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A *Streptomyces sindenensis* Strain LS1-128 Exhibiting Broad Spectrum Antimicrobial Activity

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Abstract: A *Streptomyces* sp. LS1-128 (closely related to *S. sindenensis*) isolated from the sediments of Loktak Lake, the largest freshwater lake in Eastern India, shows broad spectrum antimicrobial activity with significant inhibition zones against *B. pumilus* MTCC 1607 (25 mm), *E. coli* MTCC 739 (20 mm), *B. sphaericus* MTCC 2303 (19 mm), *B. subtilis* MTCC 121 (14 mm) and *C. albicans* MTCC 227 (11 mm). LS1-128 is an aerobic, gram-positive actinomycete with off-white to brown substrate mycelium with no diffusible pigment. Spores are arranged in rectiflexible chains in the aerial mycelium. It grows well in ISP2, ISP3, ISP4, ISP5, ISP6, ISP7, *Streptomyces* Agar and Actinomycete Isolation Agar. It can degrade starch, casein and urea and has good growth on almost all carbon sources tested including L-Arabinose, D-Fructose, D-Galactose, D-Glucose, D-Mannitol, D-Rhamnose, Salicin and D-Xylose. Based on the phenotypic and genotypic characteristics, LS1-128 was found to be most closely related to *Streptomyces sindenensis* (99.927% homology). The other close relatives are *S. parvus*, *S. badius* and *S. globisporus*). LS1-128 with promising antimicrobial activities obtained from Loktak Lake sediment has been putatively designated as *Streptomyces sindenensis* strain LS1-128.

Key words: Actinomycetes, *Streptomyces sindenensis* strain LS1-128, *Streptomyces*, lake, sediment, Loktak Lake, antimicrobial

INTRODUCTION

Actinomycetes especially *Streptomyces* sp. are rich sources of bioactive natural products with potential applications as pharmaceuticals and agrochemicals (Atta, 2009; Atta *et al*., 2009; Manteca *et al*., 2008). They are found widely in nature and in most ecological niches (Takahashi and Omura, 2003). Actinomycetes are potential sources of many bioactive compounds (Lakshmipathy and Kannabiran, 2009). They are prolific producers of secondary metabolites: antibiotics, herbicides, pesticides and anticancer agents (Atta and Ahmad, 2009; Osada, 1998; Saadoun and Gharaibeh, 2003). More than 6000 compounds are produced by *Streptomyces* sp. and many of them have commercial importance as anti-infectives (antibiotics, antiparasitic and antifungal agents), anticancer or immunosuppressant agents (Takahashi and Omura, 2003).

Streptomyces is the largest prokaryotic genus with 562 valid species currently (Euzeby, 2009). They are representative antibiotic producing prokaryotic group, while their morphological differentiation and genetic properties (linear chromosomes and genomic instability) have basic science interests (Locci, 1989; Chen *et al*., 2002).

Streptomyces sp. are widely recognized as industrially important organisms for their ability to elaborate different kinds of novel secondary metabolites (Bibb, 2005). *Streptomyces* sp. are prolific producers of useful bioactive compounds (Tanaka and Omura, 1990), providing 75-80% of the naturally occurring antibiotics discovered till date. They are known to elaborate a wide diversity of natural products including antibiotics, antifungal agents, plant growth factors, enzymes and enzyme inhibitors, antiparasitic, anticancer and immunomodulating agents (Berdy, 1995; Okami and Hotta, 1988; Omura, 1986; Bonjar, 2004). *Streptomyces* sp. or strains with novel antibiotics may still exist in nature (Okami and Hotta, 1988). They are gram positive bacteria with distinct features such as high DNA G+C content, presence of LL-Diaminopimelic acid (LL-DAP) and the absence of characteristic sugars in the cell wall (Anderson and Wellington, 2001). They also produce extensively branched substrate and aerial mycelia (Locci, 1989). They produce a wide variety of various bioactive compounds, such as antibiotics, enzymes, anticancer and agroactive compounds (Okami and Hotta, 1988; Berdy, 1995).

As exploration of the usual terrestrial sources are nearly exhausted, there is the imperative need to access various habitats such as soils, paddy fields, lake mud and

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Fig. 1: LS1-128

water, forest and cave soils (Takahashi and Omura, 2003; Jiang and Xu, 1996; Xu *et al*., 1996; Kim *et al*., 1998).

Some reports say that actinomycetes of freshwater habitats can produce bioactive compounds (Okami, 1986); so more studies on aquatic actinomycetes are warranted (Rifaat, 2003). Also, there is the crying need for new antimicrobials and antifungal agents.

Actinomycetes from freshwater habitats have been relatively neglected (Goodfellow and Haynes, 1984). There is also the crying need for new antibiotics and antifungal agents.

In the ongoing search/continuing study of bioactive actinomycetes in various habitats in Manipur, part of Indo-Burma Biodiversity Hotspot (Myers *et al*., 2000), we had isolated a large number of actinomycetes esp. *Streptomyces* sp., from lake sediments of Loktak Lake, the largest freshwater lake in Eastern India (Sanasam and Ningthoujam, 2005a, b).

The aim of the present investigation was to screen a subset of our actinomycete collection for antagonistic activities against bacteria and fungi of medical and agricultural importance and characterize the bioactive strains. During this study, we found a strain, LS1-128 (Fig. 1), with interesting broad spectrum activities against selected bacterial and fungal pathogens. This study deals with bioactivity of the strain and polyphasic characterization of the antagonistic strain to establish its taxonomic position.

MATERIALS AND METHODS

Media and reagents: Microbial growth media were obtained from Himedia (Mumbai, India) or prepared in the Lab according to standard protocols. All other reagents and chemicals were of the highest grade available.

Loktak Lake: Loktak Lake is one of the largest freshwater lakes in Eastern India. It has been covered under Ramsar convention in 1990 (Ningombam and Bordoloi, 2007).

The lake's ecology is in precarious state due to shallowing by siltation and pollution by incoming streams and rivers and from human activites and agricultural runoff. Its condition worsened after launching of a hydroelectric project in the lake about 30 years ago.

Sampling: Sediment samples were collected from the bottom of Loktak Lake at various depths and stored in refrigerator till processing. They were processed soon after collection.

Isolation of lake actinomycetes: The strain was isolated from sediments of Loktak Lake, the largest freshwater lake in Eastern India using Starch Casein Nitrate Agar (Kuster and Williams, 1964). It is maintained on Bennett's agar slants (Jones, 1949) at 28 and 4°C.

Indicator organisms: The test organisms *Bacillus pumilus*, *B. sphaericus*, *B. subtilis*, *E. coli* and *Candida albicans* were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. *Pseudomonas aeruginosa* DN1 is a lab isolate and part of the collection of Microbial Biotechnology Research Lab (MBRL), Manipur University (MU), Canchipur, India.

Antimicrobial screening: The antimicrobial assay was done by Bauer *et al*. (1966) method against *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *B. pumilus* MTCC 1607, *B. sphaericus* MTCC 2303, *E. coli* MTCC 739, *P. aeruginosa* DN1, *C. albicans* MTCC 227 and *A. niger* MTCC 1344 obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India, except for DN1 which is a lab collection.

Phenotypic characterization: The morphology of the spore chain and the spore surface were examined by light microscopy of 14 days old culture on inorganic salts-starch agar. The cultural properties of the strain were evaluated according to the guidelines of the International *Streptomyces* Project (ISP) as described by Shirling and Gottlieb (1966).

Cell wall chemotype analysis: The isomeric form of Diaminopimelic acid (DAP) in the whole cell hydrolysates were analysed by TLC (Staneck and Roberts, 1974).

Genotypic characterization: 16S rDNA amplification and sequencing were carried out as described by Kim *et al*. (1998). The almost complete 16S rDNA sequence was submitted to EzTaxon server (Chun *et al*., 2007), which

contains manually curated databases of type strains of prokaryotes for sequence analysis. Related strains were selected for alignment by CLUSTAL W program and were done phylogenetic analysis according to the neighbor-joining method (Saitou and Nei, 1987) using the MEGA version 4.1 (Tamura *et al*., 2007). To determine the support of each clade, bootstrap analysis was performed with 1000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

Strain LS1-128 Fig. 1 shows significant activity against 5 of the 8 test organisms tested (Table 1).

Physiological and biochemical characteristics: The phenotypic characteristics of the strain LS1-128 is shown in Table 2.

Cell-wall chemotype analysis: Whole-cell hydrolysates contained LL-DAP as diagnostic acid of the cell-wall peptidoglycan, establishing the strain to be belonging to *Streptomyces*.

Phylogenetic analysis: The almost-complete 16S rRNA gene sequence (1363 nt) of strain LS1-128 shows that the organism belongs to the genus *Streptomyces*. A phylogenetic tree was constructed for LS1-128 based on the 16S rDNA sequences of the strain and other related *Streptomyces* sp. The strain shows closest affinity with *Streptomyces sindenensis* (Fig. 2) with sequence homology of 99.927%.

Description of *Streptomyces* sp. strain LS1-128: Strain LS1-128 produced rectiflexibilis spore chains in the aerial mycelium. The colour of the substrate mycelia was off-white to brown. No diffusible pigments were produced. Strain LS1-128 grew well on yeast extract malt extract agar (ISP2-HiMedia), oatmeal agar (ISP3-HiMedia), inorganic salts/starch agar (ISP4-HiMedia), glycerol asparagine agar (ISP5-HiMedia), peptone/yeast extract iron agar (ISP6-HiMedia), tyrosine agar (ISP7-HiMedia), *Streptomyces* Agar (HiMedia) and Actinomycete Isolation Agar (HiMedia). Starch, Casein and urea are degraded. Good growth are found on almost all carbon sources tested including L-Arabinose, D-Fructose, D-Galactose, D-Glucose, D-Mannitol, D-Rhamnose, Salicin and D-Xylose. Whole cell hydrolysates contained LL-DAP as diagnostic acid of the cell-wall peptidoglycan. Cell wall fatty acid analysis shows that it contains glucose, galactose and ribose.

Table 1: Antimicrobial activity profile of LS1-128

Test organisms	Inhibition zone (mm diameter)
S aureus MTCC 96	-
B subtilis MTCC 121	14
B pumilus MTCC 1607	25
B sphaericus MTCC 2303	19
E. coli MTCC739	20
P. aeruginosa DN1	-
C albicans MTCC 227	11
A niger MTCC 1344	-

Table 2: Characteristics of LS1-128

Characteristics	Strain types	Characteristics	Strain types
Colour of aerial mycelium	White	**Hydrolysis**	
Production of diffusible pigment	-	Casein	+
Melanin Pigment production	-	Starch	+
Degradation		Urea	+
DNA	-	Growth at	
Gelatin	-	4°C	-
Hypoxanthine	-	45°C	-
Xanthine	-	**Growth in the presence**	
Utilization		4% NaCl	-
Adonitol	-	7% NaCl	-
L-Arabinose	+	10% NaCl	-
D-Fructose	+	13% NaCl	-
D-Galactose	+	**Growth in the presence**	
D-Glucose	+	**Crystal violet (0.0001% w/v)**	
Meso-inositol	-	Hypoxanthine	-
D-Mannitol	+	Phenol (0.1)	-
L-Rhamnose	+	1% Tween 80	-
Salicin	w	**Enzyme activity**	
Sucrose	-	Lecithinase	-
D-Xylose	w	Lipolytic	-
Nitrate reduction	-	Pectinolytic	+
		Proteolytic	-

+: Positive; -: Negative; w: Weakly positive

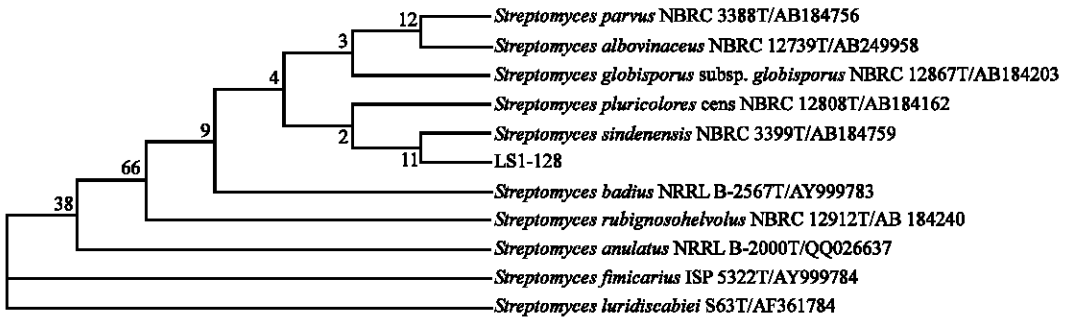

Fig. 2: Phylogenetic analysis of LS1-128 with 10 nearest homologs

However, whether LS1-128 is a novel *Streptomyces sindenensis* strain or a novel species could only be ascertained by DNA-DNA hybridization and other molecular studies with related strains from the sub-clade. There are limited reports on bioactivity of *Streptomyces sindenensis*. For example, Praveen *et al.* (2008) reported actinomycin-D production by a new isolate of *Streptomyces sindenensis* but their strain was obtained from soil sample collected from steel plant effluents in Uttar Pradesh, India.

Zarandi *et al.* (2009) obtained a strain of *S. sindenensis* from soils in Karman Province, Iran with biocontrol activity against *Magnaporthe oryzae* (causal agent for blast disease in rice). To the knowledge, this is the first report of a bioactive strain of *Streptomyces sindenensis* recovered from lake sediments.

Actinomycetes play major roles in C cycle in aquatic habitats and wetlands, due to their ability to grow at the low C concentrations and degrade recalcitrant organic matter (Kuznetsov, 1970). Therefore, they form promising sources for the survey of actinomycetes as they are well known as excellent decomposers.

However, exploration of actinomycete diversity in lakes, rivers, ponds, wetlands and other aquatic ecosystems are relatively still neglected. There are as yet scant reports on lake actinomycetes especially those from freshwater lakes. However, some groups have reported the predominance of *Streptomyces* in freshwater lake sediments and of *Micromonospora* in lake water (Terkina *et al*., 2002, 2006). Similar findings have also been reported for marine sediments (Jensen *et al*., 1991, 2007). Of late, novel drugs such as salinosporamide have been discovered from marine actinomycetes such as *Salinispora* (related to *Micromonospora*) (Jensen *et al*., 2007).

Terkina *et al*. (2002) found that *Streptomyces* and *Micromonospora* were the predominant genera in Lake Baikal using starch-containing agar media who observed that lake water was dominated by *Streptomyces* (66% of all water isolates) and sediments by *Micromonospora* (59% of all sediment isolates).

Rifaat (2003) also found that, of 114 strains from River Nile, *Streptomyces* were prevalent in water and *Micromonospora* in sediments and several *Streptomyces strains* had antimycotic activity.

Freshwater habitats have been recently stressed as promising sources of bioactive metabolites (Cross, 1981). Terkina *et al.* (2002, 2006) reported that several lake Baikal actinomycetes inhibited the growth of several pathogens including antibiotic-resistant microbes. Elliah *et al.* (2002) observed that *Streptomyces strains* from Krishna river sediments in India had antibacterial and antifungal activities. Rifaat (2003) in his studies of actinomycetes from River Nile in Egypt, reported that several *Streptomyces strains* had significant antimycotic activity. New actinomycete strains or species have also been recovered from lakes and other aquatic environments. For example, Lango *et al.* (1999) identified *Streptomyces galbus* from sediment of La Caldera in Spain.

LS1-128 was isolated from sediments of Loktak Lake in Manipur, India. We had obtained 172 lake sediment actinomycetes using SCNA medium. A majority of them were found to be *Streptomyces* and nocardioform actinomycetes. This differs from the findings of Terkina *et al*. (2002, 2006) who observed predominance of *Micromonospora* in lake Baikal sediments and *Streptomyces* in lake Baikal water. Similar findings were also found by Rifaat (2003). But this may be a reflection of the trophic status of the contrasting lakes; whereas Lake Baikal is an oligotrophic lake, Loktak Lake is highly eutrophic, polluted by inlet streams and rivers loaded with wastes and agricultural runoff.

Nocardioform actinomycetes have earlier been reported from aquatic habitats, with their presence as possibly linked with anthropogenic activities as suggested by some researchers (Rowbotham and Cross, 1977; Yamamura *et al*., 2003).

It is not surprising that we got a large number of actinomycete isolates from Loktak lake sediments as actinomycetes comprise up to 30% of the microbial population in lakes, the largest fraction that are culturable being *Micromonospora* and streptomycetes (Terkina *et al*., 2002; Sponga *et al*., 1999; Takizawa *et al*., 1993; Jiang and Xu, 1996; Jensen *et al*., 1991).

The present study indicates that Loktak Lake can be a promising source of bioactive metabolites and of novel strains or species of actinomycetes. Detailed characterization of LS1-128 and its bioactive metabolite (s) and screening of our remaining lake actinomycete isolates will now be the target of the further studies.

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REFERENCES

Anderson, A.S. and E.M.H. Wellington, 2001. The taxonomy of *Streptomyces* and related genera. Int. J. Syst. Evol. Microbiol., 51: 797-814. DOI: 10.1099/ijs.0.02314-0. PMID: 11411701. http://ijs.sgmjournals.org/cgi/reprint/51/3/797.

Atta, H.M., 2009. An antifungal agent produced by *Streptomyces olivaceiscleroticus* AZ-SH514. World Applied Sci. J., 6: 1495-1505. http://idosi.org/wasj/wasj6(11)/7/pdf.

Atta, H.M., S.M. Dabour and S.G. Desoukey, 2009. Sprasomycin antibiotic production by *Streptomyces* sp. AZ-NIOFD1: Taxonomy, fermentation, purification and biological activitites. American-Eurasian J. Agric. Environ. Sci., 5: 368-377. http://idosi.org/aejaes/jaes5(3)11.pdf.

Atta, M.A. and M.S. Ahmad, 2009. Antimycin-A antibiotic biosynthesis produced by *Streptomyces* sp. AZ-AR-262: Taxonomy, fermentation, purification and biological activities. Aust. J. Bas. Sci., 3: 126-135. http://www.insinet.net/ajbas/2009/126-135.pdf.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496. PMID: 5325707. http://www.ncbi.nlm.nih.gov/pubmed/5325707.

Berdy, J., 1995. Are actinomycetes exhausted as a source of secondary metabolites? In: Proceedings of the 9th International Symposium on the Biology of actinomycetes, New York: Allerton. part I, pp: 3-23.

Bibb, M.J., 2005. Regulation of secondary metabolism in *Streptomyces*. Curr. Opin. Microbiol., 8: 208-215. DOI: 10.1016/j.mib.2005.02.016. PMID: 15802254. http://www.jic.ac.uk/STAFF/mervyn-bibb/pdfs/Biib2005.pdf.

Bonjar, S., 2004. Broadspectrin, a novel antibacterial from *Streptomyces* sp. Biotechnol., 3: 126-130. http://scialert.net/.

Chen, C.N., C.H. Huang, H.H. Lee, H.H. Tsai and R. Kirby, 2002. Once the circle was broken: dynamism and evolution of *Streptomyces chromosomes*. Trends Genet., 18: 522-529. DOI: 10.1016/S0168-9525(02)02752-x. PMID: 12350342. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6TCY-46JYKWV-1-9&_cdi=5183&_user=2252484&_orig=search&_coverDate=10%2F01%2F2002&_sk=999819989&view=c&wchp=dGLbVzb-zSkzV&md5=8c903935962735c2635094055f9794f1&ie=/sdarticle.pdf.

Chun, J., J.H. Lee, Y. Jung, M. Kim, S. Kim, B.K. Kim and Y.W. Lim, 2007. EzTaxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int. J. Syst. Evol. Microbiol., 57: 2259-2261. DOI: 10.1099/ijs.0.64915-0. PMID: 117911292. http://ijs.sgmjournals.org/cgi/content/full/57/10/2259.

Cross, T., 1981. Aquatic actinomycetes: A critical survey of the occurrence, growth and role of actinomycets in aquatic habitats. J. Applied Bacteriol., 50: 397-423. PMID: 7019182. http://www.ncbi.nlm.nih.gov/pubmed/7019182.

Elliah, P., K.V.V. Raju, S.N. Bapi, K. Adinarayana, G. Adinarayana, V. Saisha, S. Madhavi and J. Premkumar, 2002. Bioactive actinomycets from Krishna river sediments of Andhra Pradesh. Hindusthan Antibiotics Bull., 44: 1-4. PMID: 15061588. http://www.ncbi.nlm.nih.gov/pubmed/15061588.

Euzeby, J.P., 2009. List of prokaryotic names with standing in nomenclature. http://www.bacterio.net.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the boothstrap. Evolution, 39: 783-791. http://www.jstor.org/stable/pdfplus/2408678.pdf.

Goodfellow, M. and J.A. Haynes, 1984. Actinomycetes in Fresh Water Sediments. In: Ortiz-Ortiz, L., L.F. Bojalil and V. Yakoleff (Eds.). Biological, Biochemical and Biomedical Aspects of Actinomycetes. Academic Press, New York, London, pp: 453-472.

Jensen, P.R., P.G. Williams, D.G. Oh, L. Zeigler and W. Fenical, 2007. Species-specific secodary metabolite production in marine actinomycetes of the genus *Salinispora*. Applied Environ. Microbiol., 73: 1146-1152. DOI: 10.1128/AEM.01891-06. PMID: 17158611. http://aem.highwire.org/cgi/content/abstract/73/4/1146.

Jensen, P.R., R. Dwight and W. Fenical, 1991. Distribution of actinomycetes in near-shore tropical marine sediments. Applied Environ. Microbiol., 57: 1102-1108. PMID: 2059035. http://aem.highwire.org/cgi/content/abstract/57/4/1102.

Jiang, C. and L. Xu, 1996. Diversity of aquatic actinomycetes in lakes of the middle plateau, Yunnan, China. Applied Environmental Microbiology. PMID: 16535213. http://aem.highwire.org/cgi/content/abstract/62/1/249.

Jones, K.L., 1949. Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristics. J. Bacteriol., 57: 141-145. PMID: 16561658. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC385488/pdf/jbacter00630-0027.pdf.

Kim, S.B., C. Falconer, E. Williams and M. Goodfellow, 1998. *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. Int. J. Syst. Bacteriol., 48: 59-68. DOI: 10.1099/00207713-48-1-59. PMID: 9542077. http://ijs.sgmjournals.org/cgi/content/abstract/48/1/59.

Kuster, E. and S.T. Williams, 1964. Selection of media for isolation of *Streptomyces*. Nature, 202: 928-929. DOI: 10.1038/202928a0. http://adsabs.harvard.edu//abs/1964Nature.202..928K.

Kuznetsov, S.I., 1970. Microlora of lakes and its geochemical activity. Lenigrad: Nauka, pp: 1-9.

Lakshmipathy, D.T. and K. Kannabiran, 2009. A morphological, biochemical and biological studies of halotolerant *Streptomyces* sp. Isolated from saltpan environment. Am. J. Inf. Dis., 5: 207-213. http://www.akademik.unsri.ac.id/download/journal/files/scipub/ajid53207213.pdf.

Lango, Z., L.G. Toth, L. Kovacs and L. Cruz-Pizarro, 1999. Identification and biochemical characterization of *Streptomyces galbus* from the sediment of the high mountain lake La Caldera (Sierra Nevada, Spain). Folia Microbiologia, 44: 651-656. DOI: 10.1007/BF02825656. http://www.springerlink.com/content/r727672127pn8t34/.

Locci, R., 1989. *Streptomyces* and Related Genera. In: Williams, S.T., M.E. Sharpe and J.G. Holt (Eds.). Bergeys Manual of Systematic Bacteriol., Williams and Wilkins, Baltimore, USA, 4: 2451-2508.

Manteca, A., R. Alvarez, N. Salazar, P. Yague and J. Sanchez, 2008. Mycelium differentiation and antibiotic production in submerged culture of *Streptomyces coelicolor*. Applied Environ. Microbiol., 74: 3877-3886. DOI: 10.1128/AEM.02713-07. PMID: 18441105. http://aem.asm.org/cgi/reprint/74/12/3877.

Myers, N., R.A. Mittermeier, C.G. Mittermeier, Da G.A.B. Fonseca and J. Kent, 2000. Biodiversity hotsopts for conservation priorities. Nature, 403: 853-858. DOI: 10.1038/35002501. PMID: 10706275. http://web.ipb.ac.id/~mujizat/jurnal/Myers%20et%20al%202000.pdf.

Ningombam, B. and S.B. Bordoloi, 2007. Amphibian fauna of Loktak Lake, Manipur, India with ten new records for the State. Zoos' Print J., 22: 2688-2690. http://www.zoosprint.org/ZooPrintJournal/2007/May/2688-2690.pdf.

Okami, Y. and K. Hotta, 1988. Search and Discovery of New Antibiotics. In: Goodfellow, M., S.T. Williams and M. Mordarski (Eds.). Actinomycetes in Biotechnology, Academic Press, London, pp: 33-67.

Okami, Y., 1986. Marine microorganisms as a source of bioactive agents. Microbial. Ecol., 12: 65-78. DOI: 10.1007/BF02153223. http://springerlink.com/content/141648432508206 7/fulltext.pdf.

Omura, S., 1986. Philosophy of new drug discovery. Microbiol. Rev., 50: 259-274. PMID: 3534537. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC373071/pdf/microrev00054/0037.pdf.

Osada, H., 1998. Actinomycetes: how fascinating microorganisms. Actinomycetol., 12: 85-88. DOI: 10.3209/saj.12-85. http://www.jstage.jst.go.jp/articles/saj/12/2/85/_pdf.

Praveen, V., C.K.M. Tripathi, V. Bihari and S.C. Srivastava, 2008. Production of actinomycin-D by the mutant of a new isolate of *Streptomyces sindenensis*. Brazilian J. Microbiol., 39: 689-692. DOI: 10.1590/S1517-83822008000. PMID: 400017. http://www.scielo.br/pdf/bjm/v39n4/arq17.pdf. www.ramsar.org.

Rifaat, H.M., 2003. The biodiversity of actinomycetes in the river Nile exhibiting antifungal activity. J. Mediterranean Ecol., 4: 5-7. http://www.jmecology.com/pdf/2003/3-4/01rifaat.pdf.

Rowbotham, T.J. and T. Cross, 1977. Ecology of Rhodococcus coprophilus and associated actinomycets in fresh water and agricultural habitats. J. Gen. Microbiol., 100: 231-240. DOI: 10.1099/00221287-100-2-23. http://mic.sgmjournals.org/cgi/reprint/100/2/231.pdf.

Saadoun, I. and R. Gharaibeh, 2003. The *Streptomyces flora* of Badia region of Jordan and its potential as a source of antibiotics against antibiotic-resistant bacteria. J. Arid Environ., 53: 365-371. DOI: 10.1006/jare.2002.1043. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WH9-47WBS1R-4&_user=2252484&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_searchStrId=1085948701&_rerunOrigin=scholar.google&_acct=C000056760&_version=1&_urlVersion=0&_userid=2252484&md5=a2944145616b77418b07a530d5000b28.

Saitou, N. and M. Nei, 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425. PMID: 3447015. http://mbe.oxfordjournals.org/cgi/reprint/4/4/406.pdf.

Sanasam, S. and D. Ningthoujam, 2005a. Diversity of actinomycetes in slected soils of Manipur and their antibiotic potential. J. Assam. Sci. Soc., 45: 44-47.

Sanasam, S. and D. Ningthoujam, 2005b. Diversity of soil actinomycetes in selected biotopes of Manipur and screening for antibiotic activities. Microbial Diversity: International Conference, April 16-18. Department of Microbiology, South Campus, Delhi University, New Delhi, India.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* sp. Int. J. Syst. Bacteriol., 16: 313-340. DOI: 10.1099/00207713-16-3-313. http://ijs.sgmjornals.org/cgi/content/abstract/163/313.

Sponga, F., L. Cavaletti, A. Lazzarini and A. Bofghi *et al*., 1999. Biodiversity and potentials of marine-derived microorganisms. J. Biotechnol., 70 (1-3): 65-69. DOI: 10.1016/S0168-1656(99)00059-0.

Staneck, J.L. and G.D. Roberts, 1974. Simplified Approach to identification of aerobic actinomycetes by Thin-Layer Chromatography. Applied Microbiol., 28: 226-231. PMID: 4605116. http://aem.asm.org/cgi/content/abstract/28/2/226.

Takahashi, Y.O. and S. Omura, 2003. Isolation of new actinomycete strains for the screening of new bioactive compounds. J. Gen. Applied Microbiol., 49: 141-154. DOI: 10.2323/jgam.49.14. PMID: 12949697. http://www.jstage.jst.go.jp/article/jgam/49/3/141/_pdf.

Takizawa, M., R.R. Colwell and R.T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. Applied Environ. Microbiol., 59: 997-1002. PMID: 16348922. http://aem.asm.org/cgi/reprint/59/4/997.pdf.

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599. DOI: 10.1093/molbev/msm092. PMID: 17488738. http://mbe.oxfordjournals.org/cgi/reprint/24/8/1596.

Tanaka, Y. and S. Omura, 1990. Metabolism and products of actinomycetes. An introduction. Actinomycetology, 4: 13-14. DOI: 10.3209/saj.413. http://www.jstage.jst.go.jp/article/saj/4/1/13/_pdf.

Terkina, I.A., V.V. Parfenova and T.S. Ahn, 2006. Antagonistic activity of actinomycetes of Lake Baikal. Applied Biochem. Microbiol., 42: 173-176. PMID: 16761573. http://www.springerlink.com/index/5103500479034026.pdf.

Terkina, I.A., V.V. Drukker, V.V. Perfenova and T.Y. Kostornova, 2002. The biodiversity of actinomycetes in Lake Baikal. Microbiol., 71: 404-408. DOI: 10.1023/A:1015871115187. PMID: 12138765. http://www.springerlink.com/index/1EV9CJPJXH2CHJJA.

Xu, L.H., Q.R. Li and C.L. Jiang, 1996. Diversity of soil actinomycetes in Yunnan, China. Applied Environ. Microbiol., 62: 244-248. PMID: 16535212. http://aem.asm.org/cgi/reprint/62/1/244.

Yamamura, H., M. Hayakawa, Y. Nakagawa and Y. Iimura, 2003. Species diversity of nocardiae isolated from lake and moat sediment samples. Actinomycetology, 17: 44-46. DOI: 10.3209/saj.17_44. http://wwww.jstage.jst.go.jp/article/saj/17/2/44/_pdf.

Zarandi, M.E., G.H. Shahidi Bonjar, F. Padasht Dehkaei, S.A. Ayatollahi Moosavi, P. Rashid Farokhi and S. Aghighi, 2009. Biological control of rice blast (*Magnaporthe oryzae*) by use of *Streptomyces sindenensis* isolate 263 in greenhouse. Am. J. Applied Sci., 6: 194-199. http://www.doaj.org/doaj?func=abstract&id=422751. http://www.scipub.org/fulltext/ajas/ajas61194-199.pdf.