Int.J.Curr.Microbiol.App.Sci (2016) 5(11): 455-458

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 5 Number 11 (2016) pp. 455-458
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.511.052

Partial Sequencing of Serendipitously isolated Antifungal Producer, *Pseudomonas tolaasii* Strain GD76 16s Ribosomal RNA Gene

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Keywords

16s r RNA gene, *Pseudomonas tolaasii* GD 76.

Article Info

Accepted: 23 October 2016
Available Online: 10 November 2016

A B S T R A C T

Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains due to their general ability to produce a diverse array of potent antifungal metabolites. Present study provides partial 16 S rRNA gene sequence of an antifungal metabolite producer and recently identified *Pseudomonas tolaasii* GD76; which is serendipitously isolated from a contaminated YPD agar plate on to which it was streaked from casein agar plate where it was showing a pronounced proteolytic activity.

Introduction

Recently, the rise of antimicrobial-resistant bacteria has provided motivation for novel bioactive compound discovery, as it has been recognized by the World Health Organization as a threat to human health (Demain, 1999; Maryna *et al*., 2007). Among the underexplored bacterial taxa, *Pseudomonadales* certainly deserve the spotlight (Maryna *et al*., 2007). Despite being long known as prolific producers of specialized metabolites, their systematic screening has been hampered by difficulties in their cultivation. Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites (Ute Hentschel *et al*., 2002).

DNA extraction and quantification

DNA Extraction was carried out using HiPurA Bacterial Genomic DNA Purification Kit (Himedia, MB505). Loopful of culture was suspended in 200µl of lysozyme solution (2.115 x 106 unit/ml) and incubated at 37^0C for 30 min. 20 µl of RNase A solution was added and incubated for 2min at room temperature. Then 20 µl of Proteinase K solution (20mg/ml) was added followed by 200µl of lysis solution C1. The mixture was vortexed and incubated at 55^0C for 10 min. DNA was precipitated by adding 200µl of ethanol to the lysate and mixed by vortexing. Lysate was then loaded on HiElute Miniprep Spin column and centrifuged at 10,000 rpm for 3 min. Flow

through liquid was discarded and the column was transferred to new 2 ml collection tube. 500 µl of Prewash Solution was then added to the HiElute Miniprep Spin column and centrifuged at 10,000rpm for 3 minutes. Flow through liquid was discarded and 500 µl of Wash Solution was added to the column. Centrifuged at 10,000rpm for 3 minutes. 200µl of elution buffer was then added to the column and incubated at room temperature for 5 minutes. Centrifuged at 10,000 rpm for 3 minutes. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -20^{0}C for further use. PCR amplification: The DNA isolated from bacteria was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). The PCR reaction mix contained 2.5µl of 10X buffer, 1µl of each primer, 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA and 8.5µl nuclease free water. The PCR amplification cycle consist of, a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 50°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C. The reagents used are procured from GeNei.

Gel electrophoresis

Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The size obtained was approx. 850bp for 16S rRNA region.

DNA sequencing

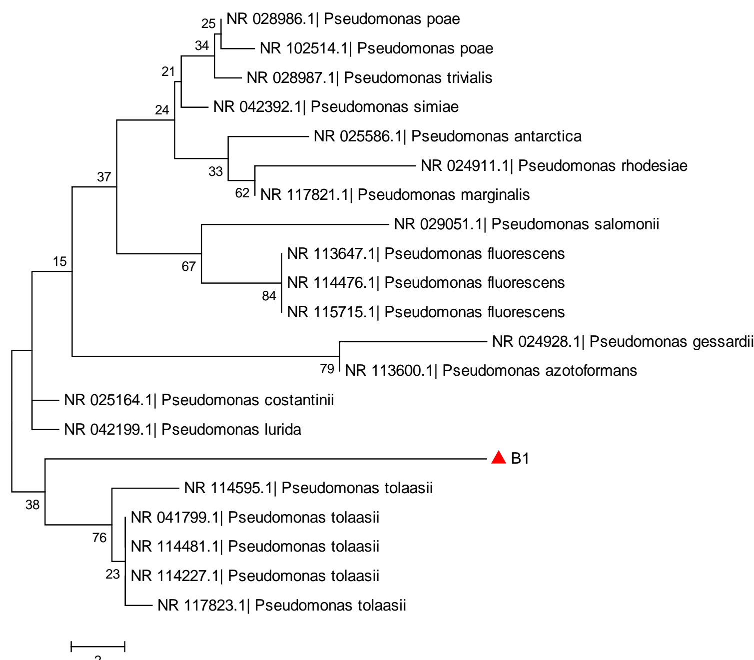
The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). 100µl of PCR-A buffer was added to the 25µl of reaction. The sample was mixed and transferred to column placed in 2ml collection tube and centrifuge at 10,000 rpm for 1min. the filtrate was discarded.700µl of W2 buffer was added to the column and centrifuge at 10,000rpm for 2min. This step was repeated twice. The column was transferred to a new tube. 25µl of Eluent was added into the column and incubated at room temperature for 2min. Then centrifuge at 10,000rpm for 5min. It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product 519F - 5'CAGCAGCCGC GGTAATAC3' sequencing primer was used.

Bioinformatics analysis

The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Center for Biotechnology Information (NCBI). The BLAST results were used to find out evolutionary relationship of bacteria. Altogether twenty sequences, including sample were used to generate phylogenetic tree. The tree was constructed by using MEGA 5 software (Felsenstein, 1985; Tamura *et al*., 2011).

Maximum Parsimony analysis of taxa

The evolutionary history was inferred using the Maximum Parsimony method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates).



The tree is drawn to scale; with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. The analysis involved 21 nucleotide sequences. There were a total of 1542 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

Nucleotide sequence accession numbers

This sequence has been deposited at NCBI – Nucleotide/ GenBank under the accession no. KU533778. The version described in this paper is the first version, accession no.KU533778.1.

The molecular phylogeny of sample was determined by analyzing 16S rRNA gene sequences. On the basis of the position of sequence of the given bacterial samples in the phylogenetic tree, B1 showed closest similarity to *Pseudomonas tolaasii.*

Acknowledgments

We thank Principal, New Arts Commerce & Science College Ahmednagar, MS India, for providing the Microbiological laboratory support. We also thank to Dr. Archana Thakur, and the Codon Bioscience, Panjim Goa India for genome sequencing. We also like to extend our thanks to Miss. Sampada Hajare and Miss. Snehal Rokade to give constant support in entire work.

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How to cite this article:

Desai, D.A., G.P. Kukreja, C.J. Raorane and Patil, S.B. 2016. Partial Sequencing of Serendipitously isolated Antifungal Producer, *Pseudomonas tolaasii* Strain GD76 16s Ribosomal RNA Gene. *Int.J.Curr.Microbiol.App.Sci.* 5(11): 455-458.
doi: http://dx.doi.org/10.20546/ijcmas.2016.511.052