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PHYSIOLOGICAL FACTORS INFLUENCING THE PRODUCTION OF ANTIBACTERIAL SUBSTANCE BY FRESH WATER ACTINOBACTERIA

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ABSTRACT

The influence of the physiological factors on the production of antibacterial metabolite by two actinomycetes strains, LAM1 and LAM2 isolated from fresh water systems of Karimnagar were studied.LAM1 showed highest antibacterial activity after seven days of incubation while LAM2 after ten days of incubation at 30°C. Optimization of antibacterial metabolites in batch cultures has been carried out. All the strains were able to grow in all the tested carbon sources. However maximum zone of inhibition was observed when cultures supplemented with glycerol as a carbon source followed by glucose and starch. Cultures containing fructose and maltose did not show any zone of inhibition. The cultures supplemented with sodium nitrate and potassium nitrate have shown highest zone of inhibition followed by peptone. The presence of iron and manganese could play an important role in the promotion of antibiotic production.

KEY WORDS Fresh water, Incubation, Zone of inhibition, Antibiotic, Actinomycetes.

INTRODUCTION

Actinomycetes are a group of morphologically diverse, Gram positive bacteria having in common DNA with high GC content in the range of 63-78% [1, 2, 3, 4]. They are widely distributed in a variety of natural and man made environments, constituting a significant components of the microbial population in soil and water [5, 6]. Diversity and bioprospecting studies on actinomycetes are mainly pertaining to terrestrial and marine ecosystems and less importantly from fresh water systems [7]. There is an increasing realization of the potential for fresh water systems as sources of actinomycetes that produce useful bioactive compounds, Cross [8] reported fresh water fresh water habitats as a promising source of bioactive actinomycetes. Actinomycetes of fresh water origin produce novel and useful bioactive metabolites [9]. The risk of antibiotic resistant pathogenic strains dictates an increasing need for the survey of unexplored and underexplored habitats for novel antibiotic producing actinobacterial strains [10, 11]. The focus is increasing towards fresh water systems for novel bioactive strains especially actinobacteria [12].

Actinomycetes are the source of important and useful antibacterial metabolites that are used to control bacterial diseases in human, animals and plants. [13, 14, 15]. More than half of commercial antibiotics were derived from actinomycetes [16].The ability of actinobacterial cultures to form antibacterial compounds is not a fixed property but can be enhanced or minimized under different physiological condition [17, 18]. Hence the composition of the medium and the metabolic capacity of the culturing microorganism greatly affect the production of secondary metabolites [18]. Changes in the nature and type of carbon, nitrogen and metal ions sources have been reported to affect the antibacterial metabolites synthesis in actinomycetes [19]. In addition to media components, temperature, pH and incubation period also influence the production of secondary metabolites [20, 21].

This work describes the production of antibacterial metabolite produced by two actinomycete isolates (LAM1 and LAM2) isolated from fresh water systems of Karimnagar, Andhra Pradesh. Improvement of antibacterial metabolite production was achieved by optimization of the cultural conditions and by formulating a defined medium for the biosynthesis of antibacterial metabolites by actinomycetes.

MATERIALS AND METHODS

The actinomycetes cultures LAM1 and LAM2 used in this study were isolated from fresh water systems of Karimnagar, Andhra Pradesh. A total of 144 water and sediment samples were collected from three freshwater systems of Karimnagar, Andhra Pradesh, India viz: Lower Manair Dam, Manakondur Pond and Kothapally Pond regularly every month during July 2006 to June 2008. Water samples were collected in a sterile one liter conical flask and brought to the laboratory by closing with sterile cotton plug. Sediment samples were collected in a sterile petri dish by using sterile spatula.

ctinomycetes from these collected samples were isolated by Double Agar Layer (DAL) method on actinomycetes isolation agar containing cycloheximide (50µg/ml) to minimize fungal contamination [22]. All plates were incubated at 30°C for 1-2 weeks. The actinomycetes colonies that appear on petri plates were counted from 5^{th} day onwards up to 14^{th} day.

A total of 24 different actinomycetes were collected from these samples viz: eleven from Lower Manair Dam (LAM1 to LAM11), seven from Manakondur Pond (MAM1 to MAM7) and six from Kothapally Pond (KAM1 to KAM6). All isolates were sub cultured and maintained in agar slants. The isolated actinomycetes strains were tested for their antibacterial activity against ten test bacteria namely *Bacillus subtils* (MTCC 431), *Proteus vulgaris* (MTCC 426), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 424), *Enterobacter aerogenes* (MTCC 111), *Salmonella typhi* (MTCC 733), *Escherichia coli (*MTCC 40), *Sarcina lutea* (MTCC 1541), *Shigella flexneri* (MTCC 1457) *and Klebsiella pneumonia* (MTCC 7162). Among, eight isolates showed good antagonistic activity against test bacteria. Two of these eight isolates (LAM1 and LAM2) were showed very potent antagonistic activities which were selected and identified. The characterization of LAM1 and LAM2 was done by following the guide lines adopted by the international *Streptomyces* Project [23]. Colors were assessed on the scale adopted by Kornerup and Wanscher [24].

They were maintained on starch nitrate agar slants and kept in a refrigerator at 4°C until further use. LAM1 is proved to be a producer of an antibacterial metabolite with a molecular weight of 323.58 and LAM2 is proved to produce an antibacterial metabolite with a molecular weight of 824.32.

Culture of LAM1 and LAM2 for antibacterial metabolite production:

For studying antibacterial metabolite production by actinomycetes strains, starch nitrate medium was used as basal medium. It was composed of (g/l) : Starch, 10.0, NaNO3, 2.5, K2HPO4, 1.0, KH2PO4, 1.0, MgSO4.7H2O, 0.5, KCl,0.5, trace salt solution 1.0 ml(CuSO4.5H2O,0.64 g/l , FeSO4.7H2O,0.11 g/l, MnCl.4H2O, 0.79 g/l and ZnSO4.7H2O, 0.15 g/l) , Distilled water, 1.0 liter. Medium pH was adjusted to 7.0 before autoclaving using 0.1 N NaOH or 0.1 N HCl solutions. 100 ml of this medium was dispensed in 250 ml conical flask and sterilized at 121°C for 15 minutes. Each flask was inoculated with spore suspension taken from one week old slant cultures. The flasks were incubated in rotary shaker incubator at 30°C for 7-10 days. After incubation period, the cells were separated from the culture filtrate by centrifugation at 5000 r.p.m for 15 minutes, washed twice with distilled water and then dried at 70°C, dry weight is measured and recorded. The antibacterial metabolites were extracted from culture filtrate by solvent extraction method and antibiotic assay was carried out using test bacteria. The test bacteria used for testing of actinomycetes isolates for antimicrobial activity include: *Bacillus subtils* (MTCC 431) and *Escherichia coli (*MTCC 40).

Influence of some cultivation factors on the production of antibiotic by active isolates:

In order to test the influence of cultivation factors on the growth and production of antibacterial metabolites by LAM1 and LAM2, starch nitrate broth was used as basal medium and the process was carried out.

Temperature, pH, and incubation period:

The effect of cultural conditions like different temperatures(15°C, 20°C, 25°C, 30°C, 35°C, 40°C, and 45°C) initial pH (3,4,5,6,7,8,9,10,11) and incubation period (2-10 days) on growth and antibacterial metabolites production was studied separately by inoculating the spore suspension of the strain into the fermentation medium and then incubated in a rotary shaker.

Carbon and nitrogen sources:

In order to investigate the influence of carbon and nitrogen sources on the antibacterial activity of active isolates, basal medium was used. Various carbon and nitrogen sources were used to replace the carbon and nitrogen sources in basal medium, while all other components were kept constant. The carbon and nitrogen sources were sterilized separately and added just prior to inoculation. Glucose, fructose, maltose, sucrose, lactose, starch and glycerol were added separately as carbon sources into the basal medium at 1% concentration. Different nitrogen sources such as KNO3, NANO3, tryptone, peptone, caseine, ammonium nitrate and ammonium sulphate were provided separately in to the basal medium at 1% concentrations. The respective biomass and antibacterial metabolite production was recorded.

Metal ion sources:

The influence of metal ions was studied by replacing the metal ions by one metal ion at different concentrations. The metal ion sources such as CuSO4.5H20, FeSO4.7H20, MnCl2. 4H20 and ZnSO4.7H20 were added separately as metal ions sources in to the basal medium. The antimicrobial activity was evaluated by measurement of inhibition zone of the target organism after 24 hours of incubation time at 30°C.

RESULTS

The isolated actinomycetes were screened with regard to their potential to generate bioactive compounds. The most potent producer strains LAM1,

Table 1. Antibacterial activity of LAM1 and LAM2 strains.

Isolates	Test Organisms (inhibition zone in mm)									
	B.s.	S.a.	S.l	E.c.	K.p.	P.v.	P.a.	S.t.	S.f	E.a
LAM 1	20	18	17	12	13	15	15	17	9	14
LAM 2	15	12	13	12	11	7	13	5	8	7

B.s - *Bacillus subtilis,* S.a- *Staphylococcus aureus* S.l- *Sarcina lutea* E.c- *Escherichia coli* , K.p- *Klebsiella pneumonia,* P.v-*Proteus vulgaris,* P.a.-*Pseudomonas aeruginosa,* S.t- *Salmonella typhi,* S.f-*Shigella flexneri,* E.a- *Enterobacter aerogenes*

Table 2: Morphological and biochemical characteristics of LAM1 and LAM2 strains

Characteristic	**LAM1**	**LAM2**
Morphological		
Aerial mycelium color	White	Yellowish white
Substrate mycelium color	White	Yellow
Colony diameter(mm)	3	4
Colony margin	Filamentous	Filamentous
Colony elevation	Flat	Convex
Spore chain	Spiral	Hook like
Spore surface	Warty	Smooth
Biochemical		
Indole production	-	-
Methyl red	-	-
Voges proskaur	-	-
Citrate utilization	+	+
H2S production	-	-
Nitrate reduction	-	-
Melanin production	+	+
Starch hydrolysis	+	+
Gelatin hydrolysis	+	+
Lipid hydrolysis	-	-
Casein hydrolysis	+	+
Carbon source utilization		
Starch	+	+
Dextrose	+	+
Fructose	+	+
Maltose	+	+
Nitrogen source Utilization		
D-Alanine	+	+
L-Arginine	+	+
L-Phenylalanine	+	+
L-Tyrosine	+	+

Table 3: Influence of different carbon source on the growth of actinomycetes and production of antibiotics

Actinomycete strain	Carbon source																			
	GLUCOSE			FRUCTOSE			MALTOSE			SUCROSE			LACTOSE			STARCH			GLYCEROL		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
LAM1	2.1	19	15	1.6	-	-	2.0	-	-	1.7	15	13	1.0	12	8	3.7	24	19	3.9	26	20
LAM2	1.4	14	9	0.9	-	-	2.2	-	-	2.5	16	11	1.6	15	12	2.6	16	14	3.4	21	17

A-Dry weight of actinomycetes cells in mg/ml,B-Zone of inhibition in mm against *B.subtilis,C*-Zone of inhibition in mm against *E.coli*

Table 4: Influence of different nitrogen sources on the growth of actinomycetes and production of antibiotics

Actinomycete strains	Metal ion source											
	CuSO4			FeSO4			MnCl2			ZnSO4		
	A	B	C	A	B	C	A	B	C	A	B	C
LAM1	3.3	21	16	3.9	26	18	3.6	23	17	1.8	18	15
LAM2	3.1	18	15	3.3	23	16	3.9	25	19	2.1	20	18

A- Zone of inhibition in mm against *E.coli* Dry weight of actinomycetes cells in mg/ml,Zone of inhibition in mm against *B.subtilis*

Table 5: Influence of metal ions on the growth of actinomycetes and production of antibiotics

Actinomycete strains	Metal ion source											
	CuSO4			FeSO4			MnCl2			ZnSO4		
	A	B	C	A	B	C	A	B	C	A	B	C
LAM1	3.3	21	16	3.9	26	18	3.6	23	17	1.8	18	15
LAM2	3.1	18	15	3.3	23	16	3.9	25	19	2.1	20	18

A- Dry weight of actinomycetes cells in mg/ml,B- Zone of inhibition in mm against *B.subtilis,C*- Zone of inhibition in mm against *E.coli*

LAM2 were selected and identified. The characterization LAM1 and LAM2 was done by following the guide lines adopted by the International *Streptomyces* Project [23]. Two of twenty four actinomycetes cultures LAM1, LAM2 were found to exhibit various degrees of activities against Gram-positive and Gram-negative bacteria (Table 1).

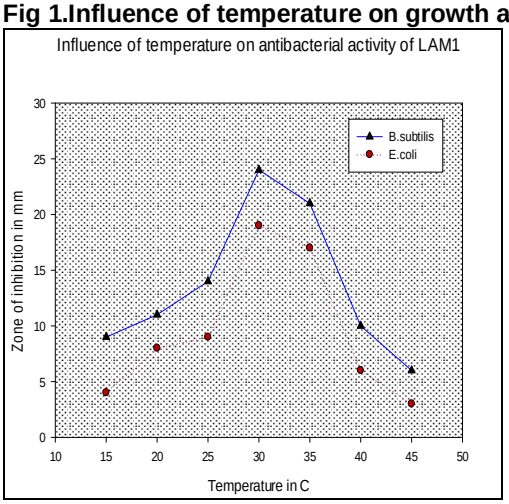
The most potent antagonistic actinomycetes strains were selected and characterized. The cultural characteristics of LAM1 showed that the aerial mycelium is white and substrate mycelium is also white and no diffusible pigments were observed. The cultural characteristics of LAM2 showed that the

aerial mycelium is yellow and substrate mycelium is yellowish white and no diffusible pigments were produced. The physiological and biochemical characteristics of LAM1 and LAM2 were summarized in Table 2 which indicated that the strain LAM1 belongs to Streptomyces group and LAM2 belongs to *Micromonospora*.

Influence of some cultivation factors on the production of antibiotic by active isolates:

Optimizations of antibiotic production on batch cultures of active isolates were carried out by using starch nitrate broth as a basal medium and the suitability of various cultivation factors were evaluated and correlated.

Fig 1.Influence of temperature on growth and production of antibiotic.



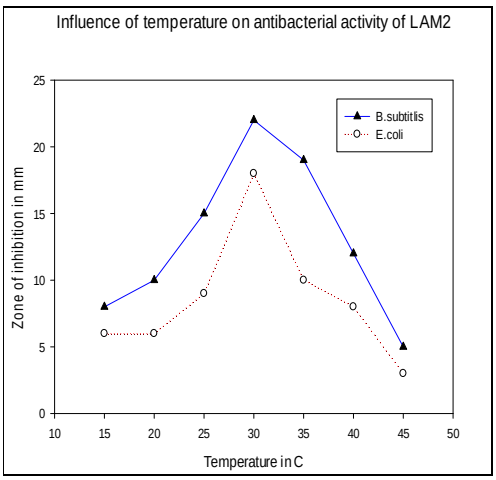
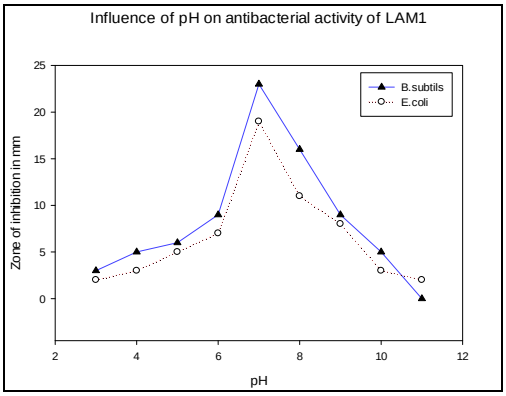


Fig 2: Influence of pH on the growth of actinomycetes and production of antibiotics



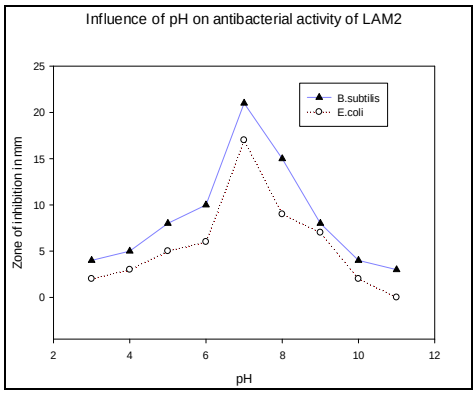
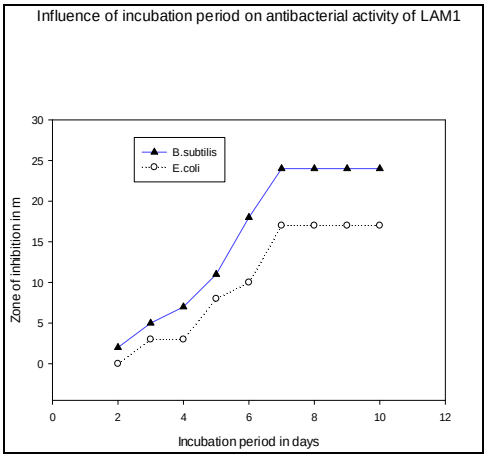
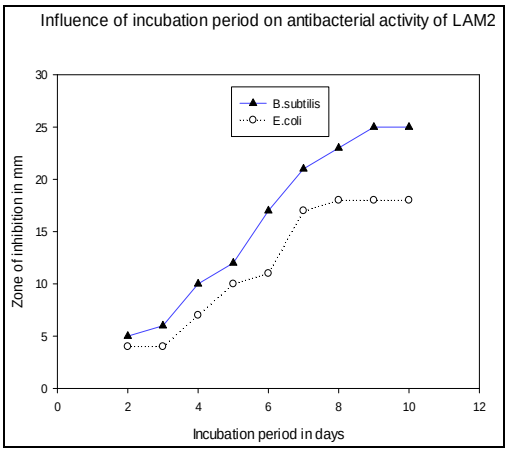


Fig 3: Influence of incubation period on the growth of actinomycetes and production of antibiotics





Temperature:

The effect of temperature on the production of antibacterial metabolites by LAM1 and LAM2 was tested by performing the fermentation at different temperatures and by studying the zone of inhibition. The results are indicated in the fig 1. LAM1 showed maximum antibacterial activity at 30°C with the highest biomass of 3.4 mg/ml. The strain LAM2 also showed maximum antibacterial activity at 30°C with highest biomass of 3.3 mg/ml. The results indicate that the optimum temperature for the growth and production of antibacterial metabolite by LAM1 and LAM2 is 30°C.

pH:

The effect of pH on the production of antibacterial metabolites by LAM1 and LAM2 was tested by performing the extraction of antibacterial metabolites at different pH from fermentation broth and by studying the zone of inhibition. LAM1 and LAM2 showed maximum antagonistic activity and growth at pH of 7 (fig. 2).

Incubation period:

The influence of incubation period on the production of antibacterial metabolites by LAM1 and LAM2 was tested by performing the fermentation for different durations and by studying the zone of inhibition. The results are indicated in the fig 3. Maximum growth and antibacterial activity was shown by LAM1 and LAM2 after seven days and nine days of incubation periods respectively.

Influence of carbon and nitrogen source:

Optimization for the growth and antibacterial metabolite production by LAM1 and LAM2 was carried out in batch cultures. The strain LAM1 and LAM2 were cultured in the basal medium with different carbon sources and their effect on the growth and antagonistic activity was studied. Both the strains were able to grow in all the tested carbon sources (Table 3). However maximum zone of inhibition was observed when cultures supplemented with glycerol as a carbon source followed by glucose and starch. Cultures containing fructose and maltose did not shown any zone of inhibition. The utilization of glycerol, glucose and starch for growth and production of antibiotic by LAM1 and LAM2 indicate the presence of an active uptake system for these substrates.

Off all the tested nitrogen sources, LAM1 showed maximum growth and antibacterial activity when cultures are supplemented with sodium nitrate or potassium nitrate followed by peptone and LAM2 showed maximum growth and zone of inhibitions when peptone is used as nitrogen source followed by sodium nitrate and potassium nitrate. (Table 4).The results indicate that sodium nitrate and potassium nitrate served as good nitrogen sources for LAM1 and peptone served as ideal nitrogen source for LAM2.

Influence of metal ions:

Influence of different metal ions on the growth and production of antimicrobial components was tested by replacing the different metal ions in the production medium and results were indicated in the table 5. The results given in the table showed that iron plays an important role in the promotion of antibiotic production by LAM1 and manganese plays an important role on the growth and antibiotic production by LAM2.

DISCUSSION

Actinomycetes are unparalleled sources of bioactive metabolites including antibiotics, plant growth factors and other secondary metabolites [25, 26].They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds [27]. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics including many of medical importance have been isolated from actinomycetes [9]. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genus *Streptomyces* and *Micromonospora* [28].

Antibiotics are low-molecular-mass products of secondary metabolism, non essential for the growth of producing organisms, but very important for human health. They have unusual structures and are most often formed during the late growth phase of the producing microorganisms [29]. Their production arises from intracellular intermediates, which are condensed into more complex structures through defined biochemical pathways. Their synthesis can be influenced by manipulating the type and concentration of nutrients formulating the culture media [17, 18, 30]. In order to achieve high product yields, it is a pre requisite to design a proper production medium in an efficient fermentation process [31]. From the results it was evident that maximum growth and antibacterial metabolite production was obtained at 30°C for LAM1 and LAM2 which clearly indicates the mesophilic nature of both the isolates. Previous reports [32] illustrate that optimal temperature range between 26°C to 35°C for antibacterial metabolites by *Streptomyces* strains and *Micromonospora* strains. The metabolic activities of actinomycetes are very much sensitive to the initial pH of the fermentation broth [16]. The highest zone of inhibition was observed at a pH 0f 7 for LAM1 and LAM2 which indicate that the optimum level of pH for antibiotic production is 7.

The period of incubation also has profound effect on growth and antibacterial metabolite production by actinomycetes [33]. The maximum zone of inhibition was exhibited after seven days of incubation by LAM1 and nine days of incubation by LAM2.

The effect of the carbon source has been the subject of continuous studies for both industry and research groups. Glucose and other carbohydrates have been reported to interfere with antibiotic synthesis and this effect depends on the rapid utilization of the preferred carbon source [29].Maximum zone of inhibition was observed when the glycerol was used as carbons source followed by glucose and starch. This indicated the presence an active uptake system for these compounds in the isolates. The results of this study revealed that the level of antibacterial production may be greatly enhanced by the nature of nitrogen source supplied in the culture medium. Similar results have been reported by many investigators [34, 35, 36, 37]. The results indicate that sodium nitrate and potassium nitrate served as good nitrogen sources for LAM1 and peptone served as ideal nitrogen source for LAM2. The growth and antibacterial metabolite production is also influenced by the nature of metal ion source used in the fermentation broth [38]. The investigation showed that iron plays an important role in the promotion of antibiotic production by LAM1 and manganese played an important role on the growth and antibiotic production by LAM2.

CONCLUSION

The present study determined the optimal culture conditions for growth and antibacterial metabolite production by two fresh water actinomycetes strains. The initial pH, incubation period, incubation temperature were found to have significant effect on the growth and antibiotic production by LAM1 and LAM2. Use of different nitrogen sources in the fermentation medium proved to be beneficial and increased the yield of antibiotic. The type of carbon sources effect the antibiotic production. Therefore, the fermentation of antibiotic can be done under optimized parameters to achieve a very good yield.

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REFERENCES

1. Stacketrandt, E. and Woese¸ C.R. (1981). Towards phylogeny of actinomycetes and related organisms.*Curr.Microbiol*.34: 296.
2. Goodfellow, M. and Cross, T. (1984).The biology of the Actinomycetales. Academic press, London. 7.
3. Embly, T.M. and Stacketrandt, E.(1994). The molecular phylogeny and systematic of actinomycetes. *Annu.Rev.Microbiol*.48: 257.
4. Madigan, M.T., Martinko, J.M. and Parker, J. (2005). Antibiotics: Isolation and characterization in Brook Biology of Microorganisms, 8th edition. Prentice Hall International Inc. New Jersey : 440.
5. Watve, M.G., Tickoo, R., Jog, M.M. and Bhole, B.D. (2001). How many antibiotics are produced by the genus *Streptomyces*? *Archives of Microbiology*. 176: 386.
6. Balagurunathan, R. and Radhakrishnan, M. (2007). Actinomycetes: Diversity and their importance in Microbiology: Applications and current trends. P.C. Trivedi (editor), Pointer publishers, Jaipur, India: 297.
7. Radhika, S., Bharathi, I., Radhakrishnan, M. and Balagurunathan, R. (2011). Bioprosopecting of fresh water actinobacteria. J*. Pharm.Res*. 4: 2584.
8. Cross, T. (1981). Aquatic actinomycetes: A critical survey of occurrence, growth and role of actinomycetes in aquatic habitats. *J. Appl. Bact*. 50: 397.
9. Okami and Hotta. (1988).Search and discovery of new antibiotics. Actinomycetes in Biotechnology. Academic press. San Diego, C.A.: 33.
10. Martin, J.F. (1982). Antibiotics: Chemotherapeutics and antimicrobial agents for disease control. John Wiley and sons. New York.
11. Ningthoujam, D.S., Suchitra, S., and Nimaichand, S. (2009). Screening of actinomycete isolates from niche habitats in Manipur for antibiotic activity. *Ameri. J. Biochem. Biotech*.5 (4): 221.
12. Ningthoujam, D.S, Suchitra, S., and Nimaichand, S. (2011). Studies on bioactive actinomycetes in a niche biotope, Nimbul River in Manipur, India. *Microbial.Biichem.Tech.*1.
13. Demain, A.L. and Fang, A. (1995). Emerging concepts of secondary metabolism in actinomycetes. *Actinomycetologica*. 9: 98.
14. Ubukata, M., Shiraish, N., Kobinata, K.T., Yamaguchi, I., Osada, H. and Isono, K. (1995). New macrolide antibiotic from *Streptomyces violaceusniger*, taxonomy, fermentation, isolation and biological activities. *J.Antibiot*. 48: 289.
15. Jones, G.H. (2000). Actinomycin production persists in a strain of *Streptomyces antibioticus* phenoxazinone synthase. *Antimicrobial agents and chemotherapy*. 44 (5): 1322.
16. Yang, S.S. and Ling, M.Y. (1989). Tetracycline production with sweet potato residue by solid state fermentation. *J. Biotech. Bioeng*. 33: 1021.
17. Waksman, S.A. (1961).The actinomycetes, classification, identification and description of

genera and species. The Baltimore. The Williams and Wilkins company.2: .
18. Slavica, I., Sandra, K., Vlada, B., Vejikovic, Dragisa, S. and Gordana D.G. (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: 1337.
19. Barratt, E.M. and Oliver, S.G. (1994). The effects of nutrient limitation in the synthesis of stress proteins in *Streptomyces lividanans*. *Biotechnology letters* 16(12): 1231
20. Higashide (1984). The macrolides: Properties, biosynthesis and fermentation. *Biotechnology of industrial antibiotics*. 451.
21. Maha, A.H., Moustafa, Y., Naggar, E.L. and Wafa, Y.S. (2001). Proceedings of First international conference (*Egyptian journal of Biology*). 3: 1.
22. Okazaki, T. and Okami. (1972). Actinomycetes in sagami bay and their antibiotic substances.
23. J.*Antibiot*. 25: 461.
24. Shirling, E.B. and Gottlieb, D. (1966). Methods of characterization of *Streptomyces* species. *Int.J.Syst.Bacteriol*. 16: 313..
25. Kornerup, A. and Wanscher, J.H. (1978). Methuen hand book of color. Methuen, London, U.K.
26. Omura, S. (1986). Philosophy of new drug discovery. *Microbiol.Rev*. 50: 259.
27. Shahidi, B.G.H., Fooladi, M.H., Mahadavi, M.J. and Shahgasi, A. (2004).Broad spectrum, a novel antibacterial from *Streptomyces* strains in bio control of *Pythium aphanidermatum*. *Res.J.Biol.Sci.* 2:232.
28. Narendrakumar, Ravikanth, S., Mishra, S.k., Singh, A.K. and Pachouri, U.C. (2010).Isolation and screening of soil actinomycetes as a source of antibiotics against bacteria. *Int. J. Microbiol. Res*. 2 :12.
29. Pandey, B., Ghimire, P. and Agarwal, V.P. (2004). Climate, health, ecology, management and conservation. International conference on the great Himalayas.
30. Sergio, S., Adan, C. and Angela, F. (2010).Carbon source regulation of antibiotic production. *J. Antibiot*. 63: 442.
31. Vasavada, S.H., Thumar, J.T. and Singh, S.P. (2006). Secretion of potent antibiotic by salt tolerant and alkalophilic actinomycete *Streptomyces sannanensis* strain RTJ-1. *Current science* 91: 1393.
32. Macedo, J.A., Sette, L.D. and Sato, H.H. (2007).Optimization of medium composition for transglutamate production by a Brazilian soil *Streptomyces* sp. *Electr.J.Biotechnol*.10 (4).
33. Yang, S.S and Swei, W.J. (1996). Oxytetracycline production by Streptomyces in solid state fermentation of corncob. *World.J.Microbiol.Biotech.*12: 43.
34. Srinivasan, M.C., Laxman, R.S. and Deshpande, M.V. (1991). Physiology and nutritional aspects of actinomycetes: an overview. *World J. Microbial Biotech*. 7: 171.
35. Khoua, S., Librihi, A., German, P. and Leferbevre, G. (1991). Cephamycin C biosynthesis in *Streptomyces cattleya*: nitrogen sources regulation. *J. Appl. Microbiol. Biotech*. 35: 253.
36. Mansour, F.A., Shirbiny, S.A. and Metwaly, N.A. (1996). Demethyl tetracycline biosynthesis by *Stretpomyces aureofaciens* sub-species viridulans as influenced by medium composition. *Egyptian J. Microbiol.* 31: 221.
37. Vahidi, H., Kobarfard, F. and Namjoyan, .F (2004). Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*. *Afr. J. Biotech*. 3: 606.
38. Yu, J., Liu, Q., Liu, X., Sun, Q., Yan, J. (2008). Effect of liquid culture requirements on antifungal antibiotic production by *Streptomyces rimosus* MY02. *Biores. Technol*. 99: 2087.
39. Kishimoto, K., Park, Y.S., Okabe, M. and Akiyama, S. (1997). Effect of ferrous ion on aminoacid metabolism in mildiomycin production in *Streptoverticillum rimofaciens*. *J.Antibiot*. 50: 206.