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Isolation and Biochemical characterization of thermophilic *Streptomyces* from soil of Raipur District of Chhattisgarh, India

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ABSTRACT

The present study was aimed to isolate and characterize the thermophilic *Streptomyces* from soil of Raipur district of Chhattisgarh. The samples were collected from 16 blocks of Raipur district, mainly from the compost and garbage soil. Isolation of thermophilic *Streptomyces* was carried out from the samples using serial dilution spread plate method with starch casein agar medium. Temperature was maintained at 45^{o}C for isolation of thermophilic *Streptomyces.* Total 42 thermophilic *Streptomyces* were isolated and characterized on the basis of colour of spore mass, reverse side colour, aerial and substrate mycelia formation and production of diffusible pigments. Out of 42 isolates, 20 were selected on the basis of temperature optima for further studies. The selected isolates were further characterized on the basis of biochemical tests and antimicrobial activity against eight microbial strains *i.e. Escherichia coli, Stapylococus aureus, Pseudomonas aeruginosa, Bacillus subtilis*, *Aspergillus niger, Penicillium notatum, Penicillium chrysogenum* and *Candida albicans.* The growth of different *Streptomyces* isolates were recorded in presence of different carbon and nitrogen sources, different concentrations of NaCl and at different pH. The growth of different isolates of *Streptomyces* was also observed in presence of different chemical inhibitors *viz*. potassium tellurite, sodium azide, phenol and crystal violet. All isolates were identified using PIB Win (Probabilistic identification of bacteria) software.

Key words: thermophilic *Streptomyces*, biochemical tests, PIB Win software.

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INTRODUCTION

Temperature is one of the most important environmental factor influencing the growth and survival of organisms. It can affect living organisms in either of two opposing ways. As the temperature rises, chemical and enzymatic reaction in the cell proceed at more rapid rates and growth becomes faster. However, above a certain temperature, proteins, nucleic acid, and other cellular components may be irreversibly damaged. As the temperature is increased. Within a given range, growth and metabolic function increase up to a point where inactivation reactions set in. Above this point, cell functions fall sharply to zero. Thus, we find that for every organism there is a minimum temperature below which growth no longer occurs an optimum temperature at which growth is most rapid, and a maximum temperature above which growth is not possible [3].

The actinomycetes are gram-positive organisms that tend to grow slowly as branching filaments. Actinomycetes are the most abundant organism in soil. They break down proteins cellulose and other organic matter[5]. The genus *Streptomyces* was proposed by Waksman and Henerici [17]. The genus currently accommodates aerobic Gram-positive actinomycetes that are highly oxidative, form extensively branching substrate hyphae, aerial hyphae bearing long spore chains, contain L-DAP and glycine but no characteristic sugar in the cell wall [9] and have a DNA base composition within the range of 69 to 73 mol% G+C.

The *Streptomycetes* produce numerous compounds (secondary metabolites), within which antibiotics are of commercial relevance. As corresponds to their habitat, these bacteria are nutritionally quite versatile and the most produce extracellular hydrolytic enzymes that permit the utilization of high molecular weight biopolymers such as proteins, polysaccharides, fats and other substrates [13].

The present study aims to isolate and identify (cultural, morphological, physiological and biochemical) thermophilic *Streptomyces* from soil of Raipur district of Chhattisgarh, India. The organisms can be exploited for isolation and characterization of important bio-molecules for different uses.

MATERIALS AND METHODS

Collection and pretreatment of soil samples

Soil samples were collected from 16 blocks of Raipur district of Chhattisgarh state (22^{o} 33' N to 21^{o} 14'N latitude and 82^{o} 6' to 81^{o} 38' E longitude). Samples were collected at random from the soil at depths between 10-20 cm especially from compost and manure containing soil to isolate thermophilic *Streptomyces.* Collected soil samples were pretreated at 45^{o}C in oven for removal of contamination in the form of lower bacteria, fungal spores, mites *etc* [19].

Media and cultural condition

Starch casein agar media (g/l: starch 10, vitamin free casamino acid 0.3, KNO_3 2, NaCl 2, $MgSO_4$. $7H_20$ 0.05, $CaCO_3$ 0.02, $FeSO_4$. $7H_20$ 0.01 and agar 18) supplemented with cyclohexamide 25µg/ml was used for the isolation of thermophilic *Streptomyces* by spread plate technique after serial dilution. The inoculated plates were incubated at 45°C for7 to 10 days. After incubation, colonies were purified using streak plate technique and kept at 4°C for further investigation.

Cultural characteristics

Streptomyces isolates were grown in culture plates containing starch casein agar medium at 45 ± 1°C for 3 days. After the incubation cultural characteristics such as colours of substrate and aerial mycelia, growth pattern, pigment production on tyrosine agar and production of diffusible pigments were observed [20-21].

Morphological characteristics

Inclined cover slip culturing was used for observing *Streptomyces* morphology. Inoculated agar plates with coverslips inserted at an angle of 45° were incubated at 45°C. After incubation time was over coverslips were withdrawn and mounted upper surface down with lacto phenol cotton blue using as a mounting agent. The spore chain morphology was examined by light compound trinocular microscope (Leica, DMLS) [4].

Effect of temperature on growth of *Streptomyces sps.*

Starch casein broth medium was prepared and transferred in 150 ml of Erlenmeyer flask, each containing 35 ml of starch casein broth medium, having 7.2 pH. 1 ml of fresh spore suspension of test *Streptomyces sps.* was transferred into broth medium. Cultures were incubated at temperatures of 27°C, 37°C, 45°C, 50°C, 55°C, 60°C, 65 ± 1 °C for ten days. Cultures were filtered after ten days with the help of sterilized preweighed Whatman No. 1 filter papers and dried in an oven at 50 °C for 24 h. dried filter papers were reweighed along with the mycelium, till a constant weight is obtained.

Cell wall analysis

The cells were killed with formalin (final concentration, 1%) for 24 h at room temperature and collected by centrifugation. The cells were washed once in distilled water and once in 95% ethanol and then dried by overnight heating in a hot air oven at 45°C [16]. The dried cells were analyzed for diaminopimelic acid (DAP) by paper chromatography following the method of Becker *et al.* [2].

Physiological and biochemical Characteristics

The physiological and biochemical characteristics were determined according to the methods of Shirling and Gottlieb[15] and Williams *et al.*[20-21] *Streptomyces* isolates were incubated at 45^{o}C and examined after 3-7 days. Degradation activity of xanthine and elastin was performed by the method of William's *et al.* [19] and results were recorded after 3-7 days. Lipolytic and lecithinase activities were performed according to the method of Nitsch and Kutzner [12]. Hydrolysis of pectin was detected by method of Hankin *et al* [6]. Hydrolysis of hippurate was tested using method of Ziegler and Kutzner [22]. Nitrate reduction and Hydrogen sulfide production were performed and results were recorded after 4-6 days. Effect of different concentration of NaCl (1-9%) and different pH (4-11) were recorded on the growth of different *Streptomyces* isolates. The growth of different isolates of *Streptomyces* was observed in presence of different chemical inhibitors *viz*. Potassium tellurite (0.01%, 0.001%), sodium azide (0.01%, 0.02%), phenol (0.1%) and crystal violet (0.0001%) [20-21].

Utilization of carbon and Nitrogen sources

The ability of test *Streptomyces* for the utilization of different carbon sources *i.e.* sucrose, D-melebiose, D-melizitose, L-rhamnose, mannitol, meso-inositol, xylitol, adonitol, dextan, raffinose, were studied using the method as suggested by Shirling and Gottlieb[15]. Carbon sources were added on the basal mineral salt agar medium at 1.0% (w/v) and result were recorded after 3-14 days. The ability of *Streptomyces* to utilize, different nitrogen sources i.e., L-histidine, L-phenyl-alanine, L-cysteine, DL-α-amino-n-butyric acid, L-valine, and L-hydroxyproline were investigated using the method as given in Bergey's Manual of Systematic Bacteriology, Volume IV[18]. Nitrogen sources were added to the basal medium at 0.1% (w/v). Results were recorded after 6 days.

Antimicrobial activity

The antimicrobial activities of *Streptomyces* culture filtrates were performed by the agar diffusion method (MTCC 1998) against eight test organism i.e. *E. coli* (MTCC 1667), *S. aureus* (MTCC 96), *P. aeruginosa* (MTCC 4682), *B. subtilis* (MTCC 1789), *A. niger* (MTCC 872), *P. notatum, P. chrysogenum* (MTCC 689) and *C. albicans* (MTCC 1637).

Resistance to antibiotics

The growth of each *Streptomyces* isolate was also tested for their ability to grow in the presence of 4 antibiotic susceptibility discs viz. neomycin (30 µg), olendomycin (15 µg), penicillin G (10 units), rifampicin (5 µg), obtained from Hi-Media Pvt. Ltd., Mumbai, India. After incubation of 7 days the presence and size of inhibition zones around the discs of the antibiotics were noted. The growth response of test *Streptomyces* was recorded as resistant (R) intermediate (I) and sensitive (S) to an antibiotic by comparison with data given for each of the antibiotic by the Hi-Media Pvt. Ltd., Mumbai, India [1].

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Identification of isolated cultures

Purified isolates of actinomycetes were identified using PIB Win (Probabilistic identification of bacteria) software. This software is based on various cultural, morphological, and biochemical characteristics. A probabilistic identification matrix for *Streptomyces* is based on 50 characters like spore chain morphology, pigmentation, antibiosis, antibiotic sensitivity, growth tolerances and nutritional requirements [8].

RESULT AND DISCUSSION

Isolation and characterization of isolates

Total 42 actinomycetes were recorded from soil of Raipur district of Chhattisgarh (Table 1). All isolates exhibited growth in starch casein agar medium.

Table 1: Occurrence of *Streptomyces* from soil of Raipur district of Chhattisgarh.

S. no.	Name of Blocks (sampling site)	Colour and texture of soil	No. of samples		No. of *Streptomyces* Isolates
			Tested	Positive	
1.	Abhanpur	Black and brown garbage and compost soil	2	2	4
2.	Arang	Dark brown compost soil	1	1	1
3.	Balodabazar	Dark brown and yellowish compost soil	2	2	3
4.	Bhatapara	Dark brown compost soil	1	1	3
5.	Bhilaigari	Dark brown ground soil	1	1	1
6.	Chhura	Dark brown ground soil	1	1	2
7.	Dharsiva	Dark brown compost soil	2	1	3
8.	Deobhog	Light brown ground soil	1	1	2
9.	Gariyaband	Dark brown compost soil	1	1	3
10.	Kasdol	Light brown ground soil	1	1	2
11.	Mainpur	Dark brown field soil	1	1	2
12.	Palari	Light brown and grayish field and compost soil	2	1	3
13.	Raipur	Light and dark ground, garbage and compost soil	4	4	6
14.	Rajim	Light brown ground soil	1	1	2
15.	Simga	Black compost soil	2	1	3
16.	Tilda	Light brown compost soil	1	1	2
	Total		24	21	42

The temperature limits for growth of 42 isolates were recorded in starch casein broth medium. Twenty isolates were selected on the basis of temperature optima. All isolates were capable to grow in the temperature ranges between 28 and 55^{o}C and showed optimum growth at 45^{o}C while one isolate i.e. TS18 (*S. phaeochromogenes)* showed good growth at 55^{o}C poor growth at 37^{o}C and no growth was found at 28^{o}C. *Streptomycetes* able to grow at 45-55^{o}C are regarded as thermophilles [7].

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Morphological, physiological and biochemical characteristics of the twenty isolates have been presented (Table.2). All the isolates were found to be gram- positive. Most of the isolates have rectiflexible spore chain morphology (Fig. 1a) and four isolates i.e.TS9, TS18, TS22 and TS42 exhibited spiral spores chain morphology (Fig. 1b).

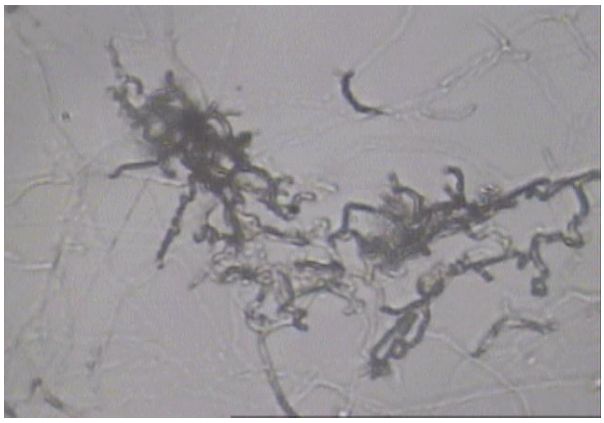

Fig. 1a: Rectiflexible spore chain of TS3 *Streptomyces exfoliates* (1000x)

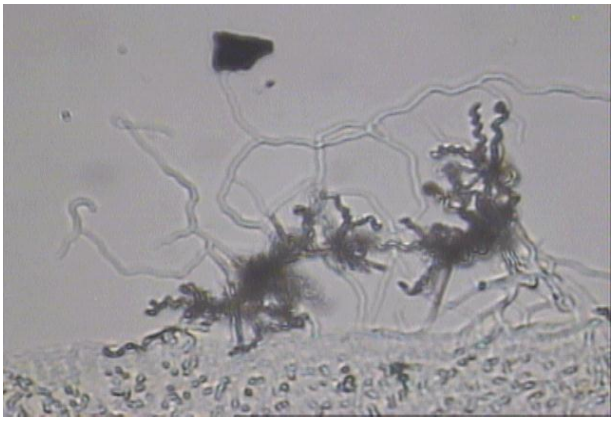

Fig. 1b: Spiral spore chain of TS18 *Streptomyces phaeochromogenes* (1000x)

Analysis of whole cell hydrolysate showed that all isolates contain LL- diaminopimelic acid indicating that they all belong to the genus *Streptomyces* (Fig. 2). Most actinomycetes isolated from any habitat are likely to be *Streptomyces* [10].

Pigment production has been observed in most of the isolates on tyrosine agar medium (Fig. 3). Most of the isolates were found positive for pectin, lecithin, lipid, and xanthine. Nitrate reduction was observed positive in TS1, TS3, TS18, TS21 TS22 and TS35, whereas remaining test isolates failed to reduce nitrate to nitrite. H_2S production was found positive in TS9, TS10, TS15, TS18, TS19, TS29, TS32, TS34, TS36, TS37 and TS42. Hydrolysis of hippurate was found positive in TS1, TS3, TS5, TS35 and TS42. Degradation of elastin was observed positive in TS1, TS3, TS18 and TS21.

Test isolates gave different growth response when tested in presence of various inhibitors. All isolates showed growth in presence of 0.001% (w/v) potassium tellurite but some

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isolates i.e., TS1, TS3, TS5, TS18, TS21, TS22, TS34, TS35, TS36, TS37, TS39 and TS42 were also exhibited growth at its higher concentration i.e., 0.01% (w/v). Most of the isolates were found to be resistant to 0.01% (w/v) sodium azide and 0.01% (w/v) phenol (Fig. 4). Crystal violet at 0.0001% (w/v) and sodium azide at 0.02% (w/v) were found effective against most of the isolates.

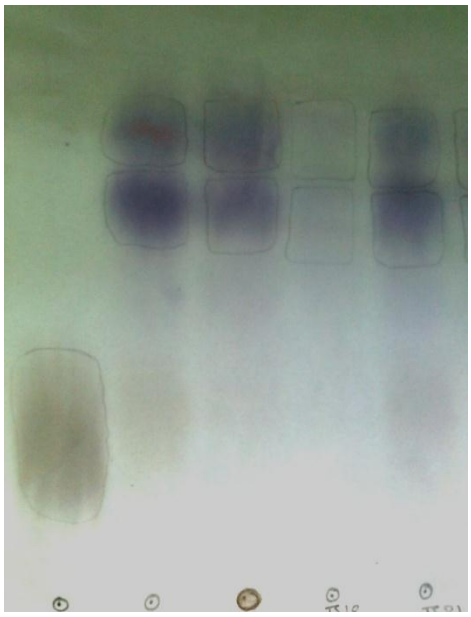

Fig 2: Cell wall analysis of *Streptomyces* by paper chromatography
1. DL- DAP (Sigma chemicals co.), 2-5 Cell hydrolysates

Fig 3: Melanin production in TS42 (*S.* olivaceoviridis) on tyrosine agar

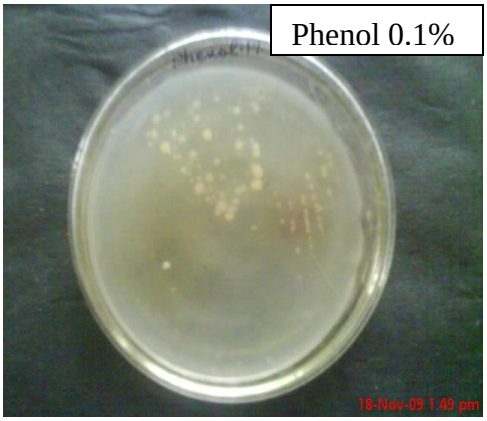

Fig 4: Effect of inhibitors on growth of *Streptomyces* (phenol 0.1%)

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In present study all the isolates grew well on the medium containing up to 2% NaCl. Five test isolates i.e., TS5, TS18, TS21, TS35 and TS39 exhibited growth at 7% NaCl and only one isolate i.e., TS39 showed poor growth in 9% NaCl. All test isolates were found to tolerate a wide range of pH from pH 4 to pH 11. Most of the isolates i.e., TS1, TS3, TS5, TS10, TS15, TS18, TS19, TS22, TS29, TS34, TS30, TS35, TS36, TS39 and TS40 recorded growth at pH 4.3

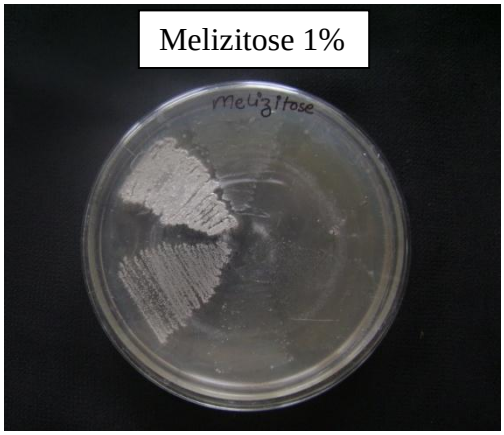

Fig 5a: Utilization of carbon source

Fig 5b: Utilization of nitrogen source

A wide range of carbon compounds were utilized by the *Streptomyces* isolated from soil of Raipur district of Chhattisgarh. The best carbon sources were mannitol, meso-inositol, L-rhamnose, D-melebiose, raffinose and sucrose. Five isolates i.e. TS18, TS22, TS29, TS30, and TS37, exhibited growth in adonitol. Five tests isolates i.e. TS10, TS15, TS18, TS19 and TS30 exhibited growth in xylitol and only three isolates i.e. TS15, TS18 and TS30 exhibited growth in D-melezitose, (Fig. 5a). All isolates exhibited growth in presence of L-histidine, and DL-α-amino-n-butyric acid (Fig. 5b)

Fig 6a: Antimicrobial activity of TS32
S. chromofuscus* against *C. albicans

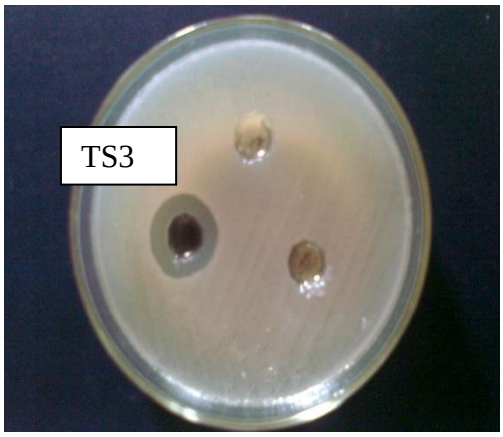

Fig 6b: Antimicrobial activity of TS3
S. exfoliates* against *B. subtilis

Seven isolates exhibited antimicrobial activity against *E. coli* (Fig. 6c and 6d) two against *B. subtilis* (Fig. 6b) and only one isolate against *C. albicans*. (Fig. 6a)

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Fig 6c: Antimicrobial activity of TS1 and TS5 against *E. coli*.

Fig 6d: Antimicrobial activity of TS35 and TS39 against *E. coli*

All isolates failed to grow in presence of Neomycin (30µg) thus indicates their sensitivity for this antibiotic. Most of the isolates were found resistant to Penicillin G (10 units) and Rifampicin (5 µg). Only two isolates i.e., TS18 and TS42 showed sensitivity to Olendomycin (15 µg) showing no growth in presence of this antibiotic.

Identification

In the present study identification of 20 isolates of *Streptomycetes* was done using PIB Win programme which provides probabilistic identification of unknown bacterial isolates against identification matrices of known strains. Probabilistic identification was based on characters like spore chain morphology, mycelial pigments, production of diffusible pigments, physiological and biochemical characteristics, resistance to antibiotics, antimicrobial activity and growth inhibitors etc. (Table 2)

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Table 2: Morphological, physiological and biochemical characteristics of thermophilic *Streptomyces*

Isolate no.	TS1	TS3	TS5	TS9	TS10	TS15	TS18	TS19	TS21	TS22	TS29	TS30	TS32	TS34	TS35	TS36	TS37	TS39	TS40	TS42
Cultural and morphological characteristics																				
Spore chain Rectiflexibiles	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
Spore chain spirals	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+
Spore mass Red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spore mass Grey	+	+	-	-	+	+	-	+	+	+	-	+	-	+	-	+	+	-	-	+
Mycelial pigment red/orange	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diffussible pigment produced	-	-	+	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-	+	+
Diffussible pigment yellow/brown	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	+
Melanin on tyrosine agar	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	+	+	+	-	+
Physiological and biochemical characteristics																				
Lecithinase activity	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+
Lipolysis	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+
Pectin hydrolysis	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+
Nitrate reduction	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-
H_2S production	-	-	-	+	+	+	+	+	-	-	+	-	+	+	-	+	+	-	-	+
Hippurate hydrolysis	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+
Elastin degredation	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Xanthine degredation	-	+	+	-	-	+	+	-	+	-	+	+	-	+	+	+	+	+	-	+
Growth at 45°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NaCl (1% w/v) growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NaCl (2% w/v) growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NaCl (5% w/v) growth	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	-
NaCl (7% w/v) growth	-	-	+	-	-	-	+	-	+	-	-	-	-	-	+	-	-	+	-	-
NaCl (9% w/v) growth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Growth at 4 pH	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+
Growth at 6 pH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 7 pH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 9 pH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 10 pH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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Growth at 11 pH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NaN3 (0.01% w/v) growth	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
NaN3 (0.02% w/v) growth	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	+	+	+	-	-
P.T.(0.001% w/v) growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P.T.(0.01% w/v) growth	+	+	+	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	-	+
Phenol (0.1% w/v) growth	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	-	+
C.V.(0.001%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Resistance to antibiotics																				
Neomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampcin	+	+	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	-	+
Olendomycin	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillin G	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Antimicrobial activities																				
E. coli	+	-	+	+	-	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-
S. aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aerogenosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. subtilis	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.chrysogenum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. notatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Utilization of nitrogen sources																				
DL-α-amino-n-butyric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-cystein	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+
L-valine	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
L-phenyl alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
L-histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-hydroxyproline	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-
Utilization of carbon sources																				
Sucrose	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Meso insitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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Mannitol	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+
Raffinose	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+
D-melizitose	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-	-	-
D- milibiose	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dextran	+	-	-	+	-	-	+	-	-	+	+	+	+	+	-	-	+	+	+	+
Xylitol	-	-	-	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-
ID Score	0.99	0.98	0.98	0.99	0.99	0.99	0.99	0.99	0.97	0.97	0.99	0.97	0.99	0.99	0.99	0.99	0.99	0.99	0.96	0.99
Identification																				

Identification TS1 *S. violaceus*, TS3 *S. exfoliates*, TS5 *S. diastaticus*, TS9 *S. chromofuscus*, TS10 *S. microflavus*, TS15 *S. microflavus*, TS18 *S. phaeochromogenes*, TS19 *S. microflavus*, TS21 *S. violaceus*, TS22 *S. cyaneus,* TS29 *S. cyaneus*, TS30 *S. cyaneus,* TS32 *S. chromofuscus*, TS34 *S. halstedii*, TS35 *S. diastaticus*, TS36 *S. microflavus*, TS37 *S. cyaneus*, TS39 *S. chromofuscus*, TS40 *S. cyaneus*, TS42 *S. olivaceoviridis.* **Legend:** + positive, - negative, NaN3 sodium azide, P.T. potassium tellurite.

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CONCLUSION

Thus, based on above observation it may be concluded that twenty thermophilic *Streptomycetes* were isolated and identified by using PIB Win software. On the basis of the results obtained from enzymatic activity (qualitative assay) cultures will be further assessed for thermostable enzyme production (quantitative assay).

The study of microorganism capable to grow in extreme condition is an important field of microbiology and biotechnology. Thermophilic representatives of the family of actinomycetes are becoming more and more important in solving certain problems of ecology, industrial microbiology, hygiene and other fields. Recently, they have been subjected to intensive investigations in search for producers of new thermostable enzymes [14].

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