American Journal of Biochemistry and Biotechnology 5 (4): 221-225, 2009
ISSN 1553-3468

Screening of Actinomycete Isolates from Niche Habitats in Manipur for Antibiotic Activity

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Abstract: Problem statement: The exhaustion of the usual terrestrial sources and the rise of resistant pathogens dictate the search for novel actinomycetes and new antibiotics. In this context, niche habitats such as caves, pristine forests, lakes, rivers, and other wetlands, high salt environments, marine ecosystems and endophytic niches are promising targets for survey of bioactive actinomycetes. **Approach:** Actinomycetes were isolated from several niche habitats in Manipur, India, on selective media such as SCNA and Chitin agar with or without antibiotics. Selected isolates were subjected to antimicrobial activity screening by Kirby-Bauer method. **Results:** 172 lake sediment (SCNA, LS1 series), 35 lake sediment (CA, LSCH series), 120 river (NRP, NRB and..series), 39 forest (AML series), 35 cave (KC1 series), 101 salt spring (NH, N3S and .. series), 46 Shirui jungle (SJ series) and 66 Shirui hill (SH series) actinomycetes isolates were obtained. Of 99 randomly selected isolates screened, 37 had antimicrobial activities against 1 or more indicator strains: 32 against Gram positive bacteria and 8 against Gram negative bacteria; 10 actinomycete strains were antimycotic and 3 had broad-spectrum antibiotic activities. About 18 potent antibacterial, 1 anti pseudomonas, 1 exclusively antifungal and 3 broad-spectrum antimicrobial actinomycetes were chosen for further studies. **Conclusion:** Niche habitats in Manipur especially wetlands show great promise for discovery of bioactive actinomycetes.

Key words: Actinomycetes, niche habitats, antimicrobial activity, Manipur

INTRODUCTION

Actinomycetes are a group of Gram-positive, high G+C, filamentous bacteria. They are excellent elaborators of biotechnological products such as antibiotics, industrial enzymes and other bioactive compounds[6,9,11,12]. They, especially *Streptomyces* species, account for more than 70% of the total antibiotic production. However the survey of streptomyces and other common terrestrial actinomycetes is nearly exhausted. This and the rise of antibiotic-resistant pathogenic strains dictates an increasing need for the survey of unexplored and underexplored niche habitats for novel antibiotic-producing Actinomycetes strains.

Intense screening of actinobacteria especially rare actinomycetes is taking place all over the world[4]. Exploration of actinomycete diversity of Manipur[15,16]-part of the Indo-Myanmar hotspot-holds promise for isolation of biotechnologically significant strains of actinomycetes and, even, novel species. Our main objective is to survey niche habitats in Manipur for isolating promising actinomycete strains and screening them for antibiotic activities.

MATERIALS AND METHODS

Soil sampling and pretreatment: Soil samples were collected from different niche habitats of Manipur comprising of lake sediments (Loktak Lake, the largest freshwater lake in North-East India), river sediments (Nambul River), cave (Khangkhui cave in Ukhrul district), forest areas (Shirui Jungle and Shirui Lily Hills in Ukhrul district and Amamlok Hills in Imphal West district) and salt springs (Ningel and Shikhong salt springs in Thoubal district). Each collection was made from 10-15 cm depth of the soil[14]. These were air-dried for 1 week[17], crushed and sieved. The sieved soils were then used for actinomycete isolation.

Enrichment and Isolation: One gram each of the sieved soil samples was treated with 0.1 g $CaCO_3$ and incubated at an ambient temperature for about a week.

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It was then suspended in 99 mL sterile distilled water and kept in an orbital shaker at 150 rpm for 30 min[5,14]. Serial dilution plating of the sample were performed (in duplicate) in SCNA (pH 7.3)[2,5,8,13] in presence or absence of 25 μg mL^{-1} nystatin and/or cycloheximide. Another sample from Loktak Lake was incubated in Chitin Agar (pH 7.0)[7,10] for isolation of rare actinomycetes. The plates were incubated at 28-30°C for 2-3 weeks. Isolated colonies were further subcultured on SCNA plates[14,18]. Selected pure isolates were then used for antimicrobial assay.

Test organisms: The test organisms used were the Gram positive bacteria *Staphylococcus aureus* (MTCC 96), *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 121) and the Gram negative bacteria *Escherichia coli* (MTCC 739) and *Pseudomonas aeruginosa* (DN1, lab isolate). The test yeast/fungus used was *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 1344).

Antimicrobial activity: Morphologically distinct actinomycete isolates were selected for antimicrobial activity screening against the pathogenic test organisms. This was done by using Kirby-Bauer method[3].

Test isolates were grown on antibiotic producing medium (GS medium) in an orbital shaker (150 rpm ambient^{-1} temperature). The fermentation broths were centrifuged after 3rd, 5th and 7th day of incubation and the supernatant were used as crude antibiotic extract of the isolates.

Test pathogens were spread on the test plates-Nutrient Agar (NA) for bacteria and Sabouraud Agar (SA) for yeast/fungi. Sterile antibiotic discs (HiMedia, 5 mm diameter)-impregnated with the crude antibiotic extract of the test isolates-were transferred to the test plates and incubated for 24-48 h. The zone of inhibition (in mm diameter) were read and taken as the activity against the test pathogen.

RESULTS

A good number of isolates were obtained from the different soil samples collected. 172 isolates were obtained from sample collected from Loktak Lake (LS1). 39 (AML), 25 (KC1), 101 (N3AH, N3S, NH1), 120 (NRB1, NRP1, NRS1), 46 (SJ) and 66 (SL) isolates were obtained from the soil samples of Amamlok Hills, Khangkhui cave, Nambul river, Ningel salt springs, Shirui Jungle and Shirui Lily Hills respectively. In the lake sample isolated using Chitin Agar (LSCH), we could get 35 isolates (Table 1).

Table 1: Actinomycete isolates from various niche habitats

Sample/collection site	No. of strain	Designation
Amamlok forest	39	AML
Khangkhui cave	25	KC1
Loktak sediments	172/35	LS1/LSCH
Nambul river bank/ plant deposit/sediment	48/40/32	NRB1/NRP1/NRS1
Ningel salt spring	68/29/20	NH1/N3S/N3AH
Shirui jungle	46	SJ
Shirui lily hill	66	SL

Table 2: List of bioactive actinomycetes from various habitats

Strain designation	No. of strain screened	No. of bioactive strain
KC1	7	2
LS1/LSCH	36	12
NRB1/NRP1/NRS1	28	12
NH1/N3S/N3AH	16	7
SJ/SL	12	4

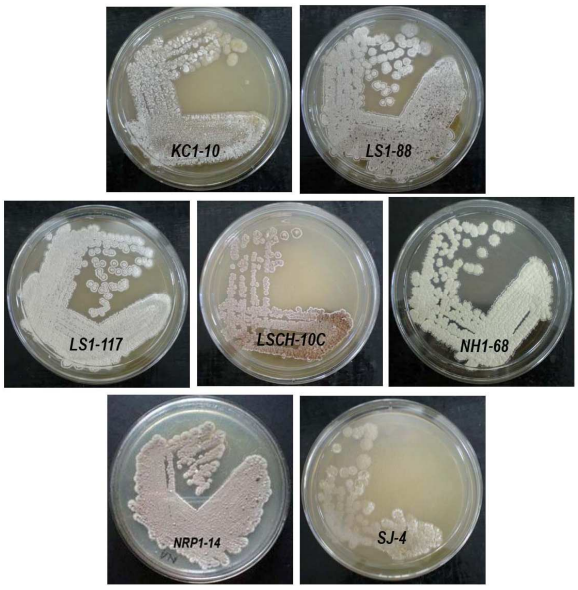

Fig. 1: Some of the bioactive actinomycete isolates from the various niche habitats of Manipur

Of the 99 isolates screened so far, 37 of them show antimicrobial activity against one or more of the test pathogens (Table 2). Of these, 32 isolates showed good antimicrobial activity against the Gram-positive bacteria, mostly against *B. subtilis* MTCC 121, 8 against Gram-negative organisms and 10 against Yeast/Fungi, with 3 showing broad spectrum of activity. Some of the representative bioactive strains are shown in Fig. 1 and 2. The antimicrobial profiles of selected actinomycete isolates are shown in Table 3.

Table 3: Antimicrobial profile of selected actinomycetes isolates

	Inhibition zone (mm)						
	Test organisms						
	Gram positive bacteria			Gram negative bacteria		Yeast/fungi	
Test isolates	MTCC 96	MTCC 106	MTCC 121	MTCC 739	DN1*	MTCC 227	MTCC 1344
KC1-7	-	13	16	-	-	-	-
KC1-10	-	14	11	15	-	13	-
LS1-11	-	-	22	-	-	-	-
LS1-81	-	-	17	-	-	-	-
LS1-88	-	-	21	-	-	-	-
LS1-117	21	16	13	-	-	-	-
LS1-145	-	-	12	-	-	-	-
LS1-172	-	-	21	-	-	13	-
LSCH-2	-	-	18	-	-	-	-
LSCH-8	-	-	-	-	-	11	-
LSCH-10C	24	23	12	-	-	12	13
LSCH-11A	-	-	21	-	-	-	-
LSCH-23	-	-	-	-	11	-	-
LSCH-31	23	18	-	-	-	-	-
N3AH-18	-	-	-	14	-	-	-
N3S-27	-	15	21	-	-	-	-
N3S-29	-	-	-	12	-	-	14
NH1-2	-	-	15	-	-	-	-
NH1-11	-	16	18	13	-	11	13
NH1-28	-	20	21	-	-	-	11
NH1-68	-	-	22	-	-	-	-
NRB1-1	13	-	17	-	-	-	-
NRB1-9	-	-	21	-	-	-	-
NRB1-20	-	-	13	-	-	-	-
NRB1-25	-	-	18	-	-	-	-
NRB1-33	-	-	21	-	-	-	-
NRP1-13	-	-	13	-	-	-	20
NRP1-14	-	-	-	-	-	19	22
NRP1-20	-	-	15	-	-	-	-
NRP1-28	-	-	17	-	-	-	-
NRP1-29	-	-	13	-	-	-	-
NRP1-34	-	-	17	-	-	-	-
NRP1-40	-	-	13	-	-	-	-
SJ-4	-	-	15	-	-	-	-
SJ-26	-	-	16	16	-	-	-
SL-7	-	13	-	14	-	12	-
SL-42	-	12	18	18	-	-	-

MTCC 96 *Staphylococcus aureus* MTCC 106 *Micrococcus luteus* MTCC 121 *Bacillus subtilis*; MTCC 739 *Escherichia coli* DN1 *Pseudomonas aeruginosa* (lab isolate); MTCC 227 *Candida albicans* MTCC 1344 *Aspergillus niger*

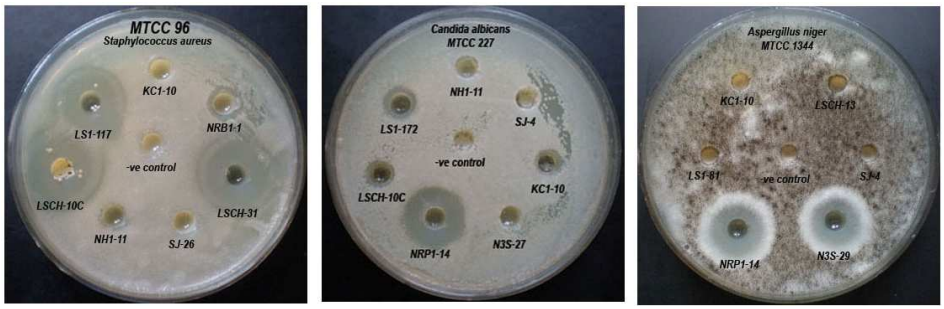

Fig. 2: Antimicrobial activity against *Staphylococcus aureus* MTCC 96, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 1344 shown by selected actinomycete isolates (Kirby Bauer method)

Among these bioactive isolates, LS1-11, LS1-88, LS1-117, LS1-172, LSCH-2, LSCH-10C, LSCH-11A, LSCH-31, N3S-27, NH1-11, NH1-28, NH1-68, NRB1-9, NRB1-25, NRB1-33, NRP1-13, NRP1-14 and SL-42 show inhibition zone of 18 mm or above. LSCH-23 is the only isolate showing activity against *Pseudomonas aeruginosa* DN1. NRP1-14 showed activity only against Yeast/Fungi. The isolate exhibiting broad spectrum of activity are KC1-10, NH1-11 and SL-7.

DISCUSSION

Actinomycetes have been intensively surveyed in several un- and underexplored environments, niche or extreme habitats in various parts of the world in the last few decades. Wang *et al*.[19] investigated the actinomycete diversity in the rainforests of Singapore. 36 actinomycete genera were obtained, among which *Streptomyces*, *Micromonospora*, *Actinoplanes*, *Actinomadura*, *Nonomuria*, *Nocardia* and *Streptosporangium* were the most abundant. Actinomycete diversity has been extensively studied in China by several groups esp. from unexplored or niche habitats. Novel strains, species and even genera have been discovered in the last few decades[20]. Similarly actinomycete diversity esp. entophytic actinomycetes have been surveyed in Thailand[21], in Jordan[22], in Egypt[23].

Several unusual or niche habitats have been explored for bioactive actinomycetes. For example interesting strains have been isolated from cave environments[24]; salt pans and other salty ecosystems. Of late, aquatic environments have yielded interesting strains and even novel genera such as Salinispora and Verrucosispora etc from which new antibiotics such as salinisporamides and abyssomycins were obtained[25]. We have found in this study that niche habitats in Manipur esp. wetlands are promising sources of bioactive actinomycetes. In contrast with marine environment, freshwater sources have been less explored. Among the potent antimicrobial isolates, several were strains isolated from Loktak and Nambul (LS1, NRB1 and NRP1 series). Bioactive actinomycetes have been reported from Lake Baikal in Russia[26], Nile river in Egypt[27], Krishna river in Andhra Pradesh, India[28]. Interestingly besides *Streptomyces* we found several nocardioform actinomycetes from wetlands in Manipur esp. Loktak lake sediments. This result contrasts with that of Terkina *et al*.[26] where majority of water isolates were *Streptomyces* and sediment isolates were predominantly *Micromonospora*. But in their case, Baikal is oligotrophic lake whereas Loktak is highly eutrophic due to polluted rivers flowing into it and anthropogenic activities around the lake. Prevalence of nocardioforms in freshwater habitats with possible linkage with anthropogenic activities were reported by Rowbotham *et al*.[29] in England and Yamamura *et al*.[30] for moat sediment actinomycetes in Japan. Further studies on actinomycete population in the plethora of wetlands in Manipur-lakes, rivers, ponds, marshes etc.-hold promise for novel strains or even species of bioactive actinomycetes. We are targeting our further investigations to cover more wetlands in Manipur using diverse selective media and pretreatment methods.

CONCLUSION

That Manipur is a goldmine of biodiversity has been amply justified by the richness of her floral and faunal diversity and now also microbial diversity. Our studies will establish the rich actinomycete diversity of the region, especially the various niche habitats of Manipur and also help conserve and utilize them in bioindustry. Further intensive studies on the actinobacterial diversity of unique biotopes in Manipur should form an important input into Indian biotech industry.

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