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MOLECULAR CHARACTERIZATION OF BACTERIA ISOLATED FROM SOIL FOR EXTRACELLULAR ALKALINE PROTEASE PRODUCTION

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ABSTRACT

Bacteria were isolated from soil samples using serial dilution method. Different isolates were then screened for their alkaline protease producing capability using skim milk hydrolysis test. The bacterial species were identified as *Bacillus cohnii* using 16S rRNA amplification and phylogenetic analysis. BLAST program and the 16S rRNA gene sequence similarity demonstrated that the query sequence was 99% similar to *Bacillus cohnii* (gb|JF689927.1) strain APT5.

Key word : *Bacillus*, alkaline protease, phylogenetic tree, gene sequence

Proteases are one of the most important industrial enzymes produced by wide range of microorganisms such as bacteria, yeasts, molds, plants and in various animal tissues [6]**.** Bacterial proteases are mostly extracellular, easily produced in larger amounts, thermostable and active at wider pH range. These properties make the bacterial proteases most suitable for wider industrial application. Alkaline proteases from the bacterial origin are the most important industrial enzymes, which contribute about 60% of the total world enzyme market [7, 4, 5]. The growth and enzyme production of the organism are strongly influenced by medium components like carbon and nitrogen sources. Besides the nutritional factors the cultural parameters like temperature, pH, incubation time were also plays a major role in enzyme production [1] and as so the optimization of media components and cultural parameters is the primary task in a biological process.

MATERIALS AND METHODS

Isolation and screening of microorganisms : The bacterium was identified and screened [2] according to the standard key of Bergey's manual of Determinative Bacteriology [3]**.** The bacteria were sub cultured and maintained on nutrient agar plates for use as inoculums for protease production by Submerged State Fermentation.

DNA extraction and Quantification : The Quantification of isolated DNA was done using UV-Vis Double Beam Spectrophotmeter.

PCR Amplification, Sequencing and Sequence Analysis : Extracted DNA were amplified for gene encoding for 16S rRNA using primår pairs forward and råvårså, The amplified PCR product was then sequenced using Sanger's Method. The percent sequence identity was determined using BLAST and phylogenetic tree construction was done by using the ClustalW2 program**.**

RESULTS AND DISCUSSION

The bacterial strains isolated from soil were screened for protease producing ability on skim milk agar. The clear zone formation around the bacterial colony confirmed the proteolytic activity of the bacterial strain due to hydrolysis of casein. Hence the strains were identified as a protease producer using 16S rRNA amplification and phylogenetic analysis.

Phylogenetic Analysis : The bacterial DNA run on 0.8% agarose gel showed bands when observed on Gel documentation system as shown in Figure 1(a).The DNA stored in Tris-EDTA buffer was quantified to 2.8 µg/ml using Spectrophotometer. DNA sample was then amplified using 16S rRNA primers and amplified bands were seen in 1.2% agarose gel as shown in Figure 1(b). Further sequencing of the PCR product was done to identify the bacterial species. The sequence (size:1420bp) obtained was then aligned using BLAST

Table-1 : Spectrophotometric analysis of isolated DNA from bacteria.

Sr. No	Sample	M factor	Ratio	DNA Conc	Protein Conc
1	BS1	1.00	1.03	1.53	3.41
2	BS2	1.00	1.02	1.38	2.97
3	BS3	1.00	1.04	5.87	12.80
4	BS4	1.00	1.02	5.56	12.70
5	BS4	1.00	0.96	1.35	3.41

**Dilution of DNA sample= 990µl (D.W) + 10 µl (sample)*

program and the 16S rRNA gene sequence similarity demonstrated that the query sequence was 99% similar to *Bacillus cohnii* (gb|JF689927.1) strain APT5. The query sequence also showed similarity with other *Bacillus* strains including *Bacillus* sp. D6 gene, *Bacillus cohnii* strain DSM 6307, Bacillus sp. NCCP-666 but have a lower score. Thus the bacterial species was identified as *Bacillus cohnii.* The phylogenetic tree showed the relatedness between other *bacillus s*trains as depicted in Figure-2.

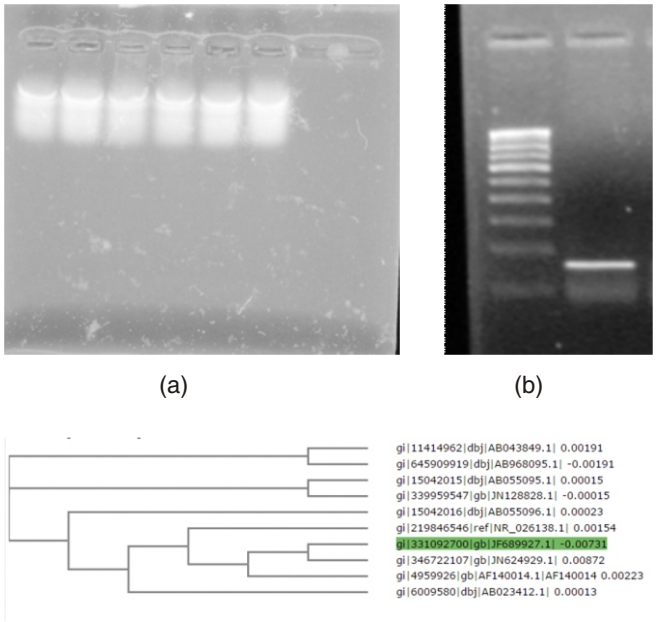

Figure-2 : Phylogenetic tree of the query sequence showing 99% similarity with *Bacillus cohnii* and depicting a relatedness to other bacillus strains

Quantification of DNA using UV-Vis Double Beam Spectrophotometer : The quality of DNA was estimated by measuring the Optical Density (O.D) or Absorbance at 260nm:280nm ratios which varied between 1.6 and 1.8. Table 1 shows the value obtained for each sample which was analyzed at 260 and 280 nm.

The bacterial isolates of the soil were cultured and screened for their protease production property. The isolates showing this property was then identified by 16S rRNA amplification and sequencing. The BLASTN result and CLUSTAL Omega helped to identify the bacterial strain as *Bacillus cohnii* strainAPT5.

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