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Original Article

Phylogenetic Analysis of Beta-Glucanase Producing Actinomycetes Strain TBG-CH22-A Comparison of Conventional and Molecular Morphometric Approac

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ABSTRACT

Actinomycetes are inexhaustible producers of commercially valuable metabolites, and are continually screened for beneficial compounds. The taxonomic and phylogenetic study of novel actinomycetes strains is mostly based on conventional methods and primary DNA structure of 16s rRNA. Although 16s rRNA sequence is well accepted in phylogeny studies, its secondary structures have not been widely used. RNA Secondary structure especially contains molecular morphometric information which is not found in primary sequences. In this scenario, a comparative study based on both conventional and 16s rRNA structure-based molecular morphometric approach was conducted for identifying the phylogeny of actinomycete isolate TBG-CH22 isolated from the Western Ghats areas in Kerala. The strain was found to a potent producer of beta-glucanase enzymes on the basis of preliminary screening. The taxonomic identification was done with conventional methods, sequence analysis, and molecular morphometric properties of 16s rRNA secondary structure. On account of conventional and primary sequence analysis, the isolate TBG-CH22 was identified as *Streptomyces* species as it delineated the evolutionary relationship of the strain within taxa. While comparing with the minimum free energy (MFE) values of 16s rRNA secondary structure of strain TBG-CH22 and five selected the phylogenetic neighbouring species, the strain *Streptomyces bikiniensis* showed lowest MFE value and confirmed the closest phylogenetic neighbour of strain TBG-CH22.

Key words: Exo-1,4-betaglucanase, Endo-1,3-betaglucanase, 16s rRNA gene, secondary structure.

Introduction

Actinomycetes, an assorted group of filamentous, sporulating, Gram-positive microbes extensively found in all environmental systems, are predominantly saprophytic and known contributors in environmental nutrient turnover processes (Mincer *et al.,* 2002). They are widely recognized for their metabolic resourcefulness by the production of economically important secondary metabolites. Considering the prominence of actinomycetes strains in terms of both biological and chemical properties, it has been continuously screened for valuable natural products and eventually it has been very critical to understand the evolutionary history of isolated strains (Alam *et al.,* 2010). Microbial systematics can be used as an effective tool for the detection of unknown microorganisms with powerful bioactivities (Ward and Good fellow, 2004). The diversity of actinomycetes strains in terms of ecology, morphology and genomic complexity with high G+C content and along with its continuous proliferation,

the phenotypic characteristic, based on taxonomical and phylogenetic studies, produces insignificant results.

Recently 16s rRNA (16s rDNA) is rigorously used to define the evolutionary history of microorganisms. Subsequently, the importance of 16S rDNA sequence-based phylogenetic studies is increased more and more in actinomycetes systematics (Anderson and Wellington, 2001). Numerous studies have attempted to practise primary DNA sequences of 16s rRNA variable region for identifying the taxonomic structure within the genus, but species differentiation persisted as a problematic task. Even though secondary structures of 16s rRNA were not considered in these studies, they play a substantial role in developing the phylogenetic relationship as they deliver "molecular morphometrics" characteristics, a meaningful information on systematics which is not commonly found in the primary sequences. Otherwise, a well-documented fact that throughout the evolution rRNA structure is tremendously conserved, *i.e.,* most of the folding, is functionally irrespective of primary sequence inconsistency (Bhattacharjee and Joshi, 2013).

RNA secondary structures are deliberated in evolutionary studies of influenza virus (Somvanshi *et al.,* 2008), satellite RNA of cucumber mosaic virus (CMV) (Fraile and Garcia-Arenal, 1991) and fungi (Zhuang and Liu, 2012). Stability of RNA secondary structures predicted its thermodynamic free energy values. Fidelity of secondary structure and its free energy based documentation of bacterial strains during the evolutionary periods has been reported. The higher free energy values which destabilize the RNA secondary structure during the course of evolution imply that the strains cannot withstand environmental pressures due to the formation of structural multi-loops (Singh and Somvanshi, 2009). The Western Ghats in India, one of a renowned biodiversity hotspot and a forested strip of relatively Old Mountain ranges, cover rich flora and fauna. This natural ecosystem which shelters enormous collection of unidentified microbes offers the possibility of bioprospection. In this scenario, an actinomycetes strain which produces both exo-β-1,4-glucanase and endo-β-1,3-glucanaseenzyme activities was investigated by exploring the Western Ghats areas in Kerala. A comparative study including conventional, 16s rRNA primary sequence and secondary structural based morphometrics was employed to identify the phylogenetic relationship of potent strain.

Materials and Methods

Isolation of Actinomycetes Strains from Soil Samples

Soil samples were collected from different Western Ghats areas in Kerala like Munnar, Anamalai, Wayanad, Nelliyampathy, Neriyamangalam, Chinnar, Marayoor, Kulathupuzha, Palode and Agasthyar koodam. Standard dilution plate technique was used for the isolation of actinomycetes strains. After 1% $CaCl_2$ treatment and pre-heat treatment, each dilution was plated on inorganic salt-starch agar (ISP-4) media. The purity of isolated actinomycetes strains was checked using Yeast extract-malt extract agar (ISP-2) plates and maintained by periodical subculturing.

Screening of beta glucanases activities

All actinomycetes strains were screened primarily for detecting exo-β-1,4-glucanase and endo-β-1,3-glucanase activity on a synthetic medium with Avicel (Sigma, USA) and AZCL- Pachyman (Megazyme, USA) as the substrates, respectively. The strains were spot inoculated on plates and incubated for 5 days at 28 °C. The enzyme production was indicated by the presence of respective zones around the colonies. The activity was determined by calculating the enzymatic index (EI) of strains using the expression, EI=Diameter of hydrolysis zone (Øh) / Diameter of colony (Øc).

Characterization using conventional method

Morphological characters like colony appearance, texture, shape, odour, oxygen relationship and motility were observed on starch casein agar. Microscopic examination of spore chains was done by

coverslip culture method (Kawato and Shinolue, 1959). The mycelium structure and spore arrangement were observed through Nikon Eclipse CiEpi-fluorescent light microscope, USA (40x). The morphology of spore and spore-bearing hyphae were identified using scanning electron microscope FEI QUANTA 200, Netherland. Cultural characteristics were studied using different ISP media (Shirling and Gottlieb, 1966). Biochemicaland physiological characterizations of isolates were carried out as described by Williams *et al.* (1983).

DNA extraction, 16s amplification and sequencing

The genomic DNA was extracted as described by Murray and Thompson (1980) with some modifications. DNA quantity was estimated using Biophotometer (Eppendorf). The 16S rRNA region was amplified with 8-27 F (5'- AGA GTT TGA TCC TGG CTC AG -3'), 1495 R (5'- CTA CGG CTA CCT TGT TAC GA -3') and 338 F (5'-ACT CCT ACG GGA GGC AGC -3') bacterial universal primers (Weisburg *et al.,* 1991). Amplifications were carried out in 25 µl reaction volume consisting of Go Taq® G2 Hot Start Green Master Mix 2X, 12.5 µl; 10 µM of each primer, 1 µl; 50-100 ng template DNA, 1 µl and H2O, 9.5 µl in a Biorad (USA) thermal cycler using the PCR conditions 95 °C for 2 min (initial denaturation), 95 °C for 1 min (denaturation), 58 °C for 30 sec (annealing) and 72 °C for 1.30 min (extension). The total number of cycles was 35, with the final extension of 72 °C for 5 min. The PCR product was purified using Wizard® SV gel and PCR clean-up system (Promega, USA) and the sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). The presence of chimeric artefacts within the obtained sequence was analyzed using DECIPHER GPL V3.0 (Wright *et al.,* 2012).

Phylogenetic Tree Construction

Sequences were assembled using BIOEDIT Program. Phylogenetic neighbours were identified using EzBioCloud server (https://www.ezbiocloud.net/) (Yoon *et al.,* 2017) along with homology research which was performed using BLAST search algorithm. Alignment of similar sequences and nucleotide similarity values were calculated using CLUSTAL MUSCLE programme (Edger, 2004). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.,* 1987) and the evolutionary tree was inferred from the Neighbour-joining (NJ) method (Saitou and Nei, 1987) using MEGA version 7.0 (Kumar *et al.,* 2016).The tree topologies were evaluated by performing bootstrap analysis based on 1,000 replications (Felsenstein, 1985).

Sequence conversion, alignment comparison and 16s rRNA secondary structure prediction

16s rDNA sequences were converted to RNA sequences using open source sequence conversion tool (http://in-silico.net/tools/biology/sequence_conversion). The converted sequences were saved as FASTA format and a threshold dot-plot alignment of TBG-CH22 with which each neighbouring sequences were drawn using the dotmatcher program from EMBOSS tools (Rice *et al.,* 2000). Physical and kinetic properties of 16s rRNA sequences were analysed using silico tool OligoCalc (Kibbe, 2007). Secondary structures of 16s rRNA sequence with a minimal free energy value of selected isolate and its five closest phylogenetic neighbours were predicted using MFOLD version 4.7 at a default temperature of 37 °C, Na+=1M and Mg++ = 0 M (Zuker, 2003).

Result and Discussion

Actinomycetes isolation and screening of beta glucanase activities

Numerous actinomycetes like colonies were observed on ISP4 agar media. All colonies were subcultured, purified and screened for beta-glucanase activity. Plate assay screening for both exo-β-1,4-glucanase and endo-β-1,3-glucanase activities which resulted 72 strains intensely produced both β-

glucanase enzyme. The strain TBG-CH22, isolated from Chinnar region of Western Ghats area in Kerala, produced substantially high exo-1,4-*β*-glucanase and endo-1,3-*β*-glucanase activity, as the zone of hydrolysis with enzymatic indexes of 6.5 and 8.5 respectively after incubation of 5 days at 28 °C (Figure 1). Preceding studies revealed the worthy climatic and soil conditions in Western Ghats areas increasingly influence the enrichment of beta-glucanase producing actinomycetes strains (Edison *et al.,* 2017).

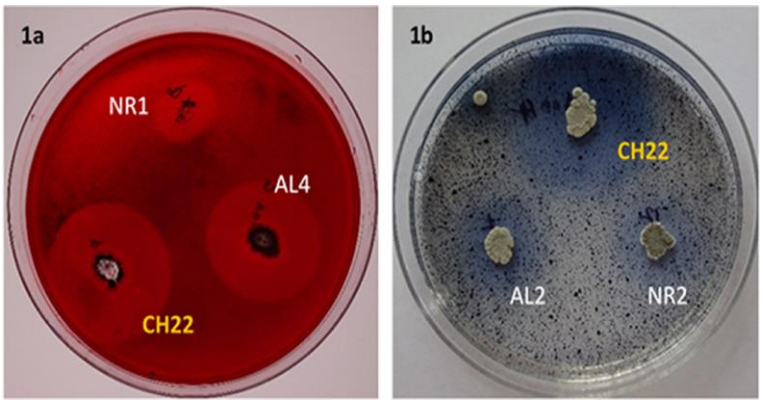

Figure 1. Plate assay primary screening of exo-*β*-1,4-glucanase (1a), endo-*β*-1,3-glucanase (1b)

Characterization using conventional method

Strain TBG-CH22 had well-distinguished morphological properties of genus *Streptomyces*. The 14-day-old culture of isolate starch casein agar showed good growth and well-developed aerial and vegetative hyphae. The microscopic examinations of the isolate observeds mooth, rod-shaped spores with rectus–flexibilis sporophores (Figure 2). The isolate grew well on media including ISP 2, ISP 4, and nutrient agar and developed dark grey to yellow aerial hyphae on all media tested. The substrate mycelium differed from pale yellow to brown yellow. The strain also produced blackish pigment in ISP6 medium. The isolate was gram-positive and utilized D-fructose, Mannitol, and D-xylose as the carbon source. It produced positive results in nitrate reduction and H_2S production. Growth was observed at a temperature range of 20-40 °C, at a wide range of pH 4–12 and in presence of 0.5 -1% NaCl. The strain is susceptible to kanamycin, ampicillin and chloramphenicol and resistant to tetracycline.

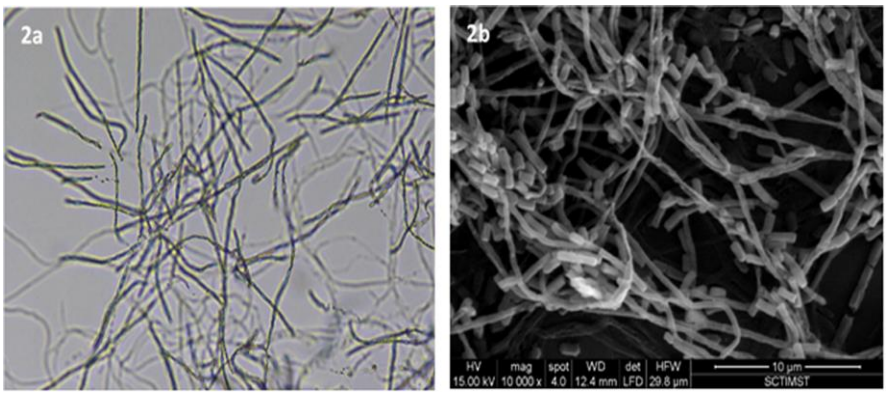

Figure 2. Light microscopic view (2a) and Scanning electron microscopic view (2b) of strain TBG-CH22

16s amplicon sequencing

PCR reaction using 16s rRNA universal primers yielded a band of approximately 1500 bp in 1.0% agarose gel (Figure 3). Sequencing PCR produced an almost complete sequence of 16s rRNA gene (1,437

nucleotides). The obtained sequence quality was good and not deciphered with any chimeric sequences. Comparison of the sequence with corresponding sequences of related organisms using BLAST algorithm clearly demonstrated that the strain TBG-CH22 contained nucleotides which are designated for genus *Streptomyces* and the sequence similarity value ranged in between 98.3–97.2%. If the unknown strain shows percentage similarity in between 99 to 95% with its reference strain the isolate would be allocated to the corresponding genus, but if it is 99 or more, the strain would be assigned to the reference species (Bosshard *et al.,* 2003). Application of PCR and DNA sequencing methods clarify the closely related taxon of organisms with enhanced authenticity than other conventional methods (Clarridge, 2004).

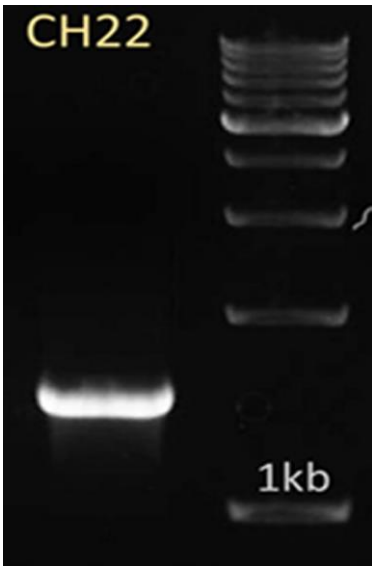

Figure 3. 16s rRNA sequence amplicon of Streptomyces sp strain TBG-CH22. Lane 1: TBG-CH22 and lane 2: 1kb ladder.

Phylogenetic tree construction and sequence alignment comparison

The phylogenetic tree derived from neighbour-joining method showed a relationship with the strain TBG-CH22 to its closely related *Streptomyces* type strains. The outer branches of tree illustrated the evolutionary relationship and inner branches reflecting a degree of relation between different sequences (Singh *et al.,* 2009). Predicted phylogenetic tree mainly divided into 5 distinct clades, where the position of TBG-CH22 located in clade 1 and produced consistent clustering with *Streptomyces* sp. like *S. tanashiensis* LMG 20274^T (AJ781362), *S. nashvillensis* NBRC 13064^T (AB184286), *S. violaceorectus* NBRC 13102^T (AB184314), *S. bikiniensis* NRRL B-1049^T (JNWL01000107) and *S. vietnamensis*GIMV4.0001^T (DQ311081) (Figure 3). The phylogenetic analysis through tree construction confirmed the evolutionary relationship of the isolate TBG-CH22 within the taxa.

16s DNA sequences of TBG-CH22 and related sequences are converted to RNA sequences. Alignment of the sequence of TBG-CH22 with each of its selected neighbouring sequences was analysed using dotmatcher program (Figure 4), endorsed to identify the regions of similarity of all possible alignments between two sequences which can visualize using dot-plots. The 'x' coordinate denotes the position of TBG-CH22 and 'y' coordinate for its neighbouring sequences. The continuous diagonal line indicates the uninterrupted identical part of both sequences. Each dot on the plot corresponds to the matched base symbol. Thus, TBG-CH22 showed an identical configuration of alignments with all the selected neighbouring sequences. The alignment result evidently indicated that all of the selected sequences are closely related to the sequence of TBG-CH22.

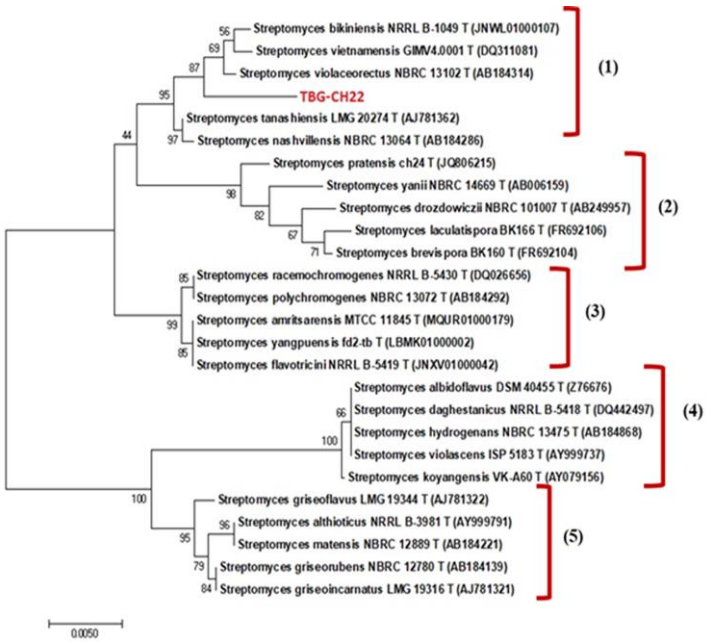

Figure 4. Phylogenetic tree showing the evolutionary relationship of strain TBG-CH22. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.08739040 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

16s rRNA secondary structure prediction

The insilico physical-kinetic analysis of RNA sequences displayed slight variations in melting temperature and GC content (Table 1). The strain TBG-CH22 and *S. bikiniensis* showed comparable basic melting temperature (88.8 °C) and slightly varied in others. The GC content of *S. bikiniensis* and *S. vietnamensis* are adjacent to TBG-CH22. The predicted 16s rRNA secondary structure of all strains are shown (Figure 5), each residue is recognized by a base pairing and the hydrogen bonds are represented as dots between the base pair. The RNA secondary structure of TBG-CH22 16s rRNA gene has minimum free energy (MFE) value of -567.84 Kcal/mol as compared to five closest phylogenetic neighbours based on similarity search conducted using EzBioCloud server. MFE is predicted by summing the discrete energy contributions of base pair stacking, bulges, hairpins, multi-branch loops and internal loops (Chaudhary and Singh, 2013). Accordingly, MFE value can be used as an overall morphometric entirety value of 16s rRNA secondary structure. All predicted secondary structures have different free energy values and insignificant variances in loop structures. Structural energy is anutmost essential factor vastly influencing structural stability (Zuker, 1994). Comparing the stem and loops structures of TBG-CH22 with that of the neighbouring strains displayed slight structural differences, it strongly evidences the novelty of the strain. While comparing the stains, S. *bikiniensis* (dG= -587.67 Kcal/mol) has minimum free energy and *S. vietnamensis* (dG= -572.64 Kcal/mol) has maximum free energy with TBG-CH22. rRNA secondary structure with lowest free energy values delivers close relationship with its most primitive organisms while the structure with highest free energy values shows less stable in the evolutionary period (Somvanshi and Seth, 2010; Singh and Somvanshi, 2009). Based on this *Streptomyces* sp. strain TBG-CH22 are closely related to *S. bikiniensis*. Predicted evolutionarily conserved secondary structure of 16s rRNA swith minimum free energy levels confirmed the closest phylogenetic neighbour of strain. The RNA secondary structure is evolutionarily more conserved than its primary DNA sequence, providing its significant use in phylogenetic studies since the RNA function mainly depends on the secondary structure and its interaction with molecules (Bhattacharjee, 2012).

Table 1. Physical and kinetic properties of rRNA sequences

Sl.No.	GenBank Accession No	Strain	Length	Melting Temperature T_m		GC%	Mol.wt (g/M)
				Basic	Salt Adjusted		
1	KY465499	TBG-CH22	1437	88.8	102.5	59.49	446769.3
2	AJ781362	*Streptomyces tanashiensis*	1448	88.6	102.5	58.90	450202.6
3	AB184286	*Streptomyces nashvillensis*	1447	88.5	102.5	58.80	449857.4
4	AB184314	*Streptomyces violaceorectus*	1447	88.7	102.5	59.08	449810.3
5	JNWL01000107	*Streptomyces bikiniensis*	1448	88.8	102.5	59.32	450109.4
6	DQ311081	*Streptomyces vietnamensis*	1419	88.2	102.5	59.33	441088.6

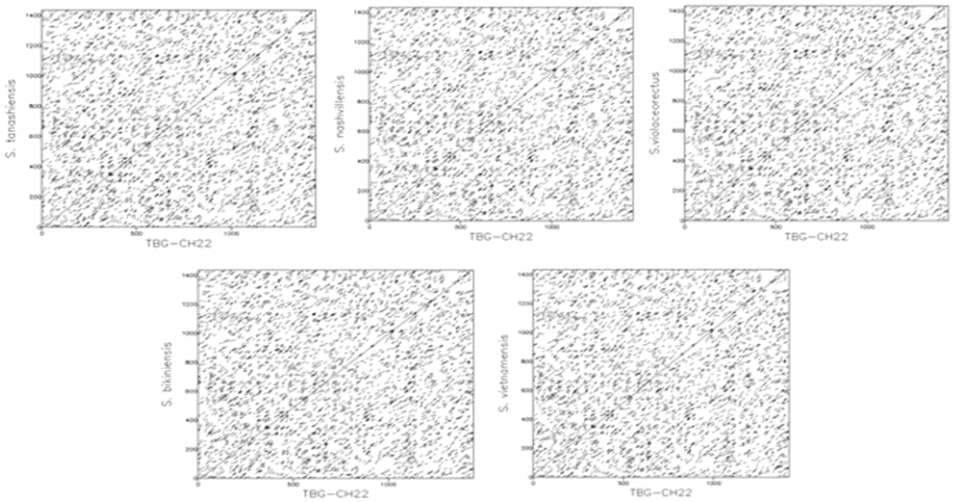

Figure 5. Threshold dot-plot indicate continuous sequence similarity between 16s rRNA sequence of TBG-CH22 with its neighbouring sequences

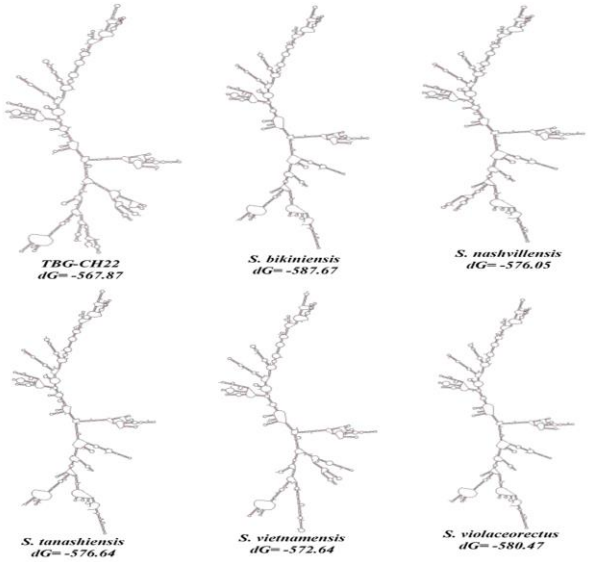

Figure 6. Predicted secondary structure of 16s rRNA with minimal free energy values (MFE) of strain TBG-CH22 and neighbouring strains

Nucleotide sequence accession number

The 16s rRNA gene sequence of *Streptomyces* sp. TBG-CH22 was deposited in the National Centre for Biotechnology Information (NCBI) Gen Bank database with the accession number KY465499.

Conclusion

The function of 16S rRNA gene has not been changed over the time, signifying that the indiscriminate sequence changes are more precise for the measurement of evolutionary development. The length of the gene (~1500 base pairs) is sufficient to release the information by bioinformatics tools. The present study uses conventional characterization methods, linear DNA sequence-based phylogeny and secondary structure based molecular morphometric studies. The conventional methods revealed that the isolated strain TBG-CH22 is an actinomycete (order actinomycetales). Linear sequence-based phylogenetic analysis confirmed the evolutionary relationship of isolate within taxa as the strain TBG-CH22 comes under the species *Streptomyces*. Predicted evolutionarily conserved secondary structure of 16s rRNAs with minimum free energy levels recognised the closest phylogenetic neighbour of strain. It evidently established that only RNomics based molecular morphometric studies assist to identify the exact phylogenetic position of *Streptomyces* sp. The impact and potential contribution of RNomics based phylogenetic studies documented the precise taxonomical identification of actinomycetes strains which, as confirmed by the molecular study of 16s rRNA, can be regarded as a promising tool for actinomycetes species-level identification.

References

Alam, MT, Merlo, ME, Takano, E, Breitling, R, (2010). Genome-based phylogenetic analysis of *Streptomyces* and its relatives. *Mol Phylogenet Evol*, 54:763-72.

Anderson, AS, and Wellington, EM, (2001). The taxonomy of *Streptomyces* and related genera. *Int J Syst Evol Microbiol,* 51:797-814.

Bhattacharjee, K, Banerjee, S, Joshi, SR, (2012). Diversity of *Streptomyces* spp. in Eastern Himalayan region – computational RNomics approach to phylogeny. *Bioinformation,* 8:548-554.

Bhattacharjee, K, Joshi, SR, (2013). Phylogenetic rearrangement of Streptomyces spp. on the basis of internal transcribed spacer (ITS) region using molecular morphometrics approach. *Indian J Biotechnol,* 12:67-79.

Bosshard, PP, Abels, S, Zbinden, R, Böttger, EC, Altwegg, M, (2003). Ribosomal DNA sequencing for identification of aerobic gram-positive rods in the clinical laboratory (an 18-month evaluation). *J Clin Microbiol,* 41:4134-40.

Chaudhary, A, Singh, HS, (2013). Secondary structure and phylogenetic utility of the ribosomal large subunit (28S) in monogeneans of the genus Thaparocleidus and Bifurcohaptor (Monogenea: Dactylogyridae). *J Parasit Dis,* 37:74–83.

Clarridge, JE, (2004). Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. *Clin Microbiol Rev,* 17:840–862.

Edgar, RC, (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res,* 32:1792–1797.

Edison, LK, Shiburaj, S, Pradeep, NS, (2017). Exploration of Western Ghats Regions in Kerala for Isolating Beta- Glucanase Producing Actinomycetes Strains. *Trends Biosci,* 10:8453-8456.

Felsenstein, J, (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 39:783-791.

Fraile, A, García-Arenal, F, (1991). Secondary structure as a constraint on the evolution of a plant viral satellite RNA. *J Mol Biol,* 221:1065-1069.

Kawato, M, Shinolue, R (1959). A simple technique for the microscopical observation. Memoirs of the Osaka University Liberal Arts and Education. 1-1 Yamadaoka Suita, Osaka Japan, pp. 114.

Kibbe, WA, (2007).OligoCalc: an online oligonucleotide properties calculator. *Nucleic Acids Res,* 35:W43-46.

Kumar, S, Stecher, G, Tamura, K, (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol,* 33:1870-1874.

Mincer, TJ, Jensen, PR, Kauffman, CA, Fenical, W, (2002). Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl Environ Microbiol,* 68:5005-11.

Murray, MG, Thompson, WF, (1980). Rapid isolation of high molecular weight plant DNA. *Nucl Acids Res,* 8:4321-4325.

Rice, P, Longden, I, Bleasby, A, (2000). EMBOSS: The European molecular biology open software suite. *Trends Genet,* 16:276-277.

Saitou, N, Nei, M, (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *MolBiolEvol,* 4:406-425.

Shirling, EB, Gottlieb, D, (1966). Methods for Characterization of Streptomyces Species. *Int J Syst Evol Microbiol,* 16:317-327.

Singh, V, Praveen, V, Khan, F, Tripathi, CKM, (2009).Phylogenetics of an antibiotic producing Streptomyces strain isolated from soil. *Bioinformation,* 4:53–58.

Singh, V, Somvanshi, P, (2009). Computational modeling analyses of RNA secondary structures and phylogenetic inference of evolutionary conserved 5S rRNA in the prokaryotes. *J Mol Graph Model,* 27:770–776.

Somvanshi, P, Seth, PK, (2009). Predicted RNA secondary structures for the conserved regions in dengue virus. *Bioinformation,* 3:435–439.

Somvanshi, P, Singh, V, Arshad, M, (2008).Modeling of RNA Secondary Structure of Non Structural Gene and Evolutionary Stability of the Influenza Virus Through In Silico Methods. *J Proteomics Bioinform,* 1:219-226.

Tamura, K, Nei, M, Kumar, S, (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci USA,* 101:11030-11035.

Ward, A, Goodfellow, M, (2004). Taxonomy as a road map for search and biodiscovery, *Microbiology,* 25:13-15.

Weisburg, WG, Barns, SM, Pelletier, DA, Lane, DJ, (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol,* 173:697-703.

Williams, ST, Goodfellow, M, Alderson, G, Wellington, EMH, Sneath, PHA, Sackin, MJ, (1983). Numerical classification of Streptomyces and related genera. *J Gen Microbiol,* 129:1743-1813.

Wright, ES, Yilmaz, LS, Noguera, DR, (2012). DECIPHER, A Search-Based Approach to Chimera Identification for 16S rRNA Sequences. *Appl Environ Microbiol,* 78:717-725.

Yoon, SH, Ha, SM, Kwon, S, Lim, J, Kim, Y, Seo, H, Chun, J, (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol*, 67:1613-1617.

Zhuang, WY, Liu, CY, (2012). What an rRNA Secondary Structure Tells about Phylogeny of Fungi in Ascomycota with Emphasis on Evolution of Major Types of Ascus. *PLoS ONE* 7: e47546.

Zuker, M, (1994). Prediction of RNA secondary structure by energy minimization. In: Griffin AM, Griffin HG (eds) Computer analysis of sequence data, Part II, vol 25, Chap CRC Press Inc., Totowa, pp 267–294.

Zuker, M, (2003).Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res,* 31:3406-15.

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