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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Isolation and screening of Streptomyces in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites

Isolement et criblage de streptomyces du sol des forêts protégées des états d'Assam et de Tripura pour des métabolites antimicrobiens

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KEYWORDS
Streptomyces spp.;
Protected forest soil;
Antimicrobial activity;
Northeast India

Abstract
Objective. — To study the antimicrobial of Antinomycets, isolated from Northeast India.
Material and methods. — A total of 110 actinomycetes strains were isolated from the soil samples collected from the protected forest soil from two States in Northeast India These were then characterized by conventional methods and assessed for their antagonistic activity preliminary against test microorganisms.
Results. — Among the 110 isolates, 65 (59.09%) strains showed antibacterial activity, 47 (42.72%) strains showed antifungal activity and 33 (30%) strains exhibited a broad-spectrum activity against both test bacteria and fungi. The production of nonpolyenic antifungal substances by promising isolates was investigated using several criteria: antibacterial activity, ergosterol inhibition, and UV—vis spectra of active extracts.
Conclusion. — These results indicate that the protected areas of Northeast India's soil microorganisms could be an interesting source of antibacterial and antifungal bioactive substances.

MOTS CLÉS
Streptomyces sp. ;
Sol de forêts protégées ;
Activité antimicrobienne ;
Nord-est de l'Inde

Résumé
But. — Étudier l'activité antimicrobienne des Actinomycètes isolés des régions du nord-est de l'Inde pour étudier leur écosystème.
Matériel et méthodes. — Cent-dix souches d'Actinomycètes ont été isolées à partir d'échantillons de sol collectés dans des forêts protégées de deux états du nord-est de l'Inde. Ils ont éte

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identifiés par des méthodes conventionnelles et testés pour leur activité antagoniste vis-à-vis de divers microorganismes.
Résultats. — Parmi les 110 souches étudiées, 65 (59,09 %) ont montré une activité antibactérienne, 47 (42,75 %) une activité antifongique et 33 (30 %) un large spectre d'activité à la fois sur les bactéries et les champignons. La production d'antifongiques non polyèniques par les isolats prometteurs a été étudiée en utilisant divers critères : activité antimicrobienne, inhibition de l'ergostérol et spectre UV—vis des extraits correspondants actifs.
Conclusion. — Ces résultats indiquent que les microorganismes du sol des régions protégées du nord-est de l'Inde peuvent être une source intéressante de substances actives contre les bactéries et les champignons.

Introduction

Despite the long list of currently available antibiotics in the market, antifungal antibiotics are a very small but significant group of drugs and have an important role in the control of mycotic diseases. Only a limited number of antifungal agents are currently available for the treatment of life-threatening fungal infections [27] The need for new, safe and more effective antifungals is a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immunocompromised host. However, many compounds, polyenes in particular, cannot be used because of their toxicity, while they are of interest in animal therapy, agriculture and industry. These antifungal agents show some limitations, such as the significant nephrotoxicity of amphotericin B [7] and emerging resistance to the azoles [4], despite several recent improvements, such as lipid formulations of polyenes with lower toxicity and new triazoles (voriconazole, rovuconazole and pasaconazole) with a wider spectrum of action, including activity against some azole-resistant isolates [9]. The search for a new, safer, broad-spectrum antifungal antibiotic with greater potency has been progressing slowly [10]. The development of new antifungal agents, preferably naturally occurring with novel mechanisms of action, is an urgent medical need.

Soil, in particular, is an intensively exploited ecological niche the inhabitants of which, to produce many useful biologically active natural products, including clinically important antibiotics. The species belonging to the genus *Streptomyces* constitute 50% of the total population of soil actinomycetes and 75—80% of the commercially and medicinally useful antibiotics have been derived from this genus [16]. The list of novel microorganisms and products derived from poorly explored areas of the world like China, Australia, Antarctica and Jordan suggests that a careful exploration of new habitats might continue to be useful [19,20,17,22].

The screening of actinomycetes from diverse ecological niches of vast untapped Indo—Burma belt of Northeast India deserves special attention to explore the potentialities of the diverse micro flora of this region, as Northeast India being a part of the Indo—Burma biodiversity hot spots [18]. This study was undertaken with an aim of highlighting the presence of actinomycetes specially the genus *Streptomyces* from different protected forest areas of Northeast India and selecting the strains with antimicrobial activity. These areas are as yet poorly studied and represent diverse and largely unscreened ecosystem and the least investigated area for the isolation of potent antibiotic-producing actinomycetes.

Materials and methods

Samples selection and used strains

From 2006 to 2007, soil samples were collected from several preserved forest ecosystems of Northeast India. These habitats included the rhizosphere of plants, mountains soil, hot spring sediment, dung and forest soils. Soil sample was collected from the ground surrounding the Kaziranga National Park (26°30—26°45″N and 93°08—93°36″E), Assam, India; Nambor Wildlife Sanctuary situated in Karbi Anglong district, Assam, India; Gibbon Wildlife Sanctuary (26°40″—26°45″N and 94°20″—94°25″E), Assam, India; Garampani Wildlife Sanctuary situated in Karbi Anglong district, Assam, India and tropical moist deciduous forest of Sepahijala Wildlife Sanctuary situated in the state of Tripura, India.

The target strains used for screening antimicrobial activity were procured from microbial type culture collection and gene bank (IMTECH, Chandigarh, India) and are: *Staphyllococcus aureus* MTCC 737, *Bacillus subtilis* MTCC 441, *Proteus vulgaris* MTCC 426, *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 741, *Candida albicans* MTCC 227, *Saccharomyces cerevisiae* MTCC 170, *Fusarium oxysporum* MTCC 284, *Fusarium moniliforme* MTCC 156 and *Aspergillus niger* MTCC 282.

Isolation, characterization and storage of the isolates (*Streptomyces* spp.)

For each collected sample, 1000 mg of the soil were suspended in 100 ml of physiological water (NaCl 9 gl^{-1}) then incubated in an orbital shaker incubator at 28 °C with shaking at 200 rpm for 30 min. Mixtures were allowed to settle, and serial dilutions up to 10^{-4} were prepared using sterile physiological water and agitated with the vortex at maximum speed. An aliquot of 0.1 ml of each dilution was taken and spread evenly over the surface of actinomycetes isolation agar (Hi-Media, Mumbai), *Streptomyces* agar (Hi-Media, Mumbai) and starch casein agar medium. The three media are added to rifampicin 2.5 μg/ml and amphotericin B 75 μg/ml to inhibit bacterial and fungal contamination, respectively. Plates were incubated at 28 °C, and monitored after 48, 72, and 96 h. Plated dilutions that gave 15—150 colonies were chosen for further isolation. Repeated streaking on *Streptomyces* agar (Hi-Media, Mumbai, India) plates led to purify bacterial colonies that showed a *Streptomyces* like appearance.

Actinomycetes colonies were recognized on the basis of morphological characteristics by light microscopy (G X10). Representative colonies were selected and streaked on new plates of starch casein agar medium. Agar plates were inoculated with the strains, incubated at 28 and 42 °C until good growth was observed. For strain maintenance, *Streptomyces* agar (Hi-Media, Mumbai) medium was used. The isolated strains are conserved at 4 °C during two months, and in a freezer at −20 °C in the presence of glycerol (15%, v/v) for a longer period.

Streptomyces colonies were characterized morphologically and physiologically following the methods given in the international *Streptomyces* project (ISP) [23]. The isolates were identified as species belonging to the genus *Streptomyces* by analyzing their morphological characteristics [15] and by biochemical tests [13]. The isolates were identified morphologically to the genus level by comparing the morphology of spore bearing hyphae with entire spore chain as described in Bergey's Manual [15]. This was done by using cover slip method in which sterile square cover slips were inserted at an angle of 45° in sterile CSPY agar medium in petridishes. Individual cultures were transferred to the intersection of the medium and coverslip. The cover slips were removed after seven days of incubation, air-dried and observed under high power magnification. Morphological characters were noted. All the *Streptomyces* spp. will be available at the Biotechnology Division, RRL (CSIR), Jorhat, Assam, India.

In vitro screening of isolates for antimicrobial activity

Preliminary screening for antibiotic production was done by conventional spot inoculation method on agar medium [24]. Subsequent screening of promising isolates was done under submerged fermentation conditions. Pure actinomycetes isolates were spot inoculated on actinomycetes isolation agar medium (Hi-Media, Mumbai). The plates were incubated at 28 °C for six days, and then inverted for 40 min over chloroform in fumehood. Colonies were then covered with a 0.6% agar layer of Sabouraud's (for yeast), potato dextrose medium (for fungi) and nutrient agar medium (for bacteria), previously seeded with one of the test organisms. The antimicrobial activity was observed after 24 h incubation at 37 °C for bacteria and 48 h incubation at 28 °C for fungi and yeast.

Submerge culture

Isolates that showed activity against test organisms in agar medium were grown in submerged culture in 250 ml flasks containing 50 ml of CSPY medium. A 2 cm^2 piece of agar from each seven-day-old culture grown on actinomycetes isolation agar was used to inoculate the flasks. These cultures were grown in a rotary shaker (Clim-O Shake, Adolf Kuhner AG) at 200 rpm, 28 °C, for seven days. The resulting culture broths (approximately 50 ml) obtained following growth of each isolate in the culture media were separated from the mycelium by centrifugation at 9168 × *g* (Sigma 3K30) for 15 min. The supernatant, sterilized by filtration, was used for extracellular antimicrobial activity by agar well diffusion method against test microorganisms [8]. By using a sterile cork borer, wells were punctured in appropriate agar medium plates previously seeded with one of the test organisms. One hundred microliter of supernatant of each isolate were administrated in each well. Plates were kept at 4 °C for at least 2 h to allow the diffusion of produced antimicrobial metabolites. The diameters of inhibition are determined after 24 h of incubation at 37 °C for bacteria, 28 °C for yeasts and after 48 h for filamentous fungi and verified active substance extraction. Each experiment was repeated three times and mean value of inhibition zones was calculated.

Organic crude extracts

Crude antimicrobial compound was recovered from the culture filtrate of each active isolate by solvent extraction with ethyl acetate. Ethyl acetate was added to the filtrate in the ratio 1:1 (v/v) and shaken vigorously for 20 min. The organic layers were collected and the organic solvent was evaporated to dryness in a vacuum evaporator at 40 °C to obtain a gummy crude extract.

Screening for antifungal polyenic and nonpolyenic metabolites

Crude extract of each strain showing antifungal activity were suspended in DMSO at a concentration of 1 mg/ml prior to antimicrobial bioassay. In order to determine the effect of the antibiotic from selected *Streptomyces* isolates on the ergosterol present in the fungal cell membrane, ergosterol was used as the reversal agent to test for its ability to reverse the inhibition of *C. albicans* caused by the antibiotic [21]. Sabouraud's agar plates with 50 mg/ml ergosterol were prepared along with a control without ergosterol. The plates were seeded with *C. albicans* MTCC 227. Ergosterol inhibition was tested by disc diffusion method. Sterile filter paper discs 6 mm in diameter (Hi-Media, Mumbai, India) were impregnated with 50 μl (50 μg crude antibiotic) suspension, dried and placed onto plates previously seeded with test microorganisms. The plates were incubated at 28 °C for 24 h and observed for the zone of inhibition.

The absorption spectrum of active extracts in methanol were recorded in the UV region (210—400 nm) by using a UV—vis spectrophotometer (Cintra 40) and compared with those of known polyenic antifungal antibiotics.

Results and discussion

Isolation of actinomycetes

This study was undertaken with an aim of highlighting the presence of actinomycetes in these ecosystems and selecting the strains with antibacterial and antifungal activity. Using the selective media and cultivation conditions described previously a total of 110 different *Streptomyces* isolates were recovered from 30 soil samples that were collected from preserved forest ecosystems of Northeast India from 2006 to 2007. A higher number (94%) of actinomycetes were isolated at mesophilic temperatures (25—37 °C). Nevertheless, seven out of 96 strains were isolated at 42 °C from samples of hot spring sediment, composts and Rhinos's dung.

All of these strains were isolated on three different culture media supplemented with rifampicin (2.5 μg/ml) and amphotericin B (75 μg/ml) to inhibit bacterial and fungal

Table 1 Initial screening of *Streptomyces* isolates (by spot inoculation method in agar medium).
Tableau 1 Criblage préliminaire des isolats de Streptomyces.

Origin	Total strains isolated	Number of active isolates against bacteria (%)	Number of active isolates against fungi (%)	AFA + ABA[a]
Soil of Kaziranga National Park	35	20 (57.14)	12 (25.71)	11 (31.42)
Soil of Nambor Wildlife Sanctuary	26	17 (65.38)	11 (26.92)	9 (34.61)
Soil of Gibbon Wildlife Sanctuary	17	11 (64.70)	7 (29.41)	5 (29.41)
Sepahijala Wildlife Sanctuary	23	14 (60.86)	14 (60.86)	7 (30.43)
Garampani Wildlife Sanctuary	9	3 (33.33)	3 (33.33)	1 (11.11)
Total	110	65 (59.09)	47 (42.72)	33 (30)

[a] AFA: antifungal activity; ABA: antibacterial activity.

contamination, respectively. The majority of the strains were collected from the actinomycetes agar medium. This medium seems to be the most specific and sensitive for actinomycetes, since it contains sodium propionate, which acts as an antifungal agent and glycerol that most actinomycetes use as a carbon source. In addition, its transparency facilitates colony observation. These reported results were anticipated because earlier studies have shown the importance of the constituents of the screening media under which the producing microorganisms were cultivated [11].

The soils of Kaziranga National Park and Nambor Wildlife Sanctuary give the higher number of actinomycetes isolates (35 and 26 isolates, respectively) with respect to others soils (Table 1). Nambor Wildlife Sanctuary is an ecosystem rich in traditional, medicinal and ornamental plants where conditions are good for microbial growth. The recovery of actinomycetes in Garampani Wildlife Sanctuary was lower (nine isolates) than the other zones possibly due to the higher sulphur concentrations of this area (Table 1).

Morphological, physiological and biochemical characteristics of isolates

All isolates grew on a range of agar media showing morphology typical of *Streptomycetes* [15], since the colonies were slow growing, aerobic, glabrous or chalky, folded and with aerial and substrate mycelia of different colors. In addition, all colonies possessed an earthy odour. Some of them also produced antibiotics as reflected by zones of growth inhibition among other inhabitants of soil samples. A confirmatory identification to genus was based on acid-fastness, Gram-stain and degradation of casein, tyrosine and xantine. All *Streptomycetes* strains were acid-fast negative and Gram-stain positive.

The cultural characteristics (Pigment production), morphological characteristics and antimicrobial activities of the different *Streptomyces* isolates are presented in Table 2. All of these isolates fitted the genus description as reported by several investigators [23,29]. The colour of the substrate mycelium and aerial spore mass was varied. *Streptomyces* isolates were categorized into six colour series according to the colour of their mature sporulated aerial mycelium with white and gray colour series being the most abundant (Table 2). Melanin production was examined on peptone—yeast extract agar (ISP 6) and tyrosine agar (ISP 7) and to examine the reverse side colour and soluble pigment media used were those recommended by Shirling and Gottlieb [23]. The production of melanoid pigments was variable in all the series with the exception of the strains grouped in the blue series, where none produced melanoid pigments. Out of 110 *Streptomyces* isolates, 38 (35%) strains produced melanin, 80 (73%) strains showed distinctive reverse side pigment, and 40 (36%) strains produced soluble pigments (Table 2).

According to the shape of the spore chains observed under light microscopy, the isolates were grouped as Rectus-Flexibilis (RF), Spira (S) and Retinaculiaperti (RA) [15]. Microscopically, it was observed that the morphology of the spore

Table 2 Morphological and cultural characteristics of the *Streptomyces* isolates.
Tableau 2 Aspects morphologiques et culturaux des isolats des Streptomyces.

	Colour Series						
	White	Gray	Yellow	Red	Blue	Variable (violet, orange, pink)	Total
Number of isolates	46	32	9	3	2	6	110
Pigment production							
Melanin	15	12	10	1	0	0	38 (35%)
Reverse side	26	29	16	3	2	4	80 (73%)
Soluble	17	13	7	1	0	2	40 (36%)
Sporophore morphology							
Rectiflexible (RF)	25	10	22	2	0	5	64 (58%)
Spirales (S)	11	21	2	1	2	1	38 (35%)
Retinaculiaperti (RA)	2	6	0	0	0	0	8 (7%)
Total	38	37	24	3	2	6	110

Numbers in parentheses represent the percentage out of the total isolates.

Table 3 Origin of samples and antimicrobial activity of the *Streptomyces* isolates (by agar cup diffusion method).
Tableau 3 Origine et activité antibactérienne des isolats de Streptomyces.

Origin	Number of active isolates	Total number of active isolates against test bacteria					Activity shown in liquid medium (%)
		S. aureus MTCC 737	*B. subtilis* MTCC 441	*E. coli* MTCC 443	*P. vulgaris* MTCC 426	*P. aeruginosa* MTCC 741	
Kaziranga National Park	20	12	15	3	2	3	16 (80)
Nambor Wildlife Sanctuary	17	10	14	4	4	4	14 (82.35)
Gibbon Wildlife Sanctuary	11	7	9	1	1	1	9 (81.81)
Garampani Wild Life Sanctuary	3	2	3	0	0	0	3 (100)
Sepahijala Wildlife Sanctuary	14	8	10	2	4	2	10 (71.42)
Total	65	39 (60%)	51 (78.46%)	10 (15.38%)	11 (16.92%)	10 (15.38%)	52 (80)

chains varied depending on the species, showing the expected straight or flexuous forms, hooks, open loops and coils, which were used, among other features, to establish differences between them. Most of the isolates bore Rectus-Flexibilis sporophores and spira that are represented by 58 and 35%, respectively. Retinaculiaperti bearers were less observed 7% and were limited to only white and gray colour series (Table 2).

Antimicrobial activity

During screening of new isolates for drug discovery, many potentially interesting microorganisms might be excluded due to their morphological similarities, suggesting similar biochemical behavior; thus, many isolates are lost and only a few of them are finally tested. In this study, the total number of isolated *Streptomyces* strains (110) was screened on agar medium and a broad-spectrum of antibacterial and antifungal activity was observed in 30% of the strains and appeared promising (Table 1). Antibacterial activity was observed in 65 isolates (59.09%) and 47 isolates (42.72%) exhibited antifungal activity (Table 1). In former studies, it was shown that the isolation rate of actinomycetes with antimicrobial activity is higher than 40% [14] and in others less than 10% [12]. These results confirm that the actinomycetes are able to produce a wide variety of antibiotics with antifungal activity. We found the best percentage (60.86%) of antifungal activity in the Sipahijola Reserve in Tripura (Table 1). This protected area has soils very rich in minerals, which are excellent conditions for actinomycetes growth and is a zone protected from human activity. These conditions enhance the competition for survival and the production of powerful vital substances.

The active isolates when subjected to submerged culture showed different activity from that of primary screening in agar medium. Some isolates did not show the activity in the liquid medium. Out of 65 active isolates in primary screening in agar medium, only 52 (80%) isolates were found to exhibit antibacterial activity while other 15 (20%) isolates did not exhibit activity in broth culture (Table 3). The antibacterial activity of *Streptomyces* strains against *E. coli* and *P. aeruginosa* was almost equal (15.38%), while 78.46% were active against *B. subtilis*, 60% against *S. aureus* and 16.92% against *P. vulgaris* (Table 3). High percentage of inhibition was recorded against Gram-positive bacteria while Gram-negative test bacteria were less inhibited. Out of the 47 isolates, which had showed antifungal activity in primary screening, only 38 (80.85%) isolates exhibited significant antifungal activity in broth culture (Table 4). The highest antifungal activity of *Streptomyces* strains was recorded against *S. cerevisiae* (87.23%), followed by *C. albicans* (80.85%) > *F. moniliformae* (51.06%) > *F. oxysporum* (44.68%) and lowest for *A. niger* (31.91%), (Table 4). During the screening of the secondary metabolite, actinomycete isolates often encountered which show antimicrobial activity on agar but not in liquid culture.

Detection of polyenic and nonpolyenic antifungal activity

As shown in Table 1, only 47 strains belonging to the genus *Streptomyces* representing 42.72% of all isolates showed antifungal activity against at least one of the test organisms. In the course of screening for nonpolyenic active compounds, 21 out of 47 isolates, showing antifungal activity, inhibited both *C. albicans* MTCC 227 and *S. cerevisiae* MTCC 170 and appeared promising (Table 5). Variable inhibitory patterns were evident when filamentous fungi were used as test species. Strains Tr-5, N-9, Gb-6 and N-10 proved to inhibitory to *F. oxysporum* and *F. moniliforme* but not on *A. niger*. On the other hand, isolates 11KDT, 5K10, N-11 and Ga-DJ displayed activity against both *F. moniliforme* and *A. niger* but not on *F. oxysporum* (data not shown). The production of nonpolyenic antifungal substances by these isolates was investigated using several criteria: antibacterial activity, ergosterol inhibition and UV—vis spectra of active extracts [21]. Ergosterol present in fungal cell membrane has a very high affinity towards polyene antibiotics. Polyene drugs form complexes with ergosterol, which open channels in the fungal membrane that cause leakage of critical intracellular constituents and subsequent cell death. This behavior is exploited in a detection method developed to identify the presence of a polyene class of antibiotics [5]. Interpretation of results as follows: reduced zone in size in presence of ergosterol — polyene type of antibiotic present

Table 4 Origin of samples and antifungal activity of the *Streptomyces* isolates (by agar cup diffusion method).
Tableau 4 Origine et activité antifongique des isolats de Streptomyces.

Samples	Number of active isolates	Total number of active isolates against test fungi					Activity shown in liquid medium (%)
		C. albicans MTCC 227	*S. cerevisae* MTCC 170	*F. oxysporum* MTCC 284	*F. moniliformae* MTCC 156	*A. niger* MTCC 282	
Kaziranga National Park	12	11	12	6	7	6	10 (83.33)
Nambor Reserve Forest	11	10	10	5	5	4	10 (90.90)
Gibbon Wildlife Sanctuary	7	5	6	4	5	2	6 (85.71)
Garampani Wild Life Sanctuary	3	2	1	1	1	0	1 (33.33)
Shipahijola Wild Life Sanctuary	14	10	12	5	6	3	11 (78.57)
Total	47	38 (80.85%)	41 (87.23%)	21 (44.68%)	24 (51.06%)	15 (31.91%)	38 (80.85)

and no reduced zone in presence of ergosterol — polyene type antibiotic absent (Table 5). The UV spectral data for the ethyl acetate extract of selected active fermented broth are shown in Table 5.

The importance of polyene antibiotic in antifungal therapy prompted the use, to determine if all or some of the active substances, reported in the present investigation was polyenic in nature. As shown in Table 5, 12 isolates (10KDT, 5K10, Tr-3, N-9, 1K6, Tr-19, N-5, N-7, Gb-6, Tr-18, Gb-13 and N-10) appeared promising because of activity against *E. coli* and *B. subtilis* (cell membrane without sterols) and no marked inhibition of antifungal activity by ergosterol (target of polyenic antifungal compounds). Maximum absorbance peaks range between 215 and 270 nm and the characteristics of absorption peaks indicate a highly polyene nature. The spectral data are consistent with those obtained by Swaadoun et al. [25]. The broad-spectrum of activity shown in these isolates is possibly due to the production of different

Table 5 Results of the screening for nonpolyenic antifungal producing *Streptomyces*.
Tableau 5 Résultats du criblage des Streptomyces produisant des antifongiques nonpolyeniques.

Isolates	Origin	Antibacterial activity[a]	λ_{max} (nm)	Ergosterol effect against *C. albicans* MTCC 227[b]	
				With	Without
10KDT	Kaziranga National Park	+	268	19	20
11KDT	Kaziranga National Park	—	350	14	20
Tr-5	Sepahijala Wildlife Sanctuary	—	347	10	14
Tr-1	Sepahijala Wildlife Sanctuary	—	342	10	15
5K10	Kaziranga National Park	+	246	25	25
Tr-3	Sepahijala Wildlife Sanctuary	+	230	9	16
N-2	Nambor Wildlife Sanctuary	—	357	14	17
N-9	Nambor Wildlife Sanctuary	+	277	20	21
Ga-DJ	Garampani Wildlife Sanctuary	—	339	11	18
1K6	Kaziranga National Park	+	225	16	15
Tr-19	Sepahijala Wildlife Sanctuary	+	268	25	25
N-5	Nambor Wildlife Sanctuary	+	236	15	15
Tr-14	Sepahijala Wildlife Sanctuary	—	310	11	19
N-11	Nambor Wildlife Sanctuary		342	19	28
N-7	Nambor Wildlife Sanctuary	+	256	8	14
Tr-13	Sepahijala Wildlife Sanctuary	—	none	9	17
Gb-6	Gibbon Wildlife Sanctuary	+	246	12	15
Tr-18	Sepahijala Wildlife Sanctuary	+	275	20	19
Gb13	Gibbon Wildlife Sanctuary	+	220	8	15
5K4	Kaziranga National Park	—	none	11	17
N-10	Nambor Wildlife Sanctuary	+	245	23	22
Amphotericin B		—	362	10	18

[a] Activity against *E. coli* and/or *B. subtilis*.
[b] Inhibition zone in mm in Sabouraud's agar medium with or without 50 mg/ml.

compounds. These results are in agreement with the high percent of fresh isolates reported to be producers of antibiotic compounds [6]. The metabolites produced by nine isolates did not show an UV—vis spectrum characteristic of a polyenic structure. Of particular interest these nine isolates, were apparently not synthesize polyene like substances. These strains have been selected for additional studies to analyze the nature of the active substances synthesized by each. Taxonomic characterization of these nine *Streptomyces* species as well as isolation, purification and structure elucidation of the antifungal metabolites produced are under investigation.

Conclusion

Microorganisms produce some of the most important medicines ever developed. They are the source of lifesaving treatments for bacterial and fungal infections. In spite of the tremendous success of the past secondary metabolite research, the number of terrestrial antibiotics seems currently to approach a saturation curve with an apparent limit in the near future. The increasing number of duplications and the urgent demand for new leading structures in pharmacology have enforced the search for metabolites in so far untouched habitats.

Our interest focused on microorganisms belonging to the Actinomycetaceae family and specifically to the *Streptomyces* genus, the members of which have demonstrated interesting antimicrobial activity. It has been estimated that the genus *Streptomyces* might produce at least 1,00,000 new compounds of biological interest [28]. The present finding highlights the importance for further investigation towards the goal of obtaining novel antimicrobial agent out of the *Streptomycetes* from Northeast India's untapped habitat. These areas represent diverse and largely unscreened ecosystem and the least investigated area for the isolation of potent antibiotic-producing actinomycetes. Few scientific works were carried out on the presence, the antibiotic-producing actinomycetes in various ecosystems of Northeast India [26]. In our laboratory a *Streptomyces* strain, namely, *Streptomyce*s sp. 201 was isolated from the tea garden soil of Jorhat city, Assam, India, (26.44 N and 94.10 E) exhibited promising antifungal and antibacterial activity against a wide range of pathogens including *M. tuberculosis* [2,3]. The high nutrient levels and diverse environmental conditions in the North-eastern region of India could trigger or favor unusual metabolite biosynthesis by these isolates and thus stressing their potential as a source of novel antibiotics, which encouraged further studies to isolate and identify these novel compounds. The sea and to a lesser extent, the rainforests are almost inexhaustible, untapped reservoirs for novel compounds [1]. Hot, humid tropical climate of Indo—Burma belt harboring numbers of rain forests, lakes, hot-springs, cold-spring, rivers, waterfalls, biospheres zones is definitely conducive for the growth of various fastidious and nonfastidious microbes. Microbial strains that exist in this belt may provide us rare and novel industrial enzymes, antibiotic or metabolites, which might be more effective than the existing regime, to cure dreaded diseases.

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