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SCREENING AND CHARACTERIZATION OF ALPHA AMYLASE INHIBITOR PRODUCING ISOLATE- *STREPTOMYCES LATERITIUS*- SS4/6 USING 16SrDNA TECHNIQUE

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ABSTRACT
Alpha amylase inhibitors play an important role in reducing blood glucose levels. Inhibiting α-amylase enzyme plays a crucial part in treatment for insulin-dependent diabetes mellitus, obesity and hyperlipedemia. Amylase inhibitors are useful tools for the determination of activities of amylase isozyme and purification of amylases. Most of the amylase inhibitors were isolated from plants and microorganisms using different screening techniques. Screening of 200 marine microorganisms resulted in isolation of one actinomycete strain with the ability to secrete potent alpha amylase inhibitor was designated as SS4/6 and characterized by using 16s rDNA sequencing and phylogenetic characterization. The isolate SS4/6 was identified as *Streptomyces lateritius* by IMTECH Chandigarh with an accession number MTCC 10474. The isolate was able to produce potent alpha amylase inhibitor which can inhibit both salivary alpha amylase and pancreatic alpha amylase.

KEYWORDS: *Streptomyces lateritius*, 16SrDNA, TLC method phylogenetic tree and starch agar plate method.

INTRODUCTION
Actinomycetes represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world and especially significant for their role in the recycling of organic matter.[1,2] Nevine and Ghanem, 2000; Srinivasan *et al*., 1991). Now a day, Diabellitus mellitus is commonly prevailing disorder among the population. By the discovery of new synthetic drug molecules we can control it, but they are having severe side effects, in order to minimize those effects we can go for naturally available molecules with minimum side effects. For example plant alpha amylase inhibitors from wheat and white kidney bean and microbial alpha amylase inhibitors like Tendamistat from *streptomyces tendae* plays an important role in controlling blood glucose levels of diabetic people. The objective of the study describes the screening and characterization of the isolate-SS4/6 as alpha amylase inhibitor producing strain. Different primary and secondary screening methods like TLC plate method, Starch agar plate method and detection by using blue value method were employed. The isolte SS4/6 was characterized by IMTECH Chandigarh and allotted an accession number. The organism was characterized morphologically and by using 16s rDNA technique. phylogenetical characterization indicated that the isolate belongs streptomycete sps. The produced alpha amylase inhibitor in fermentation medium was isolated and tested for inhibition of several enzymes.

MATERIALS AND METHODS
200 strains of actinomycetes were isolated from sea sample of Tamilnadu and were mainted using YEME media. These strains tested for alpha amylase inhibitory activity. Out of 200 strains, only one promising strain was able produce salivary and pancreatic alpha amylase activity.

Techniques used for primary screening
Starch plate method[3] (Ivo Safarik, 1990), Starch agar plate method[4] (Chiaki Imada and Usio Simidu, 1988) and TLC plate method[5] (Haimin Chen *et al*., 2004).

Four α-amylases and one glucosidase preparations were used for the experiment: α -amylase from *Bacillus subtilis*, Fungal α- amylase, Human salivary α –amylase (collected from unstimulated saliva), Albino rat pancreatic alpha amylase *and* Glucosidase.

Secondary screening
Based on the results of the primary screening, the promising isolate was subjected to secondary screening by using different types of media. It was tested for extracellular amylase inhibitory activity by fermentation

in shake flask on rotary shaker at 120 rpm at 29^{O}C for actinomycetes.

Table 1: Different types of media were used for production of the inhibitor.

S.No	Production media (PM)	References
1	PM-I	[6](Koichi Katsuyama *et al*.,1992)
2	PM-II	[7a,b](Laszlo Vertesy and Tripier, 1985 a,b)
3	PM-III	[8](Sawao Murao *et al*., 1981)
4	PM-IV	[9](Sawao Murao and Naoki Oouchi, 1983)
5	PM-V	[10](Chiaki Imada and Usio Simidu, 1992)
6	PM-VI	[11](Sawao Murao and Kunio Ohyama, 1975)
7	PM VII	[12](Jin Hwan *et al*.,1985)
8	PM-VIII	[13](Volker Oeding *et al*.,1981)

Shake flask fermentation

Well sporulated isolate SS4/6 of 6 days was used for inhibitor production. About ten mL of sterile water was added to slants of above isolate. The cells were scrapped from the slant and the resultant cell suspension was transferred aseptically into 250mL Erlenmeyer flasks containing 50mL of each sterile production medium as mentioned above. Eight different types of media (widely used for α-amylase inhibitor production in the literature) were used for the isolate. The inoculated flasks were incubated on rotary shaker (120 rpm) at 29^{O}C for three days. Ten mL of broth was centrifuged at 3000 rpm for 10 minutes and the supernatant solution was tested for α-amylase inhibitory activity by modified blue value method. Shake flask fermentation was carried out in triplicate and mean values were recorded. α-amylase inhibition assay by modified blue value method[8,10] (Sawao Murao *et al*., 1981; Chiaki Imada and Usio Simidu, 1992).

α-amylase inhibition assay by modified blue value method.[8,10] (Sawao Murao *et al*., 1981; Chiaki Imada and Usio Simidu, 1992)

Inhibitory activity of cultured broth was assayed by a modified blue value method. The enzyme used for the assay was human salivary α-amylase was dissolved in 50mM Tris HCl buffer (pH7.0) (1:99) containing 5mM $CaCl_2$. The substrate used was 0.5% soluble starch solution in the same buffer. The enzymatic reaction was started by addition of 0.5mL of enzyme solution to 0.5 mL of culture filtrate; this mixture was incubated at 37^{O}C for 5 minutes. To this mixture 2mL (1.5%) of starch solution was added and the mixture was incubated at 37^{O}C for 10 minutes, then 5mL of a solution containing 1mL of 0.5N hydrochloric acid and 4mL of 1N acetic acid was added to terminate the enzymatic reaction. To 0.1mL of this reaction mixture, 5mL of 0.005% iodine solution containing 0.05% potassium iodide was added. After standing for 20 minutes at room temperature, the absorbance of the mixture at 660 nm was measured (S). The values of iodine reaction color were also measured for an enzyme control (C) without culture filtrate, a sample blank (SB) without enzyme solution and an enzyme blank (B) without enzyme solution and culture filtrate were used to calculate the percentage of the inhibition of α-amylase activity using the following equation.

$$\{[(B-C) – (SB-S)]/ (B-C)\}X\ 100\%$$

One unit of inhibitor was defined as the amount of inhibitor required to decrease the amylase activity by 50% under the above conditions. The above procedure is used to carry out the inhibition assay of fungal, bacterial, human salivary and pancreatic alpha α-amylase by modified blue value method.

Taxonomic Studies of Actinomycete Isolate -SS4/6

Selection of media for growth

Culture media used for characterization and identification of species consists of both synthetic and organic forms. Synthetic media have found extensive application in the study of morphology, physiology and cultural properties of the organism, while organic media are used for obtaining supplementary cultural evidence. Thus to furnish reliable description of authentic, extant culture of *Streptomyces*; the criteria laid down by the International Streptomycetes Project (ISP)[14] (Shirling and Gottlieb, 1966) were adopted. The International Streptomycetes Project (ISP)[15a,b,16a,b] (Shirling and Gottlieb, 1968, 18, I study, 69, 18 –I and II studies 279, Shirling and Gottlieb, 1969, 19-II, III, IV studies), Bergey's Manual of Determinative Bacteriology[17] (Buchanan and Gibbons, 1974) Bergey's Manual of Systematic Bacteriology[18a,b] (Williams *et al*., 1992-1993) and all other relevant journals.

Media used for characterization

Waksman (1958)[19] and others recommended the inclusion of the following media for characterization of actinomycetes:

Preferably sucrose nitrate salt agar or sucrose ammonium salt agar, glucose or glycerol asparagine agar and malate or citrate agar, Two or three organic media such as nutrient agar, yeast extract malt extract agar, potato glycerol glutamate agar or oat meal agar, Three or four complex natural media such as potato plug, gelatin and milk, Peptone iron yeast extract agar for H_2S production, Tyrosine medium for tyrosinase reaction and A synthetic medium for carbohydrate utilization.

Experimental procedure

In the present work morphological studies and color determination of the selected isolates were determined by following International Streptomycetes project (ISP) procedures of[14] Shirling and Gottlieb 1966). The following media as recommended by ISP were used.

Yeast extract malt extract agar (ISP-2), Oat meal agar (ISP-3), Inorganic salts-starch agar medium (ISP-, and Glycerol-asparagine agar medium (ISP-5).

Scanning electron microscopy of SS4/6

Procedure

Special morphological features like spore morphology and spore surface ornamentation (for one selected isolate) were determined by scanning electron microscope. Samples were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4^{O}C and post fixed 2% in aqueous osmium tetroxide for 4 hours. Dehydrated in series of graded alcohols and dried to critical point drying with CPD unit. The processed samples were mounted over the stubs with doubled-sided carbon conductivity tape and a thin layer of gold coat over the samples was done by using an automated sputter coater (Model –JOEL JFC-1600) at required under Scanning Electron Microscope (SEM-Model: JOEL-JSM 5600) at required magnifications as per the standard procedure at RUSKA Lab's College of Veterinary Sciences, SVVU, Rajendranagar, Hyderabad, India.

Medium used: Yeast extract-malt extract agar (ISP-2)

Method used: Cover slip method (Williams and cross, 1971)

THE 16SrDNA STUDIES

Over the last few years, cultivation-independent methodologies, particularly the sequence analysis of cloned 16S ribosomal RNA genes (16S rDNA), have proven to be powerful tools for investigating the microbial diversity of environmental samples. At least it is important in specific identification of the metabolically active microorganisms, since these are responsible for the microbial driven environmental processes. Microbial evolution, diversity and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Microbial. Ecol., 35: 1-21.) The 16s rDNA gene sequence of the strain SS4/6 was used as a query to search for homologous sequence in the nucleotide sequence data bases by running BLASTN program. The high scoring is similar to 16s rDNA gene sequences were identified from the BLASTN result and retrieved from gene bank databases. phylogenetic trees were inferred by using the neighbor joining Boot strap analysis with the help of mega 3.0 software package.

Ability to produce antibiotics

The antibiotic producing capacity of the selected isolate was studied again for confirmation by submerged fermentation technique. The antimicrobial spectra of the selected isolates were checked by cup plate method.

Ability of the isolates to resist various antibiotics

The ability of the isolates to resist various types of antibiotics was one of the criteria for identification of actinomycete strains. This test was carried out on Bennett's agar medium. The antibiotic sterilized by filtration was incorporated into the sterile molten Bennett's agar medium, mixed thoroughly into the sterile plates (6'diam). The isolates were streaked on the agar surface and incubated at 28^{O}C.The presence or absence of growth was observed on 3rd day and on 7th day. Resistance was recorded as positive; sensitive to antibiotic was regarded as negative.

The following antibiotics were used for this test (µg/mL): Penicillin-G (10 IU) Streptomycin (100) Tetracycline (50) Cephalexin (100) Gentamicin (100) and Rifampcin (50). Diluents used for the preparation of standard antibiotic solutions are according to (Indian Pharmacopoeia 3rd edition 1985 and Indian Pharmacopoeia 4th edition 1996).

RESULTS AND DISCUSSION

Starch Plate method

1. EDTA+ salivary α-amylase inhibition
2. Human Salivary α-amylase inhibition
3. Action of salivary α-amylase on starch plate
4. Albino rat pancreatic α-amylase inhibition
5. Albino rat pancreatic α-amylase inhibition

Fig. 1: Identification of α-amylase inhibitor producing isolates by starch plate method.

SS4/6 was identified as α-amylase inhibitor producing isolates by starch plate method. EDTA is a standard amylase inhibitor.

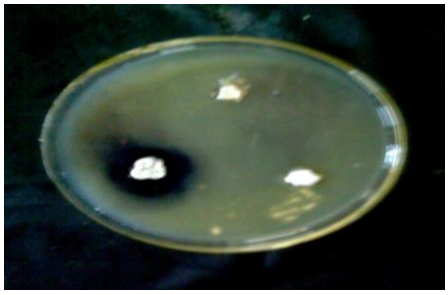

Fig. 2: Screening of salivary α-amylase inhibitor producing isolates by starch agar plate.

Among 200 actinomycetes isolates, SS4/6 was identified as the only α-amylase inhibitor producing isolate by starch agar plate method. Blue color zone was observed around the SS4/6 colony, after addition of iodine solution to the plate, indicating that SS4/6 was able to produce α-amylase inhibitor. Remaining white spots indicated that the colonies of other actinomycetes unable to produce alpha amylase inhibitor (fig:2). SS4/6 was identified as human salivary α-amylase inhibitor producing isolate by TLC plate method. Blue color zone around the sample spot indicates that it is acting against salivary α-amylase (fig:3).

Fig. 3: Identification of salivary α-amylase inhibitor producing isolate by TLC plate method.

Table 2: Determination of type of amylase inhibitor produced by the isolates.

Type of amylase	Substrate	pH	SS4/6
Human salivary α-amylase	Soluble Starch	7.0	+
Bacillus.subtilis α-amylase	Soluble Starch	7.0	-
Fungal α-amylase	Soluble Starch	7.0	-
Glucosidase	Soluble Starch	7.0	-
Pancreatic alpha amylase	Soluble Starch	7.0	+

(+ = indicates inhibition - = no inhibition).

The inhibitor produced by SS4/6 strongly inhibits the salivary α-amylase and pancreatic alpha amylase. SS4/6 isolate was unable to inhibit glucosidase, Bacillus. subtilis α-amylase and Fungal α-amylase. (using modified blue value method).

Table 3: Production alpha amylase inhibitor in different media.

Medium No	SS4/6 percent inhibition of albino rat pancreatic alpha amylase	Number of inhibitor units /mL	percent inhibition of albino rat pancreatic alpha amylase	Number of inhibitor units /mL
I	37	1.48	26	1.04
II	32	1.28	28	1.12
III	34	1.36	24	0.964
IV	38	1.52	**33**	**1.32**
V	**44**	**1.76**	22	0.88
VI	34	1.36	25	1.0
VII	31	1.24	24	0.96
VIII	37	1.48	24	0.964

Note: *It was clear from the results that the isolate SS4/6 has shown maximum inhibitory activity in medium-V. SS4/6 exhibited 44% inhibitory activity against salivary amylase and 33% against albino rat pancreatic alpha amylase activity and it was selected as the basal medium for subsequent studies.*

Micromorphology

The aerial mycelium developed moderately to good on the medium. The aerial hyphae were long and forms hooks and open loops. Sporophores are coiled and belongs to RA (Retinaculum- Apertum).

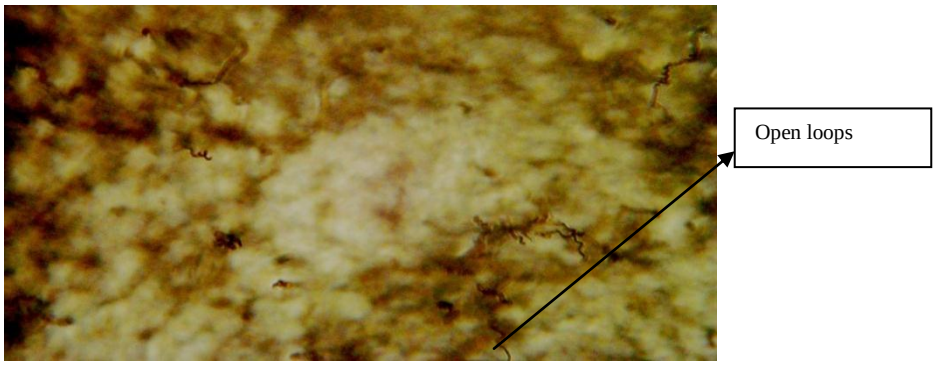

Fig. 4: Light micrograph of aerial mycelium of isolate SS4/6 at 400x magnification.

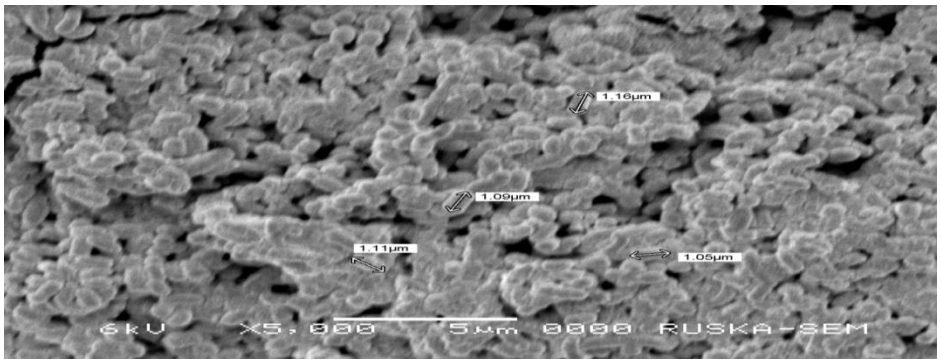

Fig. 5: Scanning electron micrograph of the isolate SS4/6 at 5000x magnification.

The morphological characteristic of isolate SS4/6 is an aerial mycelium containing hooks with open loops. On maturity, it divided and formed spiral spore chains. Each spore chain held about 10 to 20 spores. The size of each elliptical spore was about 1.0 to 1.1µm. The surface of the spore was smooth, no sclerotic granules, sporangia or zoospores were observed.

16S rDNA sequencing of SS4/6

Note: The reported identity of the sample is bound by limitations of sequence based molecular systematics

The project involved the following steps:

- DNA isolation from bacteria culture using the phenol chloroform method of Genomic DNA isolation
- Amplification of the 16SrDNA studies region using primers designed in the conserved region, the primers used were FD1 and RP2
- Sequencing of the ~1.5kb region using internal sequencing primers

16Sr DNA Sequence of Sample SS 4/6

1 tattgaggtg gaccagccgc accttccggt acggctacct tgttacgact tcgtcccaat
61 cgccagtccc accttcgaca gctccctccc acaaggggtt gggccaccgg cttcgggtgt
121 taccgacttt cgtgacgtga cgggcggtgt gtacaaggcc cgggaacgta ttcaccgcag
181 caatgctgat ctgcgattac tagcgactcc gacttcatgg ggtcgagttg cagaccccaa
241 tccgaactga gaccggcttt ttgagattcg ctccaccttg cggtatcgca gctcattgta
301 ccggccattg tagcacgtgt gcagcccaag acataagggg catgatgact tgacgtcgtc
361 cccaccttcc tccgagttga ccccggcggt ctcccgtgag tccccaacct ccgaagagtt
421 gctggcaaca cgggacaagg gttgcgctcg ttgcgggact taacccaaca tctcacgaca
481 cgagctgacg acagccatgc accacctgta caccgaccac aaggggggca ccatctctga
541 tgctttccgg tgtatgtcaa gccttggtaa ggttcttcgc gttgcgtcga attaagccac
601 atgctccgcc gcttgtgcgg gcccccgtca attcctttga gtttagcct tgcggccgta
661 ctccccaggc ggggcactta atgcgttagc tgcggcacgg acaacgtgga atgttgccca
721 cacctagtgc ccaccgttta cggcgtggac taccagggta tctaatcctg ttcgctcccc
781 acgctttcgc tcctcagcgt cagtatcggc ccagagatcc gccttcgcca ccggtgttcc
841 tcctgatatc tgcgcatttc accgctacac caggaattcc gatctcccct accgaactct
901 agcctgcccg tatcgactgc agacccgggg ttaagccccg ggctttcaca accgacgcga
961 caagccgcct acgagctctt tacgcccaat aattccggac aacgcttgcg ccctacgtat

1021 taccgcggct gctggcacgt agttagccgg cgcttcttct gcaggtaccg tcactttcgc
1081 ttcttccctg ctgaaagagg tttacaaccc gaaggccgtc atccctcacg cggcgtcgct
1141 gcatcaggct ttcgcccatt gtgcaatatt ccccactgct gcctcccgta ggagtctggg
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1261 ggtgagccat tacctcacca acaagctgat aggccgcggg ctcatcctgc accgccggag
1321 ctttcgaccc gccaagatgc cttggcaggt cagtatccgg tattagaccc cgtttccagg
1381 gcttgtccca gagtgcaggg cagattgccc acgtgttact cacccgttcg ccactaatcc
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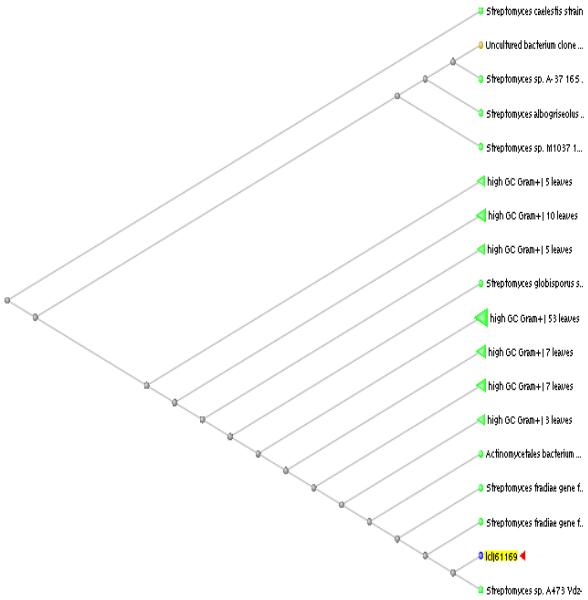

Figure 6: Phylogenitic tree for sample- SS4/6.

The 16s rDNA gene sequence of the strain SS4/6 was used as a query to search for homologous sequence in the nucleotide sequence data bases by running BLASTN program. The high scoring is similar to 16s rDNA gene sequences were identified from the BLASTN result and retrieved from gene bank databases. phylogenetic trees were inferred by using the neighbor joining Boot strap analysis with the help of mega 3.0 software package.

The sequencing results were trimmed and assembled. The assembly of the sequences is as follows

Consensus
TATTGAGGTGGACCAGCCGCACCTTCCGGTACGGCTACCTTGTTACGACTTC
GTCCC 57
16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 0
16SEQ3F_S
TATTGAGGTGGACCAGCCGCACCTTCCGGTACGGCTACCTTGTTACGACTTC
GTCCC 57
Consensus
AATCGCCAGTCCCACCTTCGACAGCTCCCTCCCACAAGGGGTTGGGCCACC
GGCTTC 114
16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 0
16SEQ3F_S
AATCGCCAGTCCCACCTTCGACAGCTCCCTCCCACAAGGGGTTGGGCCACC
GGCTTC 114
Consensus
GGGTGTTACCGACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGA
ACGTAT 171
16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S ----------GGGAACGTAT 10
16SEQ3F_S
GGGTGTTACCGACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGA
ACGTAT 171
Consensus
TCACCGCAGCAATGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGT
CGAGT 228
16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S
TCACCGCAGCAATGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGT
CGAGT 67
16SEQ3F_S
TCACCGCAGCAATGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGT
CGAGT 228
Consensus
TGCAGACCCCAATCCGAACTGAGACCGGCTTTTTGAGATTCGCTCCACCTTG
CGGTA 285
16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S
TGCAGACCCCAATCCGAACTGAGACCGGCTTTTTGAGATTCGCTCCACCTTG
CGGTA
16SEQ3F_S
TGCAGACCCCAATCCGAACTGAGACCGGCTTTTTGAGATTCGCTCCACCTTG
CGGTA 285
Consensus
TCGCAGCTCATTGTACCGGCCATTGTAGCACGTGTGCAGCCCAAGACATAA
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16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S
TCGCAGCTCATTGTACCGGCCATTGTAGCACGTGTGCAGCCCAAGACATAA
GGGGCA 181
16SEQ3F_S
TCGCAGCTCATTGTACCGGCCATTGTAGCACGTGTGCAGCCCAAGACATAA
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INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S
TGATGACTTGACGTCGTCCCCACCTTCCTCCGAGTTGACCCCGGCGGTCTCC
CGTGA 238
16SEQ3F_S TGATGACTTGACGTCGTCCCCAC---------- 365
Consensus
GTCCCCAACCTCCGAAGAGTTGCTGGCAACACGGGACAAGGGTTGCGCTCG
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16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S
GTCCCCAACCTCCGAAGAGTTGCTGGCAACACGGGACAAGGGTTGCGCTCG
TTGCGG 295
16SEQ3F_S ---------- 365
Consensus
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16SEQ2R_S ---------- 0
INS16S1RE ---------- 0
16SEQ4R_S ---------- 0
16SEQ4F_S
GACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCT
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16SEQ3F_S ---------- 365
Consensus
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GGTAA 570
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INS16S1RE ----------
GGGGGCACCATCTCTGATGCTTTCCGGTGTATGTCAAGCCTTGGTAA 47
16SEQ4R_S ---------- 0
16SEQ4F_S
CGACCACAAGGGGGGCACCATCTCTGATGCTTTCCGGTGTATGTCAAGCCTT
GGTAA 409
16SEQ3F_S ---------- 365
Consensus
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CCCCG 627
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INS16S1RE
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16SEQ4R_S ---------- 0
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16SEQ3F_S ---------- 365
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16SEQ4F_S
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AATGC 523
16SEQ3F_S ---------- 365
Consensus
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INS16S1RE
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GTTTAC 218
16SEQ4R_S ---------- 0
16SEQ4F_S GTTAGCTGCGGCACGGA---------- 540
16SEQ3F_S ---------- 365
Consensus
GGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCC
TCAGC 798
16SEQ2R_S ---------- 0
INS16S1RE
GGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCC
TCAGC 275
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
Consensus
GTCAGTATCGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCTCCTGATATC
TGCGC 855
16SEQ2R_S ---------- 0
INS16S1RE
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TGCGC 332
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
Consensus
ATTTCACCGCTACACCAGGAATTCCGATCTCCCCTACCGAACTCTAGCCTGC
CCGTA 912
16SEQ2R_S ----------CCCGTA 6
INS16S1RE
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CCGTA 389
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
Consensus
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CCTGCA 1311
16SEQ2R_S
CGCCTTGGTGAGCCATTACCTCACCAACAAGCTGATAGGCCGCGGGCTCAT
CCTGCA 405
INS16S1RE ---------- 540
16SEQ4R_S ---------- 0
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
Consensus
CCGCCGGAGCTTTCGACCCGCCAAGATGCCTTGGCAGGTCAGTATCCGGTAT
TAGAC 1368
16SEQ2R_S
CCGCCGGAGCTTTCGACCCGCCAAGATGCCTTGGCAGGTCAGTATCCGGTAT
TAGAC 462

INS16S1RE ---------- 540
16SEQ4R_S ----------GGTCAGTATCCGGTATTAGAC 21
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
Consensus
CCCGTTTCCAGGGCTTGTCCCAGAGTGCAGGGCAGATTGCCCACGTGTTACT
CACCC 1425
16SEQ2R_S
CCCGTTTCCAGGGCTTGTCCCAGAGTGCAGGGCAGATTGCCCACGTGTTACT
CACCC 519
INS16S1RE ---------- 540
16SEQ4R_S
CCCGTTTCCAGGGCTTGTCCCAGAGTGCAGGGCAGATTGCCCACGTGTTACT
CACCC 78
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365

Consensus
GTTCGCCACTAATCCCCACCGAAGTGGTTCATCGTTCGACTTGCATGTGTTA
AGCAC 1482
16SEQ2R_S
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INS16S1RE ---------- 540
16SEQ4R_S
GTTCGCCACTAATCCCCACCGAAGTGGTTCATCGTTCGACTTGCATGTGTTA
AGCAC 135
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365
Consensus GCCGCCAGCGTTCGTCCTAAGCCAGGATCAAACT 1516
16SEQ2R_S GCCGCCAGCGTTCGTCC---------- 593
INS16S1RE ---------- 540
16SEQ4R_S GCCGCCAGCGTTCGTCCTAAGCCAGGATCAAACT 169
16SEQ4F_S ---------- 540
16SEQ3F_S ---------- 365

Table 4: Top 10 sequence producing significant alignments.

Accession	Description	Max score	Total score	Query coverage	E value	Max identification
GU045551.1	*Streptomyces* sp. SCY811 16S ribosomal RNA gene, partial sequence	2659	2659	99%	0.0	99%
AL939108.1	*Streptomyces coelicolor* A3(2) complete genome; segment 5/29	2648	2648	99%	0.0	98%
GQ494993.1	Uncultured *Streptomyces* sp. clone neau49 16S ribosomal RNA gene, partial sequence	2646	2646	99%	0.0	98%
GU130099.1	*Streptomyces* sp. 220297 16S ribosomal RNA gene, partial sequence	2645	2645	99%	0.0	98%
GU045553.1	*Streptomyces* sp. SCY830 16S ribosomal RNA gene, partial sequence	2645	2645	99%	0.0	98%
EU876684.1	*Streptomyces* sp. M1019 16S ribosomal RNA gene, partial sequence	2645	2645	99%	0.0	98%
AF429397.1	*Streptomyces* sp. VTT E-99-1333 (B306) 16S ribosomal RNA gene, partial sequence	2645	2645	99%	0.0	98%
AL939119.1	*Streptomyces coelicolor* A3(2) complete genome; segment 16/29	2645	2645	99%	0.0	98%
AL939124.1	*Streptomyces coelicolor* A3(2) complete genome; segment 21/29	2645	2645	99%	0.0	98%
AL939116.1	*Streptomyces coelicolor* A3(2) complete genome; segment 13/29	2645	2645	99%	0.0	98%

Sequencing was performed with 5 different primers designed in the conserved regions on 16SrDNA, the primer names are as listed as 16SEQ2R_SS, INS16S1REV_SS, 16SEQ4R_S, 16SEQ4F_SS and 16SEQ3F_SS. Result: The sample shows 99% identity at 99% coverage with Streptomyces sps.

Table 5: Cultural characteristics of *Streptomyces lateritius* and isolate-SS4/6.

Medium	Cultural characteristics of *Streptomyces lateritius*	Cultural characteristics of SS4/6
Yeast extract-malt extract agar(ISP-2)	G : moderate AM: poorly developed and red color R : light brownish gray SP : none	G : Abundant AM: Light gray R : light brownish gray SP : none
Oat meal agar(ISP-3)	G : moderate AM: poorly developed and red color R : gray yellow SP : blue to violet	G : moderate AM: cream R : light cream SP : none
Inorganic salts starch agar(ISP-4)	G : Abundant AM: red color R : grayish yellow SP : blue to violet	G : Abundant AM: light gray R : gray SP : none
Glycerol-asparagine agar (ISP-5)	G : poor AM: no aerial mycelium R : grayish yellow SP : blue to violet	G : moderate AM: light gray R : light gray SP : none
Starch casein agar	G : abundant AM: gray R : light gray SP : blue to violet	G : abundant AM: gray R : light gray SP : none

G: growth AM: aerial mycelium R: reverse color SP: soluble pigment
Note: This report was given by IMTECH chandigarh

Table 6: Antimicrobial spectrum of SS4/6.

S.No	Test organism	Inhibition zone diameter(mm)
1	Gram-Positive bacteria *B.pumilis, B.subtilis and S.aureus*	8,10 and 16mm
2	Gram negative bacteria- *E.coli and Pseudomonas aeruginosa*	-
3	Fungi- *A.niger and P.chryosogenum*	-
4	Yeast- *Candida.albicans*	-

No activity was observed against Gram negative bacteria, fungi and yeast. Indicating the isoltae ss4/6 was able to produce narrow spectrum antibiotic.

Table 7: Resistance to various antibiotics.

S.No	Antibiotic(µg/mL)	Growth response	Result
1	Penicillin G (10 IU)	+	Resistant
2	Streptomycin (100)	+	Resistant
3	Tetracycline (50)	-	Sensitive
4	Gentamicin (100)	-	Sensitive
5	Cephalexin (100)	-	Sensitive
6	Rifampcin (50)	-	Sensitive

+ Resistant, - Sensitive. The isolated strain SS4/6 is resistant to penciliin and streptomycin and sensitive to tetracycline, gentamycin, cephalexin and rifampcin.

Growth in presence of inhibitory compounds
No growth was observed in the presence of phenol (0.1%) and growth was observed in presence of crystal violet (0.0001%) and potassium tellurite (0.0001%).

Cell wall composition analysis indicates that it contains only LL-DAP and glycine and belongs to cell wall type-I.

DISCUSSION
Both primary and secondary screening methods indicated that our isolate is a potential alpha amylase inhibitor producer. The isolate is active against human salivary alpha amylase and pancreatic alpha amylase.

Spore chain morphology of (standard isolate) *Streptomyces lateritius*
The sporophores occurred as Retinaculia aperti, but the spore chains representative of section RF are also common. Mature spore chains generally consists of 10 to 50 spores per chain; longer chains are observed some times. The spore surface is warty. *Streptomyces lateritius* produces naphthoquinone type antibiotic called granaticin, which is a broad spectrum antibiotic.[20] (Elson *et al*., 1988).

Spore chain morphology of SS4/6

It shows abundant growth on inorganic salts starch agar (ISP-4) and starch casein agar, poor growth on Glycerol-asparagine agar (ISP-5) and moderate growth on Oat meal agar (ISP-3) and Yeast extract and malt extract agar (ISP-2).

Our isolate SS4/6 and *S.lateritius* have major dissimilarities in carbon utilization pattern and growth characteristics on various ISP media. Our isolate produced narrow spectrum antibiotic, which is active against gram positive organism.

CONCLUSION

In view of large number of differences our isolate can be considered as a new variant of *Streptomyces lateritius.* Hence it is designated as *Streptomyces lateritius* var. *SS*4/6.

Future research: The produced inhibitor in the fermentation medium has to separate and purify the sample for further analysis like molecular weight determination of the compound, nature of the compound and structure of the compound.

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