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IN SILICO ANALYSIS OF ANTIBIOTIC PRODUCING ACTINOMYCETES COMMUNITIES AND THEIR RELATEDNESS TOWARDS ANTIMICROBIAL ACTIVITIES

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ABSTRACT
In the present study we have tried to explore the possibilities of applying in silico approaches for understanding the concept of relatedness between closely related species of actinomycetes. Various biochemical tests suggested the presence of SD9 as most promising isolate with potential antibiotic activity and in silico analysis by Neighbor-Joining method using MEGA software suggested a closely related overview 28 species of Streptomyces. This is well supported by the biochemical analysis and antibiotics producing characteristics along with inhibitory effects over bacteria, yeast and fungi which well indicates that the Streptomyces *griseus* could be the active species in the test isolate. Further computational analysis including BLAST search and phylogenetic analysis were performed to correlate the probable species with other species of the genera.

Introduction
Actinomycetes are morphologically diverse, gram-positive bacteria having in common DNA with an unusually high GC content. The morphological diversity ranges from the *micrococci*, which divide as cocci to members of the *actinobacteria* that form pleomorphic rods or go through a coccus-rod-coccus life cycle (1-4). These groups of bacteria possess many important and interesting features and they multiply by variations on a theme of fragmentation division. They are of considerable value as producers of antibiotics and of other therapeutically useful compounds. *Streptomyces* are particularly prolific and can produce a great many antibiotics and other class of biologically active secondary metabolites. They cover around 80% of total antibiotic production (5, 6). Moreover some biogeographical studies on actinomycetes communities were also explored for type II Polyketide gene (7) and are noteworthy in identifying gene source for various species of actinomycetes.

Computational biology approaches are very useful in studying evolutionary relationship of one species to another. Phylogenetic tree analysis is one among them. Phylogenetic analysis can be done to find out divergence or convergence of genes as well as species. The stability of tree topology obtained can then further be determined by Bootstrapping method.

In present study we have tried to predict the species relatedness of the isolated samples through phylogenetic approach on the basis biochemical characteristics similarity with other species of actinomycetes.

MATERIALS AND METHODS

Selection of isolate: Out of 24 isolates collected in our previous study, the isolates SD9, belonging to the genera *Steptomycetes*, was the most promising isolate (2) and this isolate was selected for further in silico analysis.

Characterization: The isolates were characterized on the basis of morphological characteristics and biochemical tests so that we can propose a probable species of the Actinomycetes (8, 9). This includes the nature of the isolate towards protein casein hydrolysis and other morphological features.

In Silico analysis: for in silico analysis we performed Basic Local Alignment Search Tool (BLAST) and phylogenetic analysis of our isolate species so as to

correlate it with other species of *Streptomyces*. BLAST was carried out using 16s rRNA sequence of *S.griseus* as the query sequence to find out other homologous species. Phylogenetic tree was constructed with 28 most closely related species to *S.griseus* using Neighbor-Joining method available in MEGA software (10). Also Bootstrapping analysis of the tree was performed with 500 replicates using Tajima-Nei nucleotide substitution model with MEGA software.

RESULTS AND DISCUSSIONS

Biochemical and Antagonistic properties:

The extent of hydrolysis of protein by any proteinase is limited to 10-30%. *Sterptomyces griseus* protease had very broad substrate specificity and was capable of hydrolyzing various kinds of peptide bonds. Enzymatic potential of actinomycetes are also estimated (11) and the extent of hydrolysis of protein by this protease was estimated to reach 60-90% (8). Our test isolate SD9 shows a very high activity against the protein casein indicating the presence of S.*griseus*.

Our test isolate SD9 inhibits the growth of bacterium *Staphylococcus aureus* and the fungi *Saccharomyces cerevisiae*. Antibiotic Flucoxacillin has activity against beta-lactamase producing organism such as *Staphylococcus aureus*. Many other same type of penicillin lack this activity (12). Since this antibiotic is produced by *S.griseus* it may be concluded that this isolate contains *S.griseus*. Also this point is supported by the fact that Grisein, an antibiotic which have substantial activity against *S. aureus*, is produced by certain strains of *S.griseus* (13).

The isolate SD9 was grown in a medium containing the antibiotics tetracycline and streptomycin which were used to inhibit unwanted growth. So it must have the species of *Streptococcus* which shows resistance towards the above mentioned antibiotics. *S. griseus* subspecies griseus strain NBRC 13350 has 674 and 333 genes resistant towards tetracycline and streptomycin respectively indicating its presence in the isolate (14).

Chitinase inhibitor Allosamidine is a signal molecule for chitinase production in *S. griseus*. This can inhibit the production of chitinase in *S. aureus* and this Chitinase is essential for the growth *S. cerevisiae* (15). Our isolate SD9 has an inhibitory activity against *S. cerevisiae*. This cross relationship between production and activity of Allosamidine indicate the presence of *S. griseus*.

In Silico Analysis:

Based on Biochemical and antagonistic properties we assumed that the test isolate SD9 has *S. griseus* as the active species and performed further in silico analysis to relate it with other species of the genera. Nucleotide BLAST gives a number of species homologous to *S. griseus*. Out of the total species, 28 species were selected for phylogenetic analysis (table1). Phylogenetic analysis revealed that *S. grieus* was most closely related with *S. flaveus* strain NBRC 3359, *Streptomyces* sp. AC113 and *Strepotomyces* sp. VTT E-042007 since these constitutes same monophyletic clade having a bootstrap value of 41 (fig 1).

Conclusion:

The above study indicates that in silico approaches are effective tools for predicting the relatedness between actinomycetes towards antimicrobial activities. In this way we have identified that the effectiveness of antibiotics against probable isolate can be utilized for exploring further possibilities of purification and characterization of novel metabolites from existing actinomycetal microbial community. Further research on utilizing molecular characterization and sequencing approach can be effective tools for matching these results in future.

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Streptomyces* species homologous to *S.griseus
1. Streptomyces alboviridis
2. Streptomyces argenteolus
3. Streptomyces flaveus
*4. Streptomyces globosus*NBRC15874
*5. Streptomyces griseolus*NBRC3719
6. Streptomyces griseus
*7. Streptomyces griseus*ATCC10137
*8. Streptomyces griseus*NBRC13350
9. Streptomyces luridiscabiei
10. Streptomyces microflavus NRRLB-1332
11. Streptomyces paresii
12. Streptomyces praecox
13. Streptomyces scabiei NBRC3111
14. Streptomyces spAC113
15. Streptomyces spME04-34E
16. Streptomyces spSXY49
17. Streptomyces spVTTE-042007
18. Streptomyces spVTTE-042644
19. Streptomyces spVTTE-042667
20. Streptomyces spVTTE-042674
21. Streptomyces spVTTE-062969
22. Streptomyces spVTTE-062973
23. Streptomyces spVTTE-062980
24. Streptomyces spVTTE-062982
25. Streptomyces spVTTE-062986
26. Streptomyces spYIM8
27. Streptomyces VTTE-042638
28. Streptomyces flavolimosus_CGMCC2027

Table 1. *Streptomyces* species homologous to *S.griseus* (based on BLAST result using 16s rRNA sequence of *S.griseus* as query)

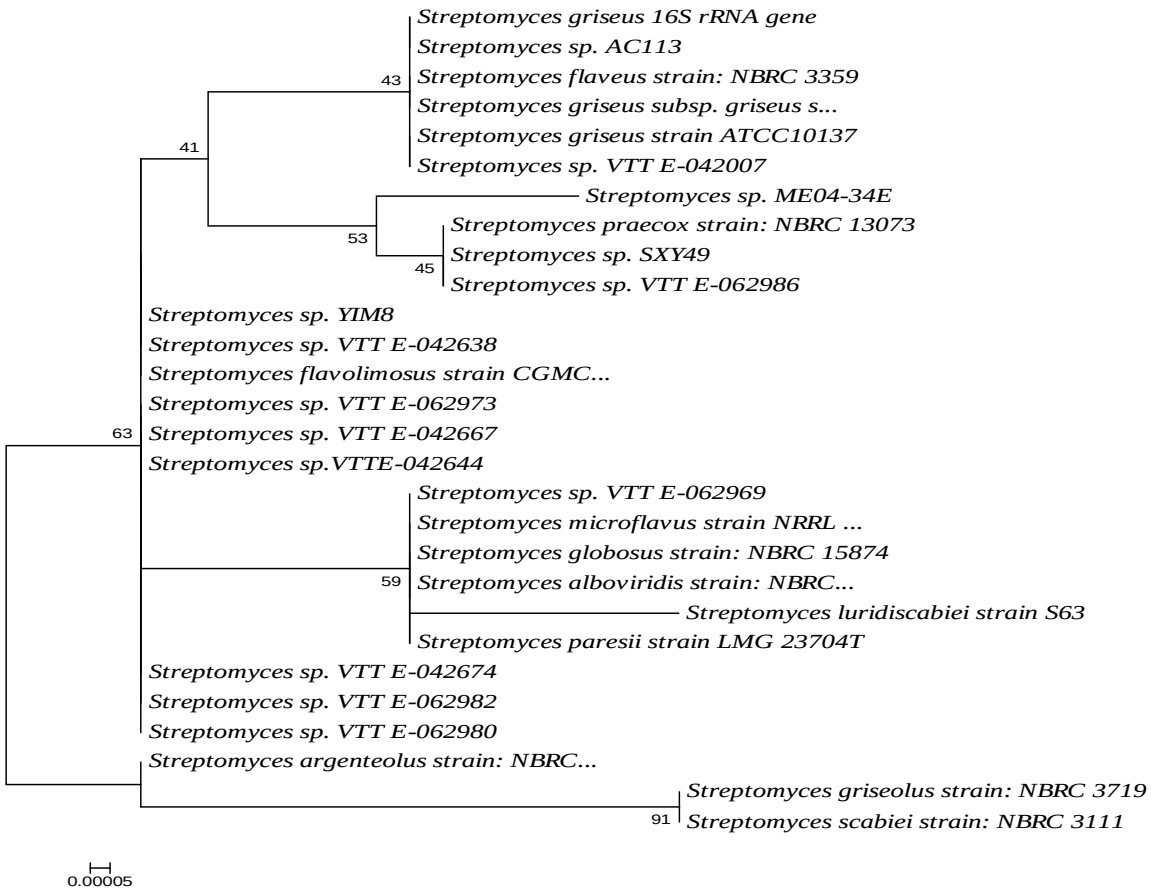

Fig 1. Phylogenetic tree of 28 species of *Streptomyces*.

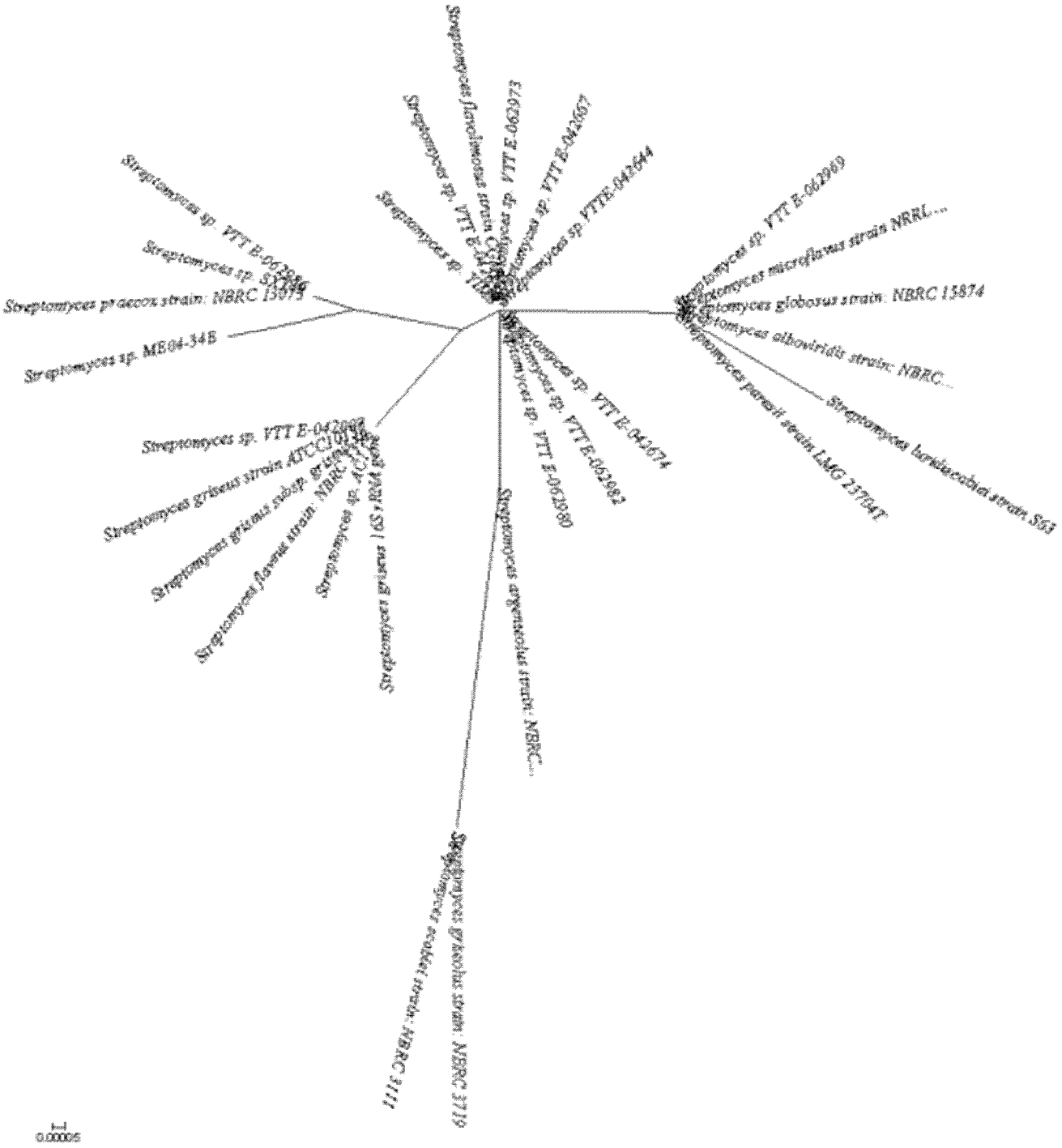

Fig 2: Phylogenetic tree showing monophyletic clade.