

Isolation and Molecular characterization of Cellulase Producing Bacteria from Soil

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ABSTRACT: Today the energy and environmental crises has been a huge obstacle for the progress of human civilization. The most predominant energy source which is the fossil fuel supply is limited and it also causes environmental effects such as green house gases. The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intensive research and industrial subject .A Total of 146 organisms were isolated based on Morphology and Biochemical characterization. Among all isolated organisms 57 bacterial species were isolated, the one cellulolytic bacterial isolates (ABS 125 A) shows maximum enzyme activity .However, sequencing of 16S rRNA region is a suitable technique for species identification and this strain studied through 16S rRNA gene sequence analysis for identification and it is identified as *P. aeruginosa*. The NCBI Gene Bank accession numbers of the 16S rRNA gene sequences for isolates ABS 125 A was JF513146.1. The aim of the present study is to isolate the efficient cellulase producing organisms from the saline soil of Akola and Buldhana District, Maharashtra, might offer scope for further research to evaluate prospects for application of cellulase enzyme producing bacteria.

I. INTRODUCTION

Cellulose is the primary structural component of the plant cell wall and the main product of photosynthesis in terrestrial plants [1]. Cellulose is the most abundant and common natural renewable biopolymer available on Earth [2]. Cellulose, being the predominant carbohydrate in nature is a polymer of glucose residues connected by -1, 4 glycosidic linkages. Cellulase hydrolyzes this -1, 4 glycosidic linkages to glucose units. Complete hydrolysis of the enzyme is accomplished by the synergistic action of three types enzymes which consists of endoglucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β -glucosidase (EC 3.2.1.21). Endoglucanase hydrolyses -1, 4 bonds in cellulose molecule, whereas exoglucanase cleaves the ends to release cellobiose and β -glucosidases converts cellobiose to glucose [3]. Which are mostly used for hydrolysis of lignocellulosic biomasses for ethanol production [4].

Sequencing of 16S rRNA is a molecular technique for characterization of bacteria and tools involved is to analyse the phylogenetic relationship of an organism [5]. For species identification various molecular methods has been raised. According to Busse *et al.*, 1996 [6], the molecular biological methods such as nucleic acid analysis, protein pattern or fatty acid profiles has been proved for identification of bacteria rapidly. Identification of bacteria at species level, DNA sequences at genus-specific might be widely used. According to some scientists [7, 8, 9] 16S-23S rRNA intergenic spacer of the ribosomal RNA operon (RRN) gene region is used for identification of strains and species. The aim of this study was, sequencing of the 16S rRNA gene region of *Pseudomonas aeruginosa* isolated from soil saline soil of Akola and Buldhana District, Maharashtra, and to study the phylogenetic relationship between the various strains of *Pseudomonas aeruginosa*.

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II. MATERIALS AND METHODS

Isolation and Screening of organisms

Bacteria were isolated from the soil sample collected from different villages of saline belt of Akola and Buldhana District, Maharashtra India. The isolated microbes were grown on basal salt media supplemented with 1% carboxy methylcellulose [10]. Cellulolytic organisms were selected on the basis of zone of clearance in congo red test and enzyme activity [11] and identified on the basis of morphological and biochemical characteristics [12]. Among these ABS 125 A was selected as a highest enzyme producer. For species identification sequencing of 16S rRNA gene region was carried out.

DNA Extraction:

1. Bacterial Genomic DNA was isolated using the Insta Gene™ Matrix Genomic DNA isolation kit. In result, 20µl of the supernatant was used per 50 µl PCR reaction.
2. **PCR Protocol:** Using below 16S rRNA Universal primers gene fragment was amplified using MJ Research Peltier Thermal Cycler.

Primer Details:

Primer Name Sequence Details Number of Base

27F AGAGTTTGATCMTGGCTCAG 20

1492R TACGGYTACCTTGTACGACTT 22

Template DNA of 1 µL were added in 20µL of PCR reaction solution. 27F/1492R primers were used for bacteria, and then PCR reaction performed with below conditions :

Initial Denaturation 94°C for 2 min and then 35 amplification cycles at 94°C for 45 sec, 55°C for 60 sec, and 72°C for 60 sec. Final Extension at 72°C for 10 min. DNA fragments are amplified about 1,400bp in the case of bacteria. Include a positive control (*E.coli* genomic DNA) and a negative control in the PCR.

3. Purification of PCR products

Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore). The PCR product was sequenced using the 518F/800R primers. Sequencing reactions were performed using a ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

4. Sequencing protocol

Single-pass sequencing was performed on each template using below 16s rRNA universal primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

Sequencing Primer Details:

Primer Name Sequence Details Number of Base

785F GGATTAGATACCCTGGTA 18

907R CCGTCAATTCMTTTRAGTTT 20

Sequence data was aligned and analyzed for Identifying the Sample.

Nucleotide sequence accession number and BLAST analysis

1. The nucleotide sequence 16S rRNA gene region data was submitted to NCBI nucleotide sequence database. Using BLAST tool, phylogentic tree, primer pairs were designed from NCBI database search tool. The 16s rRNA sequence was blast using NCBI blast similarity search tool. The phylogeny analysis of our sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment.
2. The program MUSCLE 3.7 was used for multiple alignments of sequences [13]. The resulting aligned sequences were cured using the program Gblocks 0.91b. This Gblocks eliminates poorly aligned positions and divergent regions (removes alignment noise) [14]. Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as Substitution model.
3. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering. [15].

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III. RESULTS

Screening of bacteria for Cellulase Activity.

Screenings of bacteria for their cellulase activity were carried out by the hydrolysis of substrate incorporating in the basal salt medium. After an incubation period, enzyme activities were detected by the appearance of zones around the bacterial colonies. ABS 125 A were showed the highest enzyme activity Based on the morphological and biochemical characters it was identified as *Pseudomonas aeruginosa* (Table 1). *Pseudomonas aeruginosa* was gram negative, which shows positive result on catalase and oxidase test. Hence to identify and confirm the *Pseudomonas aeruginosa* at molecular level, 16S rRNA gene region was amplified and sequenced. Genomic DNA was extracted from *Pseudomonas aeruginosa* by the standard method. PCR amplification of 16S rRNA gene region by using primer was carried out as in Fig. 1. The sequence was submitted to NCBI database and the accession number is JF513146.1. By using BLAST analysis, NCBI data gave 99% similarity. Phylogenetic tree generated by NCBI tool proves that this organism genetically related with other organisms. (Fig.2)

Table 1: Morphological And Biochemical Identification Of Pseudomonas aeruginosa

Sr. No.	Characteristics	
Morphological Identification		
1.	Gram Staining	Gram negative, Rods
2.	Colony	Small circular, pigmented,
Biochemical Identification		
1	Catalase	+
2	Amylase	-
3	Urease	-
4	Cellulase	+
5	Oxidase	+
6	Indole	+
7	Methyl Red	-
8	Vogus prauskaur	-
9	Citrate	+
10	Nitrate	+
11	Glucose	AG-
12	Lactose	AG-
13	Mannitol	AG-

+-Positive, - Negative, A-Acid, G-Gas

>Pseudomonas_contig_1

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CCCTCGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTACGCGCGGAC
GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAAGT
GGGGATCTTCGACCTACGCTATCAGATGAGCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCCTACCAAGGCGACGATCCGTA
ACTGGTCTGAGAGGATGATCAGTACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAAATATTGGACAATGG
GCGAAAGCCTGATCCAGCCATGCCCGTGTGTGAAGAAGGTCTTCGGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAA
TACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCAGCTAAGTTCGTCAGCAGCCGCGGTAAATACGAAGGGTGCAAGCGTTAATC
GGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATCCAAAACCTA
CTGAGCTAGAGTACGGTAGAGGGTGGTGAATTTCCCTGTGTAGCCGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGGC
ACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGT
CGACTAGCCGTTGGGATCCTTGTAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCCCTGGGGAGTACGGCCGCAAGGTTAAAAC
CAAATGAATTGACGGGGGCCCGACAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCT
GAGAACTTCCAGAGATGGATTGGTGCCTTCGGGAACCTCAGACACAGGTGCTGCATGGCTGTCAGCTCGTGTGCTGAGATGTTGGG
TTAAGTCCCCTAACGAGCGCAACCCTTGTCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAGTCATCATGGCCCTACGGCCAGGGCTACACACGTGCTACAATGGTGGTACAAAGGGTTGCCAAGCCCG
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GAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGA
ATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCCGCCGTCACACATGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCT
AACCGCAAGGGGACGGTTACCACGGAGTGATTCATGACTGGGGTGAAGTCGTAACAAGGAACCCGTA AAAAGTTGGCCGACCGACC
GTTTGTCCCTTGTACCCGCATCTGGGAAATTCTTACCTATACTCACTCGCTCGTCCTCGTAGCAACCAATACCCCGCTCGATTTCAAT
GTGTAGGCCGCCGCCCCCTCGAACATCGACGAAAAA AAAAAAAAAA

Fig.1: Amplified 16S rRNA gene region

- Phylogenetic tree generated from 16SrRNA sequence of the *Pseudomonas aeruginosa* isolate

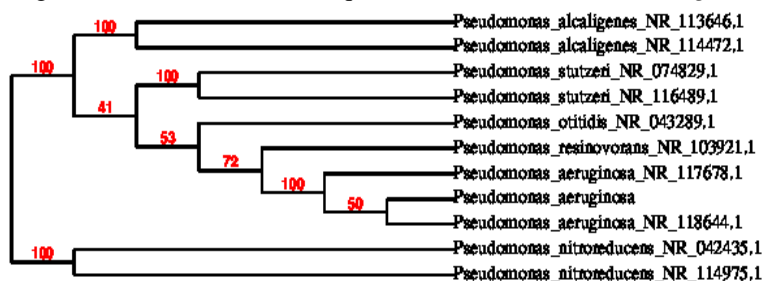


Fig 2: Phylogenetic tree

IV. DISCUSSION

Kaur and Arora (2012) [16] isolated 21 bacterial strains of these only 4 were cellulose degrading bacteria. Acharya *et al.*, in 2012 [17] also reported that *Bacillus subtilis* were most potent thermophilic cellulolytic isolates among all the isolated bacteria. Ahmed *et al.*, (2013) [18] isolated 15 cellulolytic bacteria. Among the 15 isolates one belongs to *Pseudomonas spp.*, one to *Aeromonas spp.*, one to *Pasteurella spp.*, and two belongs to *Staphylococcus spp.* and ten to *Bacillus* genus. Morphologically similar species are alike to have biochemical characters. Sequence of highly conserved gene region 16S rRNA data helps us for the prediction of correct taxonomy. Our present study was carried out to sequence 16S rRNA based on PCR amplification for identification and genetic level confirmation of cellulase producing *Pseudomonas aeruginosa* from saline belt of Akola and Buldhana District, Maharashtra. Amutha and Kokila (2012) [19] carried out a study to sequence 16S rRNA based on PCR amplification for identification and genetic level conformation of *Pseudomonas aeruginosa*. NCBI database provide more information regarding nucleotide sequences. The revealed sequence accession number is JF513146.1 in NCBI provides us taxonomy report with 99% similarity in BLAST analysis.

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