Chronology statistics such as mean sensitivity (0.51) and standard deviation (0.58) indicate high variability in ring-width patterns, largely due to high sensitivity of trees to climatic fluctuations. Existence of strong correlation between trees ($r = 0.49$) shows the presence of coherent growth pattern among trees, which is largely controlled by climate. The tree-ring chronology shows a surge in ring-width indices from AD 1100 to 1228 (Figure 2). Whether the high index values for such a century-long period are tree-specific or climate-forced is difficult to conclude at present until this part of the chronology is replicated with more tree samples.

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ACKNOWLEDGEMENTS. We thank the Department of Forests, Government of Uttaranchal for help during field trips in 2000 and 2003 for the collection of samples. J.S. and R.C. thank DST, New Delhi for financial support.

Received 1 August 2003; revised accepted 27 October 2003

Isolation, characterization and antimicrobial activity of marine halophilic *Actinopolyspora* species AH1 from the west coast of India

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A halophilic *Actinopolyspora* species AH1 was isolated from a marine sediment sample obtained from Alibag coast, Maharashtra. The strain showed good growth in medium containing 10 to 15% (w/v) NaCl and with 30 to 36°C temperature. Cultural properties of *Actinopolyspora* were studied extensively on starch casein agar and other media. Strain AH1 showed an elongated and circular shape with 20 to 30 spore-chain structures observed by slide culture technique and scanning electron microscopy. The morphological, biochemical and physiological characters of the isolate conformed to the characteristics of the genus *Actinopolyspora*, which contained only three species, viz. *A. halophila*, *A. mortivallis* and *A. iraqiensis*. However, the *Actinopolyspora* species AH1 isolated and characterized by us showed different properties compared to the known species. It showed resistance to clindamycin, vancomycin, nalixidic acid and streptomycin antibiotics. Interestingly, it showed good antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis* and antifungal activities against *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Fusarium oxysporum*, *Penicillium* species and *Trichoderma* species. This is a report of the antimicrobial activity exhibited by *Actinopolyspora* species AH1 isolated from the marine sediment.

IN recent years marine microorganisms have become important in the study of novel microbial products exhibiting antimicrobial, antiviral, antitumor as well as anticoagulant and cardioactive properties[1–3]. These active compounds may serve as model systems in the discovery of new drugs[4,5]. Halophilic species have been described as *Actinopolyspora halophila*, *Actinopolyspora mortivallis* and *Actinopolyspora iraqiensis*[6–9]. During the course of screening for antibiotics from marine actinomycetes, a halophilic *Actinpolyspora* species was isolated from Alibag, a coastal region in the west coast of India. Species AH1 isolated in the present study required 8 to 15% (w/v) NaCl for growth. This communication deals with isolation, characterization and antimicrobial activities of marine halophilic *Actinopolyspora* species AH1.

Strain AH1 showed good growth on starch casein agar (SCA) after 3 days. The medium consisted of 1% soluble starch, 0.1% casein, 0.05% KH_2PO_4, 0.05% $MgSO_4$, 3% NaCl, 50% natural sea water collected from Alibag sea coast and 50% distilled water as a base. The final pH of the medium was adjusted to 7.6 with 0.1 N NaOH before sterilization[10–13]. The inoculated plates were incubated at 28°C for 7 days. After isolation, strain AH1 was purified by streak plate method and stock culture was maintained on SCA slant at 4°C in a refrigerator[14,15]. Growth characteristics were observed using different types of media, such as modified SCA, glucose asparagine agar, glycerol asparagine agar, tyrosine agar, yeast extract–malt extract agar, nutrient agar, maltose yeast extract agar and glycerol glycine agar[16,17]. Colonies showed wrinkled appearance on the surface of SCA containing 8 to 15% (w/v) NaCl, and hence the strain was considered as a halophilic species of the genus *Actinopolyspora*. The growth was found to take place in the temperature range of 10–45°C and 28°C was found to be optimum for growth. The strain was grown on SCA at different pH values such as 3.5, 5, 7 and 9 for 8 to 10 days, and pH 7.6 was found to be optimum for

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Table 1. Cultural characteristics of *Actinopolyspora* AH1 species on different media*

Medium	Growth	Aerial mycelium	Substrate mycelium	Pigment
Starch casein agar	Good	White	Yellowish-white	Brown
Glycerol asparagine agar	Good	White	White	None
Glucose asparagine agar	Moderate	White	Yellowish-white	None
Yeast malt extract agar	Moderate	Yellowish-white	Dark orange	Pale yellow
Tyrosine agar	Good	White	Yellowish-white	Pale yellow
Nutrient agar	Moderate	White	Yellowish-brown	None
Maltose yeast extract agar	Good	White	Yellowish-white	None
Medium glycerol glycine	Good	White	Yellowish-white	None

*Each medium was prepared in artificial sea water as a base containing 3% (w/v) NaCl.

Table 2. Carbon utilization and acid production of *Actinopolyspora* AH1 and other strains*

	AH1 species		*A. halophila*		*A. mortivallis*		*A. iraqiensis*	
Carbon source	Utilization	Acid production	Utilization	Acid production	Utilization	Acid production	Utilization	Acid production
D-glucose	+	–	+	–	+	–	+	ND
D-arabinose	±	–	++	–	–	–	+	–
Sucrose	–	–	+	–	+	+	+	–
D-xylose	–	–	+	–	+	+	–	–
M-inisitol	±	–	++	–	–	–	+	–
D-mannitol	+	–	+	–	–	–	+	+
D-fructose	+	+	+	–	+	ND	+	–
L-rhamnose	+	–	++	–	–	–	–	–
Raffinose	–	–	+	–	+	–	+	–

++, Strongly positive utilization; +, Positive utilization; ±, Utilization doubtful; –, Utilization negative; ND, Not determined.
*Carbon utilization and acid production were performed using basal medium with 1% (w/v) carbon source at 28°C temperature and observed after 14 days.

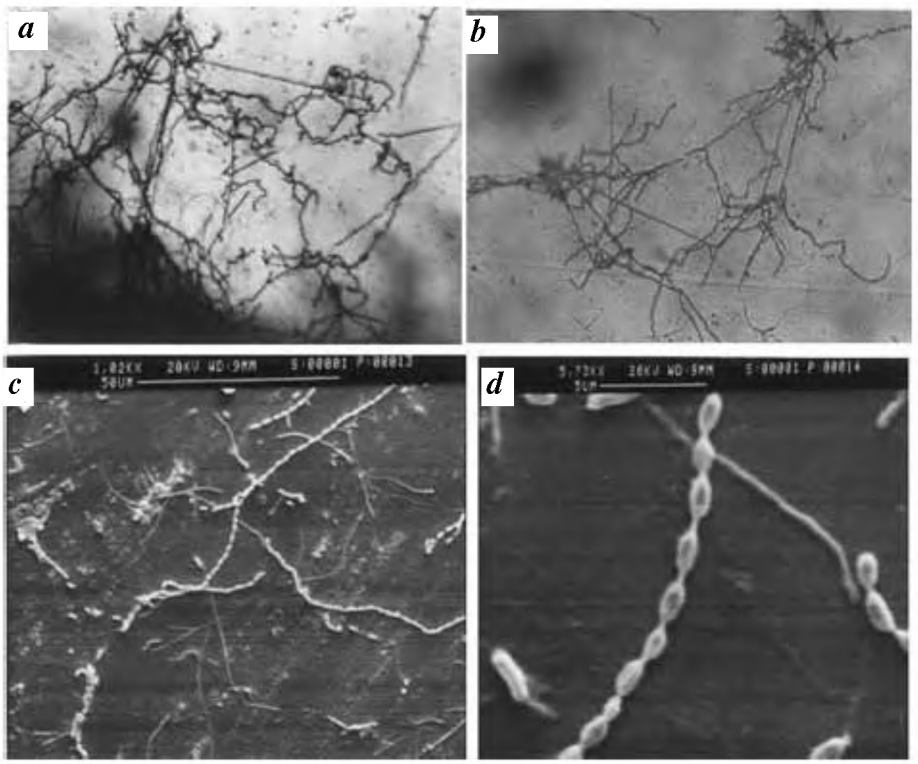

Figure 1. Spore chain structure of *Actinopolyspora* species AH1. ***a***, Light microscope × 100; ***b***, Light microscope × 400; ***c***, Scanning electron micrograph × 1020; ***d***, Scanning electron micrograph × 5730.

growth. Pigmentation, different colours[18,19] of aerial mycelium and substrate mycelium were observed for 14 days on different media at 28°C (Table 1).

The spore mass of AH1 was found to be white in colour and appeared abundant, particularly on SCA containing 10% (w/v) NaCl. Colonies were 4 to 6 mm in diameter, thin, elongated and convex. On the reverse side of the culture growth, the colonies were yellow to brown in colour. Substrate mycelia were well developed, branched and mostly unfragmented. Spores were not observed on the substrate mycelium. The fine structure was mainly studied[20–22] using compound microscope (× 100, × 400; Figure 1 *a* and *b*) and scanning electron microscope (× 1020, × 5730 Cambridge, UK; Figure 1 *c* and *d*). The sporophores were straight and long up to more than 25 spores. Spores varied in size and shape within a single sporophore. All spores showed a smooth surface and the shape varied from spherical to a short rod with rounded ends[23,24]. The spore diameter varied from 0.7 to 1.0 μm and length varied from 1.0 to 2.0 μm. A gap or small plug separated some spores. At all stages of growth, cells were found to be Gram-positive and acid-fast.

Carbon and nitrogen utilization, and biochemical tests were performed according to standard methods described for actinomycetes[24,25]. Strain AH1 used D-glucose, D-mannitol, D-fructose and L-rhamnose as a carbon source for growth; however, sucrose, D-xylose and raffinose were not utilized. Acid production was mainly observed from fructose. Carbon utilization and acid production of AH1 species and known species are shown in Table 2. L-asparagine, DL-alanine, L-cysteine, L-tyrosine and potassium nitrate were used as a nitrogen source for growth. Nitrate was reduced to nitrite. Gelatin was liquefied. Strain AH1 also produces enzymes such as amylase, protease and lipase.

Diaminopimelic acid (DAP) present in the cell wall was determined by hydrolysates of whole cells and of isolated cell walls[26–28]. Paper chromatography

Table 3. Characteristics of *Actinopolyspora* AH1 and known species of halophilic *Actinopolyspora*

Characteristics	Strain AH1	*A. halophila*	*A. mortivallis*	*A. iraqiensis*
Spore chain morphology	Long (up to 30), elongated	Long (up to 20), elongated	Short (up to 10), oval	Short (up to 15) spherical
Growth in 10% (w/v) NaCl				
Colour of colony	White	Black	Pale yellow	Pale brown
Colour of soluble pigments	Brown	Black	Brown	None
Growth in				
5% (w/v) NaCl	+	–	+	+
25% (w/v) NaCl	–	+	+	–
NaCl concentration (% w/v) for optimal growth	8–15	15–20	10–15	10–15
Growth at 42°C	+	+	+	–
Optimum temperature (°C)	32–34	37	45	30–35
Temperature for max. growth (°C)	45	43	50	40
Enzyme production				
Amylase	+	+	+	+
Gelatinase	+	+	+	ND
Nitrate reductase	+	+	–	ND
Protease	+	+	+	+
Lipase	+	+	+	+
Sensitivity to antibiotics*				
Amoxycillin (10)	S	S	R	S
Clindamycin (10)	R	S	S	R
Vancomycin (30)	R	S	S	R
Ampicillin (10)	S	R	S	ND
Carbenicillin (10)	ND	R	S	S
Tetracycline (10)	S	S	S	ND
Chloramphenicol (10)	S	S	S	S
Erythromycin (15)	S	S	S	S
Streptomycin (10)	R	R	R	ND
Nalidixic acid (30)	R	R	R	ND

+, Positive reaction; –, Negative reaction; ND, Not determined; S, Sensitive, R, Resistant.
*Concentration of antibiotics is in μg/disc.
Data of *A. halophila*, *A. mortivallis* and *A. iraqiensis* were collected from the literature.

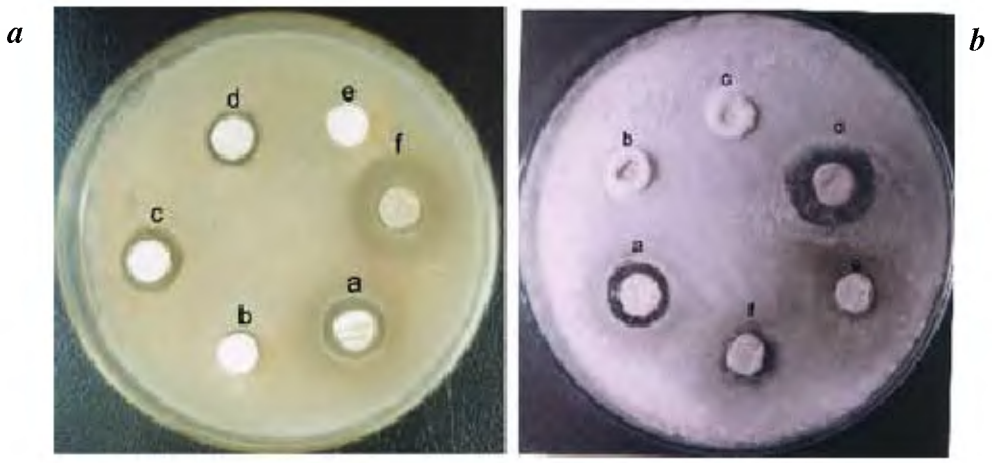

Figure 2. Antimicrobial activity of *Actinopolyspora* species AH1 employing cylinder plate method. (*a*) Against *S. aureus* and (*b*) against *Trichoderma* species. a, SCA; b, Glycerol asparagine agar; c, Glucose aspargine agar; d, Maltose yeast extract agar; e, Medium glycerol glycine and f, Tyrosine agar.

Table 4. Antimicrobial activity of marine *Actinopolyspora* species AH1*

Micro-organism	Test culture	Diameter of zone of inhibition (mm)
Bacteria	*Bacillus subtilis*	12.5
	Staphylococcus aureus	17.5
	Staphylococcus epidemidis	13.2
	Pseudomonas aeruginosa	–
	Escherichia coli	–
	Serratia marcescens	–
	Enterobacter aerogenes	–
Fungi	*Aspergillus niger*	12.0
	Aspergillus fumigatus	11.5
	Aspergillus flavus	15.8
	Fusarium oxysporum	16.2
	Trichoderma species	13.5
	Penicillum species	17.0
	Cryptococcus species	–
	Candida albicans	–

*Activity was performed using cylinder plate technique and SCA media containing artificial sea water as a base with 3% (w/v) NaCl.
This result is the mean of three experiments.
–, No activity.

showed that only meso-DAP was found to be present. The whole cell hydrolysate contained arabinose, galactose and ribose. From the DAP and sugar analysis, it was concluded that strain AH1 had a type-IV cell wall. Strain AH1 also contains a number of ester-linked phospholipids as well as glycolipids and neutral lipids. However, no ether-linked phospholipids were found (data not shown). Mycolic acids were absent[9]. The findings that strain AH1 has a chemotype-IV cell wall and absence of mycolic acids correspond to the characteristics of the genera *Actinopolyspora*, *Thermomonospora*, *Micropolyspora*, *Pseudonocardia*, *Nocardia* and *Saccharopolyspora*. It is important to note that the presence of spores in a chain occurring only on aerial mycelium eliminates all genera, except *Actinopolyspora* and *Saccharopolyspora*[27,29]. Substrate mycelium was not fragmented into rod-shaped elements, which clearly indicated that strain AH1 belongs to the genus *Actinopolyspora*. All the properties of the *Actinopolyspora* species AH1 were compared with three known species of *Actinopolyspora* (Table 3). It was observed that strain AH1 differs from known species of *Actinopolyspora*.

The strain AH1 was screened for antimicrobial activity by cross-streak method and cylinder plate method[30,31]. AH1 was grown on different modified agar media at 28°C for 7 days and then transferred onto plates containing test organisms. Plates were further incubated at 37°C for 24 h for bacteria and at 28°C for 4 days for fungi, and the zone of inhibition around the isolates was examined. AH1 showed good activity against Gram-positive bacteria like *S. aureus*, *S. epidermidis*, *B. subtilis* and fungi such as *A. niger*, *A. fumigatus*, *A. flavus*, *F. oxysporum*, *Penicillium* species and *Trichoderma* species. Diameter of zone of inhibition varied from 11.5 to 17.5 mm (Table 4), and it confirmed antimicrobial activity. AH1 did not show any antimicrobial activity against Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, *S. marcescens*, *E. aerogenes* and fungi like *C. albicans* and *Cryptococcus* species. Data clearly indicate that strain AH1 exhibited strong antimicrobial activity against Gram-positive bacteria as well as fungi. Antimicrobial activity was found to depend on the medium[32,33] used for growth. AH1 species grown on tyrosine agar showed good antibacterial activity against *S. aureus* compared to maltose yeast extract agar, SCA and glucose asparagine agar. However, species AH1 grown on glycerol glycine agar and glycerol asparagine agar did not show any antibacterial as well as antifungal activity. Strain AH1 grown on glucose asparagine agar exhibited antimicrobial activity against *S. aureus* (Figure 2*a*). However, it failed to show its activity against *Tri-*

choderma species (Figure 2*b*). AH1 species grown on maltose yeast extract agar showed good antifungal activity against *Trichoderma* species compared to SCA and tyrosine agar. Thus antimicrobial activity exhibited by *Actinopolyspora* species AH1 seems significant.

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ACKNOWLEDGEMENT. We thank the Director, Agharkar Research Institute, Pune for help in scanning electron microscope studies.

Received 3 May 2003; revised accepted 28 October 2003