of Streptomyces marinensis in solid state fermentation process. Biochemistry, 39: 529-534.

23. Farid, M.A., El-Enshasy H. E., Ei-Diwany, A. I. and El-sayed, E.A. (2000). Optimization of the cultivation medium for Natamycin production by *Streptomyces netalensis*. Journal of Basic Microbiology, 40:157-166.

24. Labrode, A. L., Cialdella, J. I., Fox J.A. and Marshall, V.P.(1985). Directed biosynthesis of paulomycine-A the effect of L-methionine, L-Threonine and alpha ketobutyric acid. Journal of Antibiotics, 38:1426-1428.

25. Tanaka, Y., Graefe, U., Yazawa. K. and Mikami, Y. (1998). Production of nocardicyclins by clinical isolates of *Nocardia pseudobrasiliensis* and *in vivo* antitumor activity of the antibiotic. ChemInform, 29: 589-591.

26. Sujatha, P., Bapiraju. K.V. and Ramana, T. (2005). Studies on a new marine Streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus*. Microbiological Research, 160: 119-126.

27. Sun, C.H., Wang, Y., Zhov, J. Q., Jin, W. Z., Yov, H. G., Zhao, L.X., Si. S.Y. and Li.X (2007). Chemomicin A: A new Angucyclinone antibiotic produced by *Nocardia mediterranei* sub sp. kanglensis 1747-64. Journal of Antibiotics, 60:211-215.

28. Kavitha, A. and Vijayalakshmi, M. (2009). Cultural parameters affecting the production of bioactive metabolites by

Nocardia Levis MK-VL-113. Journal of Applied Sciences Research, 5: 2138-2147.

29. Battacharyya, B.K., Pal, S.C. and Sen, S.K.(1998). Antibiotic production by *Streptomyces hygroscopicus* D1.5: Cultural effect. Revista de Microbiologia, 29: 49-52.

30. Atta, H. M., Bayoumi, R., El-Sehrawi, M., Aboshady, A. and Al-Huminay, A. (2010). Biotechnological application for producing some antimicrobial agents by actinomycetes isolates from Al-Khurmah Governorate. European Journal of Applied Sciences, 2:98-107.

31. Elliah, P., Srinivasulu, B. and Adinarayana, K. (2004). Optimization studies on Neomycin production by a mutant strain of *Streptomyces marinensis* in soild state fermentation process. Biochemistry, 39: 529-534.

32. Rizk, M., Tahany. A. M. and Hanaa. M. (2007). Factors affecting growth and antifungal activity of some *Streptomyces* species against Candida albicans. International Journal of Food, Agriculture and Environment, 5:446-449.

33. Mustafa, O. (2009). Antifungal and antibacterial compounds from *Streptomyces* strains. African Journal of Biotechnology, 8:3007-3017.

34. Ghosh, U. K. and Prasad, B. (2010). Optimization of carbon, nitrogen sources and temperature for hyper growth of antibiotic producing strain *Streptomyces kanamyceticus* MTCC 324. The Bioscan, 5:157-158.

35. Atta, H.M., Bahobail. A.S. and El-Sehrawi. M.H. (2011). Studies on isolation, classification and phylogenetic characterization of antifungal substance produced by *Streptomyces albidoflavus*-143. New York Science Journal, 4: 40-53.

36. Gesheva, V., Ivanova, V. and Gesheva. R. (2005). Effect of nutrients on the production of AK-111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. Microbiological Research, 160: 243-248.

37. Salvica Ilic., Sandra Konstantinovic., Vlada B Veljkovic., Dragiša S. Savic., Gordana Đ. and Gojgic Cvijovic. (2010). The impact of different carbon and nitrogen sources on antibiotic production by *Streptomyces hygroscopicus* CH-7. Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 2:1337-1342.

38. Wu, J.Y., Jenn-Wen, H., Sin-Der, S. H., Wei-Chenand, L. and Yungehuan, L. (2008). Optimization of cultivation conditions for fungi chromin production from *Streptomyces padanus* PMS-702. Journal Chineseinst. Chem. Engin, 39: 67-73.

39. Singh, L. S., Mazumdar. S. and Bora,T.C. (2009). Optimization of process parameters for growth and bioactive metabolite production by a salt-tolerant and alkaliphilic actinomycete, *Streptomyces tanashiensis* strain A2D. Journal de Mycologie medicale, 19:225-223.

40. Chattopadhyay, D. and Sen, S.K. (1997). Optimization of cultural conditions for antifungal antibiotic accumulated by *Streptomyces rochei* G 164. Hindustan Antibiotics Bulletin, 39: 64-71.

41. Han, W.C., Lee, J. Y., Park, D.H., Lim, C.K. and Hwang, B. K. (2004). Isolation and antifungal and antioomycete activity of

Streptomyces scabiei strain PK-A41, the causal agent of common scab disease. The Plant Pathology Journal, 20:115-126.

42. Hassan, M.A., Moustafa, Y., EL-Naggar and Wafa, Y. (2001). Physiological factors affecting the production of an antimicrobial substance by *Streptomyces violatus* in batch cultures. Egyptian Journal of Biology, 3:1-10.

43. Ismet, A., Vikineswary, S., Paramaswari, S., Wong, W. H., Ward, A., Seki, T., Fiedler, H. P. and Goodfellow, M. (2004). Production and chemical characterization of antifungal metabolites from *Micromonospora* sp.M39 isolated from mangrove rhizosphere soil. World Journal of Microbiology and Biotechnology, 20:523-528.

44. Ripa, F.A., Nikkon, F., Zaman, S. and Khondkar, P. (2009). Optimal conditions for antimicrobial metabolites production from a new *Streptomyces* sp. RUPA-08PR isolated from Bangladeshi soil. Mycobiology, 37:211-4.

45. Iwai, Y., Awaya, J., Kesado, T., Yamada, H., Omura, S. and Hata, T. (1973). Selective fermentation of cerulenin by Cephalosporin Caeruleus. Journal of Fermentation Technology, 51:575-578.

46. Vahidi, H., Kobarfard, F. and Namjoyan, F. (2004). Effect of cultivation conditions on growth and antifungal activity of *Mycenal leptocephala.* African Journal of Biotechnology, 3: 606-609.

47. Vilches, C., Mendez, C., Hardission, C. and Salas, J.A. (1990). Biosynthesis of oleandromycin by *Streptomyces antibioticus:* Influence of nutritional conditions and development of resistance. Journal of General Microbiology, 136:1447-1454.

48. Usha Kiranmayi, M., Sudhakar, P., Krishna, N., Yellamanda, B. and Vijayalakshmi, M. (2011). Taxonomic Characterization of Potential Bioactive metabolite producing Actinomycetes from Mangrove Sediments of Coringa. Journal of Pharmacy Research 4:12 (In Press).