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RESEARCH ARTICLE

STATISTICAL ANALYSIS OF ACTINOMYCETES ISOLATED FROM THE HARIDWAR REGION OF UTTARAKHAND (INDIA)

Garima Arya[1], Jugmendra Singh[2]

[1]Departments of Microbiology, Gurukula Kangri Vishwavidhalaya, Haridwar, India

[2]Department of Applied Sciences & Humanities, Panipat Institute of Eng. & Technology, Panipat, India
*Corresponding Author's Email: garimaaryaphd@gmail.com, jugmendra@gmail.com

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ABSTRACT

Statistical analysis of Actinomycetes isolated from biodegraded and non biodegraded building materials at Haridwar region of Uttarakhand (India). The concentrations of viable actinomycetes varied between 50-250 x10^4 and 5-150x10^4 cfu/g in the biodegradable and non degradable buildings, respectively. In all studies, the concentrations of viable actinomycetes were higher in the index (biodegradable) buildings than in the reference (non biodegradable) buildings. Phylogenetic characterization of the isolates based on 16S rRNA gene sequencing supported their assignment to 18 different actinomycete genera representing seven different suborders. The result exhibited similarity 91% with 10 strains from the uncultured known species of *Streptomyces, Nocardia* and *Micromonospora*. The 16S rRNA nucleotide sequence of *Streptomyces, Nocardia* and *Micromonospora* consisted of 749 bp each and the G-C content was 70%. These results show a high diversity of actinomycetes associated with buiding materials and the detection and identification of this group of organisms is extremely important for the deteriogenic process and the development of control methods.

Keywords: Actinomycetes; Nocardia, Micromonospora, building materials, statistical phylogenetic analysis

INTRODUCTION

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily dwell in the soil (Kutzner, 1986)[1]. These are aerobic, Gram-positive bacteria. They are one of the major groups of soil population and are very widely distributed (Kuster, 1968)[2]. The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. Actinomycetes populations are relatively lower than other soil microbes and contain a predominance of *Streptomyces* that are tolerant to acid conditions (Crandal and Sieber, 1996)[3]. One of these well-known soil microbes is actinomycetes. There are about 100 genera of actinomycetes in the soil (Lo *et al*. 2002). However, Arid soils of alkaline pH tend to contain fewer *Streptomyces* and more of the rare genera such as *Actinoplanes* and *Streptosporangium*. However, alkaliphilic actionmycetes will provide a valuable resource for novel products of industrial interest, including enzymes and antimicrobial agents (Mitsuiki *et al*., 2002; Tsujibo *et al*., 2003)[4, 5].

As biodegradative agents, microorganisms are important in the degradation of soil organic materials into humus. But some actinomycetes secrete a range of enzymes that can completely degrade all the components of lignocellulose (lignin, hemicellulose and cellulose), while others may secrete a narrower range of enzymes that can only partially achieve this degradation (Mason *et al*., 2001)[6]. With their ability to secrete these enzymes, they are effective at attacking tough raw plant tissues and softening them for other microbes. The use of chemicals to control plant disease pathogens may be harmful for both human and environment. Kaushik and Chauhan (2009)[7] expressed that since pathogenic bacterial strains are gaining drug resistance. There is need to discover novel sources of antimicrobials. Many researchers are working towards isolating actinomycetes which have the ability to degrade harmful chemicals and also those with ability to act as biocontrol agents.

MATERIAL AND METHODS

Soil sample collection

Test samples were collected from index and reference building materials in winter and rainy seasons at Haridwar, Uttarakhand (India) . Each collection was made from 1-2 cm depth of or on the surface of materials (Kutzner, 1986)[1]. These were air-dried for 1 week (Williams, 1989)[8], crushed and sieved. The sieved soils were then used for actinomycete isolation. All the samples were collected from November 2008 to January 2010.

Isolation of actinomycetes

In this investigation, modified starch-casein agar was used for isolating actinomycetes and pH of media used was set to 7.2. Cyclohexamide and nystatin (0.050 mg/ml) were added into the medium as antifungal agent proposed by Lazzarini, (2000)[9]. Dehydrated nutrient agar was used for routine culture of the bacteria under test. Samples of 1 g each were mixed with 10 ml of sterile distilled water and incubated at room temperature for 1 h on orbital shaker with vigorous shaking. Soil suspension was then pipetted and spread onto Humic acid B-Vitamin maintained for longer period by serially subculturing.

Morphology identification

Actinomycetes were streaked onto actinomycetes isolation agar. Following Sorlini and *et al.*, (1987)[10], cover slip and Gram staining techniques were employed for microscopic observation where the cover slip was stabbed onto the agar at an angle of 45° and incubated at 30 °C for 6 days. After 6 days of growth, the actinomycetes were examined. Cover slips were then taken out from the agar and put onto the prepared slides. Crystal Violet staining dye was used for this purpose (Stackebrandt and *et al.*, 1992)[11]. Slides were then viewed using a research microscope. Identification of actinomycetes to genus level was then carried out based on 'Bergey's Manual of Determinative Bacteriology', 9th edition.

Test Buildings

Test buildings were categories into two types of building: (i) Index building (ii) Reference building

Index building The index building was biodegradable and moistened. These were also categorized into three categories:

(i) Industrial buildings
- Gurukul Kangri Pharmacy
- BHEL

(ii) Religious building
- Mansha Devi temple
- Daksh Prajapati temple

(iii) Residential building
- Gurukul Kangri residential building
- Old Kangri or Punya bhumi building

Reference building

Reference building was neither moistened nor biodegraded. These all four buildings locations were selected for the isolation of isolates of actinomycetes. The samples were collected into two seasons, winter (January) and rainy season (July) by scraping off surface material and plaster to a depth of 1-5 millimetre (mm). The total samples were split into two parts. One part was used for the isolation of actinomycetes by standard cultivation techniques and another part was used for scanning electron microscopy.

RESULTS AND DISCUSSION

The Sample Collected from Sampling Sites at Haridwar Region

The biodegraded building wall material analyze in study I and II, samples were collected into two seasons (winter and rainy) from each building. Thus seven wall material sample were selected for testing (six samples from index building and one from reference building) for the comparative study there results are tabulated in Tables 1 and 2. The samples were taken from painted plaster interior and exterior wall of a dwelling part and tiles from the side wall of the buildings. The observations recorded about cfu/g of material from different sampling sites are given in table 1.

Statistical analysis of concentration of the Isolates of Actinomycetes of the Building Materials

Biodegraded building material borne concentration (cfu/g) of viable isolates of actinomycetes in biodegraded wall are summarized in Tables 1 and 2. Concentration were higher in rainy season than that of winter season this is because in winter season temperature is low about 5-15˚C, this temperature is not favourable for growth of isolates of actinomycetes. In rainy season temperature become about 25-30˚C, which is the plus point in the favor of the growth of isolates. Thus, rainy season provide a favourable environmental condition for the growth of actinomycetes and the occurrence of actinomycetes genera in the residence building not more differ from that in industrial and religious buildings (Table 1 and 2). The most common actinomycetes genera were *Streptomyces* species together with *Nocardia* and *Micromonsopora* species. Some differences in the concentrations of individual genera were observed, concentrations of microbial genera in the rainy season were higher in moistened buildings than in winter season. In this study, mean ratio between an industrial, religious and residential buildings matched with each other, was found 13 *Streptomyces* species, 09 *Nocardia* and 07 *Micromonospora* species. On the basis of their occurrence all isolates of actinomycetes were categories into three groups as shown in Table 4.

Table 1: Test Buildings: Daksh Prajapati Tample, Punya Bhumi, Gurukul Gangri, B.H.E.L., Mansha Devi Pharmacy and Gurukul Kangri Pharmacy in winter season (average of three replicates)

S. No.	Dilution	Daksh Tample cfu/g soil ± SE	Punya Bhumi cfu/g soil ± SE	Gurukul Kangri cfu/g soil ± SE	B.H.E.L. cfu/g soil ± SE	Mansa Devi Tample cfu/g soil ± SE	Gurukul Kangri Pharmacy cfu/g soil± SE
I	10^{-2}	$195x10^2$ ± 4.3	$187x10^2$ ± 4.2	$214x10^2$ ± 5.3	$123\ x10^2$ ± 4.2	$203x10^2$ ± 3.6	$118x10^2$ ± 2.5
II	10^{-3}	$161x10^3$ ± 3.9	$160x10^3$ ± 3.9	$183x10^3$ ± 5.1	$120\ x\ 10^3$ ± 4.0	$175x10^3$ ± 3.5	$116x10^3$ ± 2.4
III	10^{-4}	$149x10^4$ ± 3.1	$145x10^4$ ± 3.5	$159x10^4$ ± 4.6	$115\ x\ 10^4$ ± 3.6	$143x10^4$ ± 3.1	$111x10^4$ ± 2.0
IV	10^{-5}	$100x10^5$ ± 2.4	$108x10^5$ ± 2.7	$137x10^5$ ± 3.6	$97x10^5$ ± 3.2	$101x10^5$ ± 2.4	$103x10^5$ ± 2.8
V	10^{-6}	$68x10^6$ ± 1.8	$86x10^6$ ± 2.1	$100x10^6$ ± 3.2	$76x10^6$ ± 2.8	$82x10^6$ ± 1.9	$83x10^6$ ± 1.5
VI	Control	-	-	-	-	-	-

Table 2: Test Buildings: Daksh Prajapati Tample, Punya Bhumi, Gurukul Gangri, B.H.E.L., Mansha Devi Pharmacy and Gurukul Kangri Pharmacy in rainy season (average of three replicates)

S. No.	Dilution	Daksh Tample cfu/g soil ± SE	Punya Bhumi cfu/g soil ± SE	Gurukul Kangri cfu/g soil ± SE	B.H.E.L. cfu/g soil ± SE	Mansa Devi Tample cfu/g soil ± SE	Gurukul Kangri Pharmacy cfu/g soil ± SE
I	10^{-2}	$211x10^2$ ± 4.1	$198x10^2$ ± 4.9	$218x10^2$ ± 5.7	$129\ x10^2$ ± 6.0	$210x10^2$ ± 5.3	$122\ x10^2$ ± 4.7
II	10^{-3}	$173x10^3$ ± 3.8	$165x10^3$ ± 4.5	$184x10^3$ ± 5.2	$126x10^3$ ± 5.4	$175x10^3$ ± 5.1	$121x10^3$ ± 4.6
III	10^{-4}	$155x10^4$ ± 3.2	$150x10^4$ ± 3.4	$161x10^4$ ± 4.7	$121x10^4$ ± 5.1	$152x10^4$ ± 4.6	$114x10^4$ ± 4.0
IV	10^{-5}	$116x10^5$ ± 2.8	$109x10^5$ ± 3.1	$141x10^5$ ± 3.9	$102x10^5$ ± 4.5	$118x10^5$ ± 3.2	$107x10^5$ ± 3.8
V	10^{-6}	$90x10^6$ ± 2.4	$83x10^6$ ± 2.8	$105x10^6$ ± 3.1	$86x10^6$ ± 4.0	$95x10^6$ ± 2.6	$89x10^6$ ± 3.2
VI	Control	-	-	-	-	-	-

Table 3: Non biodegradable Gurukul Kangri residential building in rainy and winter season (average of three replicates)

S. No.	Dilution	Dilution factor	Rainy Season cfu/g soil ± SE	Winter Season cfu/g soil ± SE
I	10^{-2}	10^2	$68x10^2$ ± 2.4	$50x10^2$ ± 2.1
II	10^{-3}	10^3	$54x10^3$ ± 2.2	$33x10^3$ ± 1.9
III	10^{-4}	10^4	$31x10^4$ ± 1.7	$29x10^4$ ± 1.4
IV	10^{-5}	10^5	$24x10^5$ ± 1.2	$13x10^5$ ± 1.0
V	10^{-6}	10^6	$15x10^6$ ± 1.1	$5x10^6$ ± 0.2
VI	Control	-	-	-

Table 4: Categories of isolates of actinomycetes

Group	Genus	Isolates symbol
Group I	*Streptomyces* species	A_1, A_2, A_3, A_9, A_{10}, A_{11}, A_{16}, A_{17}, A_{21}, A_{22}, A_{23}, A_{24} and A_{29}
Group II	*Nocardia* species	A_4, A_5, A_7, A_{12}, A_{15}, A_{18}, A_{19}, A_{20} and A_{26}
Group III	*Micromonospora* species	A_6, A_8, A_{13}, A_{14}, A_{25}, A_{27} and A_{28}

Table 5: Statistical concentration of viable isolates of actinomycetes in industrial building, religious building and residential building

Total isolates 29	Industrial building		Religious building		Residential building		Study
	No. of isolates	**Range of colonies**	**No. of isolates**	**Range of colonies**	**No. of isolates**	**Range of colonies**	
***Streptomyces* species**	4	10-30	3	0-25	6	0-25	I
***Nocardia* species**	2	0-20	3	0-15	4	0-16	I
***Micromonospora* species**	-	00	1	0-10	6	0-10	I

The results of study-I are obtained on NAM, SCAM, AIAM and GAAM (Crook and Goodman, 1996)[12].

Percentage of isolates of *Streptomyces* species in industrial, religious and residential building were 10.23%, 12.48% and 22.12% respectively, and Nocardia species were 4.80%, 7.49% and 18.75% in industrial, religious and residential buildings respectively. The prevalence percentage of *Micromonospora* species in industrial building was not found but in religious and residential buildings percentage were 7.20% and 10.56%. Thus the total percentages of *Streptomyces*, *Nocardia* and *Micromonospora species* were 44.83%, 31.04% and 24.13 in the respective buildings (Table 5). The results are obtained on media AIAM, NAM and binnet agar.

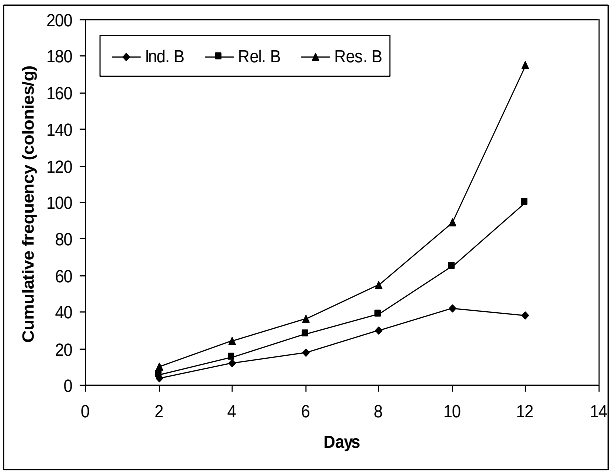

Figure 1: Shows the cumulative frequencies distribution of total concentration of viable actinomycetes in moisture damaged test buildings

Phylogentic Classification of Isolates of Actinomycetes

The alignment of the nucleotide sequence (749 base pair (bp)) of actinomycetes isolates were done through matching with the 16S rRNA reported gene sequence in the gene bank (Tamura and *et al.*, 2007)[13]. The data base of national center for biotechnological information (NCBI) basic local alignment search tool (BLAST) available at (www.ncbi-nlm-ningov) was used to compare the isolate strains with sequence of the reference species of the isolates $A_{11,}$ A_{20} and A_{27} contained in genomic database bank and produced a ethidium bromide stained gel separation pattern of actinobacteria DNA fragment coding for 16S r RNA (Figure 2).

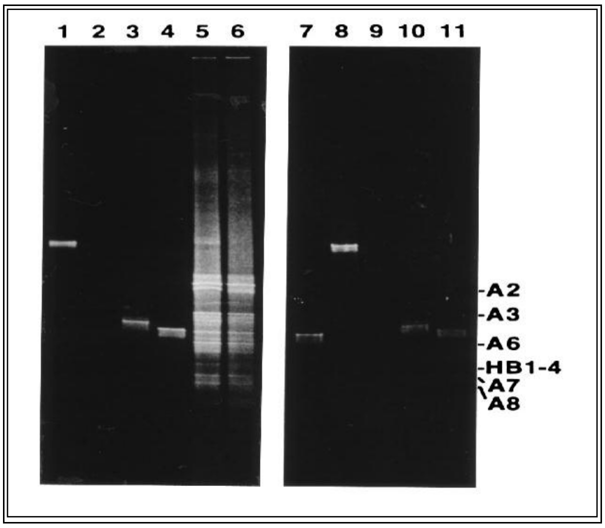

Figure 2: Gel Image of 16S rDNA amplicon

This Figure 2 represents the ethidium bromide-stained DGGE separation pattern of bacterial DNA fragment coding for the 16S rRNA. Lanes 1 to 4 and 7 to 11 show the fragments obtained from actinomycetes isolates A_{11} (lane 2, 3 and7), A_{20} (lanes 1, 8 and 10), A_{27} (lanes 4, 9 and 11), and (lanes 5 and 6, representing control DNA. A weak band was visible only in the original gel. The position of the band is marked.). Lanes 5 and 6 show the reproduced band patterns of the 16S rRNA genes which were amplified directly from DNA. Bands A2, A3, A6, A7, and A8 were excised and sequenced.

The result exhibited similarity 91% with 10 strains from the uncultured known species of *Streptomyces*, *Nocardia* and *Micromonospora*. The 16S rRNA nucleotide sequence of *Streptomyces*, *Nocardia* and *Micromonospora* consisted of 749 bp each and the G-C content was 70%. These results were in accordance to many authors who mentioned that the G-C content of the actinomycetes DNA is 69-78% (Williams *et al.*, 1989)[8]. Modern identification system are based on 16S rRNA sequence data, which have provided valuable information about test isolates (A_{11}, A_{20} and A_{27}) systematically and then have been used to identify several new isolates (Tamura *et al.*, 2007)[13].

A phylogenetic tree was constructed based on a 1216 bp alignment of the cloned PCR amplicons and known actinomycete sequences (Williams, 1989)[8]. The actinomycete 16S rDNA sequences amplified from DNA isolated from isolates of building walls affiliated with several clusters in the phylogenetic tree. The clones affiliated with three beanches, one including Nocardia *beijingensis*, *Nocardia araoensis* and *Nocardia puris* second include the *Micromonospora carbonacea*, *Micromonospora chalcea* and the third including *Streptomyces ambofaciens*, *Streptomyces rutgersensis* and *Streptomyces melanosporofaciens.* Other sequevars were also present; some of them being distantly related to sequences obtained from database. The isolate A_{11} closely related to *Streptomyces rutgersensis* and *Streptomyces sporocinereus*, isolate A_{27} closely linked to *Micromonospora matsumotoense*, *Micromonospora siamensis* and *Micromonospora mirobrigensis* and the third isolate linked to the known species of *Nocardia araoensis*, *Nocardia pneumonia* and *Nocardia beijingensis* as shown in Table 6.

Table 6: Sequence producing significant alignments of isolates of actinomycetes

Accession	Description	Max score	Total score	Query Coverage	Max. ident.
AF154129	*Nocardia* spp. Pstl 16S ribosomal RNA gene	2246	2246	100%	100%
AB108780	*Nocardia* strain IARI-CS-70 16S ribosomal RNA gene	2246	2246	100%	100%
Sample A_{20}	*Nocardia* strain PstI 16S ribosomal RNA gene	2246	2246	100%	100%
AB108779	*Nocardia* strain HaNA4 16S ribosomal RNA gene	2246	2246	100%	100%
AB108781	*Nocardia* strain PRE1 16S ribosomal RNA gene	2246	2246	100%	100%
AB092566	*Nocardia* strain PstI 16S ribosomal RNA gene	2246	2246	100%	100%
Z36936	*Nocardia* strain PstI 16S ribosomal RNA gene	2246	2246	100%	100%
AB108778	*Nocardia* spp. 08EPH131 16S ribosomal RNA gene	2246	2246	100%	100%
AB108775	*Nocardia* spp. SBS 16S ribosomal RNA gene	2246	2246	100%	100%
AJ508748	*Nocardia* strain PRE1 16S ribosomal RNA gene	2246	2246	100%	100%
X925599	*Micromonospora* spp.08EPH 16S ribosomal RNA gene	2246	2246	100%	100%
AF152109	*Micromonospora* spp. Asnl16S ribosomal RNA gene	2246	2246	100%	100%
Sample A_{27}	F75061 strain 16S ribosomal RNA gene	2246	2246	100%	100%
AJ626950	Sna Bl 16S ribosomal RNA gene	2246	2246	100%	100%
AB193565	Pvul 1 strain 16S ribosomal RNA gene	2246	2246	100%	100%
X92601	Pvul 1 strain 16S ribosomal RNA gene	2246	2246	100%	100%
X92611	KpnI 16S ribosomal RNA gene	2246	2246	100%	100%
X92604	KpnI 16S ribosomal RNA gene	2246	2246	100%	100%
X92594	AgeI strain 16S ribosomal RNA gene	2246	2246	100%	100%
M27245	AgeI strain 16S ribosomal RNA gene	2246	2246	100%	100%
AJ391820	Pvul 1 strain 16S ribosomal RNA gene	2246	2246	100%	100%
AJ781368	Sph I 1 strain 16S ribosomal RNA gene	2246	2246	100%	100%
Strain A_{11}	Sph I 1 strain 16S ribosomal RNA gene	2246	2246	100%	100%
AY508511	AgeI strain 16S ribosomal RNA gene	2246	2246	100%	100%
AJ781369	Sph I strain 16S ribosomal RNA gene	2246	2246	100%	100%
AJ391837	AgeI strain 16S ribosomal RNA gene	2246	2246	100%	100%

The evolutionary analysis was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.16216694 is shown. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 788 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. However, the results indicate the presence of several different types of isolates of *Streptomyces*, *Nocardia* and *Micromonospora* in biodegraded building material samples, suggesting higher diversity with several species.

CONCLUSION

The existing molecular techniques have proved to be reasonably efficient for the identification of actinomycetes. However, the groups found on buildings are considerably different. Currently, there are a few research groups worldwide which are studying the role of actinobacteria in the degradation of historic monuments. Certainly, this will lead to the development of rapid and reliable molecular techniques for the identification of this problematic group of actinomycetes.

It is hoped that an integrated approach of molecular and microbiological techniques will lead to a better understanding of the composition of bacterial species on ancient wall material and to new ways to restore and conserve this cultural heritage. Thus, to control moisture, to ensure long building life and good indoor air quality, three goals must be set and met:

- Control liquid water;
- Manage indoor humidity levels and condensation; and
- Select material and hygrothermal assembly designs that minimize mould growth and other moisture problems.

The role of actinomycetes in the degradation of buildings like cultural heritage cannot be neglected. Since they are saprophytes and require no more light, water, and mineral ions to grow. These microorganisms, along with bacteria and fungi, readily colonize the external surfaces of historic monuments and develop a biofilm, which, in turn, alters the appearance of the building and serves as a substrate for the growth of other biodeteriogens. Both these and the actinobacteria, themselves can cause aesthetic, chemical and physical decay. The detection and identification of this group of organisms is extremely important for the future study of the deteriogenic process and the development of control methods.

REFERENCES

1. Kutzner, KJ. The family *Streptomycetaceae*. In: Starr, M. P., Stolp, H., Trper, H. G., Balows, A., Schlegel, H. G. (Eds) The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Springer-Verlag, New York, 1986,; 2: 2028-2090.
2. Kuster, E. The actinomycetes. In: Soil Biology, eds. Burges (A.) & Raw (F.), Academic Press, London, 1968.
3. Crandall, M. S. and Sieber, WK. The national institute of occupational safety and health indoor environmental evaluation experience. Part one: Building environmental evaluations. *Applied Occupational Environmental Hygiene,* 1996; 11: 533-539.
4. Lo, C.W., Lai, N.S., Cheah, H.Y, Wong, N.K.I. and Ho, C.C. Actinomycetes isolated from soil samples from Crcker Range, Sabah. *ASEAN Review on Biodiversity and Environmental Conservation* July–September 2002.
5. Tsujibo H, Kubota T, Yamamoto M, Miyamoto K, Inamori Y. Characteristics of chitinase genes from an alkaliphilic actinomycete, *Nocardiopsis prasina* OPC-131. Appl. Environ. Microbiol. 2003, 69: 894-900.
6. Mason, M., Ball, A., Reeder, B.J., Silkstone, G., Nichollis, P. and Wilson, M.T. Extracellular Heme Peroxidases in Actinomycetes: A case of mistaken identity. *Applied and Environmental Microbiology,* 2001; *67:* 4512–9.
7. Kaushik, P. and Chauhan, A. Cyanobacteria: Antibacterial Activity. New India Publishing Agency, 101 Vikas Surya Plaza, CU Block, Pitam Pura, New Delhi 110088, 2009, pp xiii+1-198.
8. Williams, S. T. Bergey's manual of systematic bacteriology. (Ed) Murray, B., Krieg, H. The Williams and Wilkins Company, Batim, 1989; 4: 2341-2451.
9. Lazzarini, A. Cavaletti, L., Toppo, G. and Marinelli, F. Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhoek,* 2000; 78: 399-405.
10. Sorlini, C., Sacchi, M. and Ferrari, B. Microbiological deterioration of Gambara's frescos exposed to open air in Brescia, Italy. *International Journal of Biodeterioration,* 1987; 23: 167-179.
11. Stackebrandt, E., Liesack, W. and Witt, D. Ribosomal RNA and rDNA sequence analysis. *Gene,* 1992; 115: 255-260.
12. Crook, B. and Goodman, G. C. Mushroom worker's lung: A clinical, serological and environmental study of work conditions in a commercial mushroom farm. *Occupational Environmental Medicine,* 1996; 21: 45-52.
13. Tamura, K. Dudley, J. Nei, M. and Kumar, S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution,* 2007; 24: 1596-1599.

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