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Occurrence of *Streptomyces aurantiacus* in mangroves of Bhitarkanika

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ABSTRACT

Thirteen strains of *Streptomyces* were isolated from phyllosphere of nine mangrove tree species found in Bhitarkanika mangrove ecosystem of Orissa. According to physiological, biochemical data, all 13 of the isolates were taxonomically identified to the genus *Streptomyces* as a*urantiacus* species. All strains are grayish, spirals and forming amorphous colony. Almost all utilized araginose, produced H2S, resistant towards rifampicin and penicillin, urea except few strains. However, they exhibited different extracellular activity like phosphate solubilization, lipase and L asparaginase production. This is a unique report from this mangrove ecosystem as far as *Streptomyces* occurrence is concerned.

Keywords: *Streptomyces*, mangrove

INTRODUCTION

Streptomyces are of special group of bacteria belonging to actinomycetes and known for fungus like myceloid features and production of secondary metabolites & enzymes (Gandalf *et al*., 2000, Lescic *et al*., 2004). Though, several reports are available on various activity of *Streptomyces* obtained from different habitats, few reports are available on the occurrence of *Streptomyces* and their activity in mangrove ecosystem (Dhanasekaran *et al*., 2005; Siva Kumar *et al*., 2005). Mangroves are the plants of highly adaptive nature and found in brackish water of different salinity levels (Schmit and Shearer, 2004; Ananda and Sridhar, 2004). Bhitarkanika mangrove ecosystem of Orissa is one of largest mangrove ecosystem in our country and unexplored as far as the studies on *Streptomyces* is concerned. Different strains of *Streptomyces* were isolated from mangrove tree species and identified. The detailed characteristic features of these strains have been described in this paper.

MATERIALS AND METHODS

Source of materials

The leaf samples from nine mangrove plants i.e. *Heriteira fomes, Sesubium protrucastrum , Protulacia quartata, Aegeceras corniculata, Bruguiera parviflora, Agalaia cuclata, Brownloia, Sonneratia apilata, Acanthus ilicifolius* found in Bhitarkanika mangroves were collected for the isolation of *Streptomyces*.

Isolation media

The dilution plate technique was followed for the isolation of *Streptomyces* on ISP 3, ISP4 and ISP5 media (Hi.media).

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Growth characteristics

Streptomyces isolates were grown in starch casein liquid medium of 4.5 and 7.2 pH and at a 30 ºC and 37 ºC for 10 days in static culture condition. Finally their dry biomass was measured using. At the same time, change in pH, colour of filtrate, diffusible pigments were observed and recorded. The plate culture of all isolates prepared in Starch casein medium was observed for morphological characteristics like colony characteristics, coloration, margin etc.

Morphological Studies

Slide cultures of all the isolates of Streptomyces were prepared on starch casein medium and ISP 3 medium incubated at 30 ºC and 37 ºC by using cavity slides. Periodical observations regarding spore morphological, arrangements and mycelloid structure were recorded by using Nikon Japan Trinocular Research Microscope Model 50i.

Taxonomic identification

Different biochemical tests for carbohydrate utilization, nitrogen utilization, growth in different stress conditions, antibiotic resistance, amino acid degradation, enzyme activity like, amylase, protease, asparaginase, antifungal activity and phosphate solubilization were analyzed for characterization of these isolates. Finally data were used for the identification of *Streptomyces* isolates using Probabilistic identification of bacteria (PIB win).

RESULT AND DISCUSSIONS

Overall 13 isolates of *Streptomyces* were isolated from *Hertiera fomes (4), Aegeceras corniculatum (2),* and

each from *Sesubium protrucastrum, Protulacia quartata, bruguiera parviflora, Agalaia cuclata, Brownloia, Sonneratia apilata,* and *Acanthus ilicifolius.*

Colour of the colony

Three strains (ST12, ST 45 and ST 73) were greyish in colour with whitish reverse side of the colony (Table 1). It showed yellow–brown colour in culture broth of starch casein medium of pH 4.5 at 37 ºC. The strain ST 62 was whitish grey in colour and reverse side of its colony was white. It also gave white colour in culture broth of starch casein medium of pH 7.2 at 37 ºC. Similarly, ST 68 was grayish white in colour with grayish reverse side and produced light brown colour in culture broth. In exception, ST 74 was found to be Grayish along with black reverse coloration that produced yellow pigments in culture broth of starch casein medium of pH 7.2 at 37 ºC. Other remaining strains viz., ST 31, ST 32, ST 35, ST 39, ST 61, ST 66 and ST 71, were white in colour with yellowish reverse coloration that also produced yellow colour in liquid starch casein medium.

Spore chain morphology

Spore chains are of the section spirals but not rectiflexibles. Spores are in spiral arrangement forming coiled structure. From the mycelium the spores are arranged in chains.

Special Morphological characteristics

Almost all the strains have condensed round colony having amorphous growth on starch casein agar medium (Table 1). The outer margin of the colony was round. In strain ST 35 outer margin is serrated while it was regular in two strains namely ST 39 and ST 74. *S. aurantiacus* strain ST 45 showed amorphous growth with watery droplets and edge white. The outer margin of the colony was entire. Where as strain ST 61, ST 62 and ST 66 had an amorphous growth with condensed and rough surface with margin irregular. An amorphous growth and wrinkled surface with margin entire bearing dusty spores was observed in strain ST 71 and ST 73.

Growth conditions

All strains of *S. aurantiacus* were sensitive to higher temperature for its proper growth. Most of them preferred 37 º C temperatures except ST 35 that grew well at 30 ºC (Table 2). Alkaline pH was found to best for their growth in starch casein medium except ST 12 that performed well in acidic pH. Melanoid pigments are not formed on peptone yeast iron agar and tyrosine agar except ST 71 and ST 12 (Table 3). These strains were unable to tolerate potassium tellurite (0.001%) sodium azide (0.01%), NaCl (7%), phenol (0.1%). Growth was not observed in sodium citrate, sodium acetate, sodium propionate and sodium pyruvate. Surprisingly growth of *S. aurantiacus* strain ST 39 was observed in sodium citrate.

Utilization of carbon

These strains were not able to utilize many carbons sources like sucrose, mesoinositol, raffinose, manitol, D-lactose, salicin, D-melizitose, D-xylose, adonitol, methyl A-D mannoside, inositol, L-rhamnose, xylitol, D-ribose, D-mellibiose, adipate hippurate and isobutyrate . Almost all strain utilizes arabinose for their growth. Most of the strains utilized adipate and malonate. Exceptionally, D fructose. Mesoinositol and mellizitose xylan could be utilized by ST 31 and 32 . *S. aurantiacus* strain ST 39 utilized manitol and ribose. Another two strains namely ST 73 and ST 74 utilized rafinose where as ribose was metabolized by ST 71 and ST 45.

Utilization of nitrogen sources

Almost all strains didn't utilized amino butyric acid, phenyl alanine and guanine as nitrogen source but capable to utilize urea as nitrogen source except ST 31, ST 32, ST 35 and ST 39 that used potassium nitrate as nitrogen source. L cysteine was metabolized by few strains like ST 71, ST 39, ST 66, and ST 68 where as hydorxiproline and Vaqline was utilized by only ST 12. Mostly utilized alanine. Glycine and lucine and lysine were utilized by ST 35, ST 61 and ST 52.

Antibiotic susceptibility

Total four antibiotics were tested against these strains namely neomycin, rifampcin, Oleandomycin and penicillin. All are resistant to rifampcin and penicillin G except ST 31 and ST32 that had sensitivity towards penicillin G. The other three strains namely, ST 71, ST 35 and ST 66 are resistant for all antibiotic tested where as ST 61 and ST62 were sensitive towards oleandomycin and neomycin exhibited in. Three more strains viz. ST 45 , ST 73, ST 74 were resistant to neomycin and sensitive to oleandomycin while ST 68, ST 12 and ST 39 are resistant to oleandomycin and sensitive to neomycin.

Enzyme activity

Many strains namely, ST 12, ST 35, ST 73, ST 74 had shown both amylolytic and proteolytic activity where as ST 31, ST 32, ST 66 and ST 61, ST 62, ST 68 showed only lipolytic and proteoltyic activity, respectively. The amylolytic activity could be observed only in strain ST 45 where as ST 66 were responsive towards lipase, amylase and protease production.

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Table1: Morphological characteristics of *Streptomyces* strains

S. No.	*Streptomyces* strains	Colour of the colony		Growth	Nature of the colony	Other characters of the colony
		Front	Back			
1	*S. auranticus* strain ST 71	white	yellow	amorphous	margin entire and dusty.	wrinkled surface.
2	*S. auranticus* strain ST 12	grey	white	dusty	round margin	flat colony.
3	*S. auranticus* strain ST 31	white	yellow	amorphous	smooth colony	round and condensed.
4	*S. auranticus* strain ST 32	white	yellow	amorphous	smooth colony	condensed
5	*S. auranticus* strain ST 35	white	brownish	amorphous	serrated margin	smooth surface and condensed
6	*S. auranticus* strain ST 39	white	off white	amorphous	regular margin	condesed colony
7	*S. auranticus* strain ST 45	grey	black	amorphous	serrated margin	watery droplets on sides of sporulation.
8	S. auranticus strain ST 61	white	white	amorphous	irregular margin	condensed and roughsurface.
9	*S. auranticus* strain ST 62	whitish grey	white	amorphous	irregular margin	edge white with gryeish centre.
10	*S. auranticus* strain ST 66	white	yellow	amorphous	irregular margin	rough surface.
11	*S. auranticus* strain ST 68	greyish white	greyish	amorphous	round margin	slight zone formed.
12	*S. auranticus* strain ST 73	greyish	greyish	amorphous	irregular margin and dusty.	edge white.
13	*S. auranticus* strain ST 74	greyish	black	amorphous	regular margin	edge white

Table 2: Growth characteristics of *Streptomyces* strains in Starch Caesin medium

S. No.	*Streptomyces* strains	Temperature and pH											
		30						37					
		4.5			7.2			4.5			7.2		
		pH	colour	biomass	pH	colour	biomass	pH	colour	biomass	pH	colour	biomass
1	*S. auranticus* strain ST 71	4.5	white	0.00 ± 0.00	9.1	Mustard yellow	0.04 ± 0.02	4.5	white	0.00 ± 0.00	9.2	Mustard yellow	0.08 ± 0.01
2	*S. auranticus* strain ST 12	8.1	yellow	0.65 ± 0.01	8.6	yellow	0.54 ± 0.00	8.7	yellow	0.82 ± 0.00	8.8	yellow	0.31 ± 0.00
3	*S. auranticus* strain ST 31	6.1	white	0.04 ± 0.01	5.8	yellow	0.35 ± 0.01	5.2	white	0.17 ± 0.01	5.3	yellow	0.68 ± 0.01
4	*S. auranticus* strain ST 32	4.8	white	0.00 ± 0.00	7.3	yellow	0.62 ± 0.00	4.6	white	0.00 ± 0.00	8.2	yellow	0.83 ± 0.01
5	*S. auranticus* strain ST 35	8.7	light yellow	0.35 ± 0.01	9	Light yellow	0.45 ± 0.02	8.6	light yellow	0.29 ± 0.02	8.9	yellow	0.20 ± 0.01
6	*S. auranticus* strain ST 39	7	white	0.07 ± 0.01	9	Mustard yellow	0.07 ± 0.02	8.9	cream	0.08 ± 0.05	9.1	Mustard yellow	0.83 ± 0.02
7	*S. auranticus* strain ST 45	8.3	light yellow	0.23 ± 0.02	8.8	brown	0.75 ± 0.04	8.4	yellow	0.32 ± 0.01	9.1	brown	1.21 ± 0.02
8	*S. auranticus* strain ST 61	5.7	white	0.02 ± 0.01	7.6	yellow	0.01 ± 0.01	6.1	white	0.04 ± 0.01	7.5	yellow	0.12 ± 0.02
9	*S. auranticus* strain ST 62	7.5	white	0.27 ± 0.02	8.2	yellowish	0.07 ± 0.01	8.5	yellow	0.32 ± 0.01	7.1	white	0.33 ± 0.01
10	*S. auranticus* strain ST 66	5.7	white	0.00 ± 0.00	8.8	golden yellow	0.27 ± 0.01	5.8	white	0.00 ± 0.00	8.8	light yellow	0.31 ± 0.02
11	*S. auranticus* strain ST 68	5.1	transparent	0.00 ± 0.00	7.6	light brown	0.21 ± 0.01	5.3	transparent	0.00 ± 0.00	7.8	light brown	0.26 ± 0.04
12	*S. auranticus* strain ST 73	5.5	transparent	0.00 ± 0.00	9.1	Mustard yellow	0.15 ± 0.00	6.7	yellowish	0.11 ± 0.00	9.2	Mustard yellow	0.33 ± 0.01
13	*S. auranticus* strain ST 74	5.8	transparent	0.00 ± 0.00	8.3	yellow	0.94 ± 0.01	5.6	white	0.00 ± 0.00	8.6	yellowish	1.21 ± 0.01

Table 3: Biochemcial characteristics of *Streptomyces* strains

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
	ST 71	ST 12	ST 31	ST 32	ST 35	ST 39	ST 45	ST 61	ST 62	ST 66	ST 68	ST 73	ST 74
MORPHOLOGY													
Spore chain rectiflexibles	-	-	-	-	-	-	-	-	-	-	-	-	-
Spore chain spirals	+	+	+	+	+	+	+	+	+	+	+	+	+
Spore mass red	-	-	-	-	-	-	-	-	-	-	-	-	-
Mycelial pigment red/orange	-	-	-	-	-	-	-	-	-	-	-	-	-
Diffusible pigment produced	-	-	-	-	-	-	-	-	-	-	-	-	-
Diffusible pigment produced yellow/brown	-	-	-	-	-	-	-	-	-	-	-	-	-
Spore mass grey	+	+	-	-	-	+	+	-	-	-	+	+	+
OTHER TESTS													
Melanin on peptone/yeast/iron agar	+	+	-	-	-	-	-	-	-	-	-	-	-
Melanin on tyrosin agar	+	+	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	+	+	+	d	+	-	-	-	-	-	-
Hydrogen sulphide production	-	+	-	-	-	-	-	-	-	-	-	-	-
Xanthin degradation	-	-	-	-	-	-	-	-	-	-	-	-	-
Allantoin degradation	+	-	-	-	+	-	-	+	+	+	-	+	+
ANTIBIOTIC RESISTANCE													
Rifampicin resistance	+	+	+	+	+	+	+	+	+	+	+	+	+
Neomycin resistance	+	-	+	+	+	-	+	-	-	+	-	+	+
Oleandomycin resistance	+	+	+	+	+	+	-	-	-	+	+	-	-
Penicillin-G resistance	+	+	-	-	+	+	+	+	+	+	+	+	+
GROWTH													
Growth at 45 degrees resistance	+	+	-	-	+	+	+	+	+	+	+	+	+
Nacl-7% w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium azide-0.01%w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenol-0.1% w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-

Potassium tellurite 0.001% w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium acetate 0.1% w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium citrate 0.1% w/v growth	-	-	-	-	-	+	-	-	-	-	-	-	-
Sodium propionate 0.1% w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium pyruvate 0.1% w/v growth	-	-	-	-	-	-	-	-	-	-	-	-	-
CARBON SOURCE													
L-Arabinose utilization	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesoinositol utilization	-	-	+	+	-	-	-	-	-	-	-	-	-
D-xylose utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Manitol utilization	-	-	-	-	-	+	-	-	-	-	-	-	-
D-Fructose utilization	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Rhamnose utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose utilization	-	-	-	-	-	-	-	-	-	-	-	+	+
D-lactose utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
D-mellizitose utilization	-	-	+	+	-	-	-	-	-	-	-	-	-
Salicin utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
D-mellibiose utilization	-	-	-	-	-	+	-	-	-	-	-	-	-
Adonitol utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylan degradation	-	+	-	-	-	-	-	-	-	-	-	-	-
Xylitol utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl A-D-glucopyranoside	-	+	+	+	+	+	+	-	-	-	+	-	-
Methyl A-D-mannoside utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Ribose utilization	+	-	-	-	-	+	+	-	-	-	-	-	-
Isobutyrate utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Hippurate	-	-	-	-	-	-	-	-	-	-	-	-	-
Adipate utilization	+	-	-	-	+	+	-	+	+	-	+	-	-
Malonate utilization	+	+	-	-	-	+	+	-	-	-	+	-	-
NITROGEN SOURCE													

Urea degradation	+	+	-	-	-	-	+	+	+	+	+	+	+
Potassium nitrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+
AMINO ACID DEGRADATION													
D-L-a-amino n-butyric acid utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Guanine degradation	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Cysteine utilization	+	-	-	-	-	+	-	-	-	+	+	-	-
Valine utilization	-	+	-	-	-	-	-	-	-	-	-	-	-
Phenyl alanine Utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Histidine utilization	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxyproline utilization	-	+	-	-	-	-	-	-	-	-	-	-	-
D-L-alanine utilization	+	+	-	-	+	+	-	+	+	+	-	-	-
Glycine utilization	-	-	-	-	+	+	-	+	+	-	-	-	-
L-leucine utilization	+	+	-	-	+	+	+	+	+	-	+	-	-
L-lysine utilization	-	-	-	-	+	-	-	+	+	-	-	-	-
SPECIAL ACTIVITY													
Lipolysis	+	-	-	-	-	+	+	+	+	-	+	-	-
Amylase	-	+	+	+	+	-	-	+	+	-	+	+	+
Protease	+	-	-	-	-	-	+	-	-	-	-	-	-
Antifungal	-	-	+	+	+	+	+	+	+	-	+	+	-

Degradation test

All strains were not xanthin degradative and none of them were H_2S positive except ST 12. Most of the strains had degraded allantoin except ST 31, ST 32, ST 35, and ST 45 that have shown only nitrate degradation. *S. aurantiacus* strain ST 68 did not show any degradation ability.

Special activities

All the strains showed antifungal activity against *Fusarium* sp. except ST 66 and 77. Among them 3 strains (ST 39, ST 61 and ST 62) were good producer of L asparaginase in intracellular and extracellular condition. The *Streptomyces aurantiacus* strain ST 12 was a good lipase producer while ST 39 exhibited phosphate solubilization capacity by forming large clear zone in Pikovaskaya agar plates.

Table 4: Identification characteristics of *Streptomyces* strain

Strains	ID score	ID modal score	Test varied
ST12	1	0.515	Allantoin degradation, spore mass grey
ST31	1	0.265	Allantoin degradation , spore mass grey
ST32	1	0.265	Spore mass grey
ST35	1	0.515	Allantoin degradation , Sodium citrate utilization
ST39	1	0.005	Allantoin degradation
ST45	1	0.515	-
ST61	1	0.515	-
ST62	1	0.515	Spore mass grey
ST66	1	0.265	Allantoin degradation
ST68	1	0.515	-
ST71	1	1	Raffinose utilization
ST73	1	0.492	Raffinose utilization , Xylan degradation
ST74	1	0.0049	

A computerized database (Table 4) was used to compare the biological properties of all isolates of *Streptomyces* with those of Streptomyces sp. The results suggest that strains are a *Streptomycete* strongly related to *S. aurantiacus*. Though it has some similarity with the type strain *Streptomyces aurantiacus* (Nonomura, 1974) for capability to utilize arbinose and fructose, at the same time they are marginally distinctive with respect to grayish appearance, degradation of allantoin and utilization of and raffinose. All biochemical and physiological characteristics directed towards the identification of this strains as *Streptomyces aurantiacus* . However, these strains are morphologically varied with the type strain of *Streptomyces aurantiacus* (Shirling and Gottlieb, 1968). Our isolates may be new variants of *Streptomyces aurantiacus*.

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