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Identification and Characterization of Bacterium Based on Genomic Analysis, Isolated from Agro-Ecosystem of Ranchi

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Abstract: The present paper dealt with the identification and characterization of bacterium isolated and cultured from agro-ecosystem by 16s rDNA based genomic analysis. The bacterium identified was *Bacillus cereus* strain Probio-32 (Genebank Accesssion Number: GU471752.1), which is a Gram positive bacteria and closely related to *Bacillus anthracis* and *Bacillus thuringiensis*. Isolated DNA of bacterium showed amplicon band of 1500bp when resolved on agarose gel. Forward and reverse DNA sequences were 833bp and 848bp respectively, which further provides consensus sequence of 1427bp. On the query sequence of 1250bp, 161blast hits matched the alignments scores of >= 200. BLAST programme, Query coverage and expectation value of all the 10 different homogenous strains provided further evidence to the obtained strain of *Bacillus cereus* strain Probio-32.

Key words: BLAST ~ Expectation value ~ *Bacillus cereus* Strain ~ Alignment

INTRODUCTION

Microbial communities are vital in the functioning of all ecosystems, however, most microorganisms are uncultivated and their roles in natural systems are unclear [1, 2]. Analysis of the gene complement for each organism revealed the pathways for carbon and nitrogen fixation and energy generation and provided insights into survival strategies in an extreme environment [2]. Dispersed repetitive DNA sequences have been described recently in eubacteria. To assess the distribution and evolutionary conservation of two distinct prokaryotic repetitive elements consensus oligonucleotide were used in polymerase chain reaction (PCR) amplification. Oligonucleotides produced clearly resolvable bands of agarose gel electrophoresis following PCR amplification. These band patterns provided unambiguous DNA fingerprints of different eubacterial species and strains. Widespread distribution of these repetive DNA elements in the genomes of various microorganisms enables rapid identification of bacterial species and strains and be useful for the analysis of prokaryotic genomes [3]. The BLAST programs are widely used tools for searching protein and DNA databases for sequence similarities [4, 5]. The determination and analysis of complete genome sequence have recently enabled many major advances to be made in the area of microbial evolutionary biology [6]. The present communication dealt with the identification and characterization of bacterium based on genomic analysis of 16S rDNA and significant alignment as well as results on BLAST programme.

MATERIALS AND METHODS

The soil sample (Table 1, edaphic factors) collected from the agro-ecosystem of Ranchi, India and dilution plate method [7] was used for estimating the bacterial population, 1 mL inoculum of the primary suspension was taken and Czapek Dox agar media was used for culture.

More than 70% of the bacterial colonies were with irregular-undulate margin and less than 30% colonies were with circular entire margin. The elevation of irregular-undulate colony was both flat (75%) and convex (20%) with white glittering or yellow colour. The dominant irregular-undulate and flat colonies were taken for genomic analysis.

DNA was isolated from the culture. Its quality was evaluated on 1.2% agarose gel, a single band of high molecular weight DNA has been observed. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. The PCR amplicon was purified to remove contaminants by using a QIAquick purification kit

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Table 1: Characteristics of soil sample taken from Agro-ecosystem

Characteristics	Value (M ± SD; n = 3)
pH	5.81 ± 0.07
Organic Carbon (mg C g^{-1} soil)	6.57 ± 0.11
Nitrogen (mg N g^{-1} soil	0.78 ± 0.01
Phosphorous (Kg P $hec.^{-1}$ soil)	27.9 ± 0.62
Potassium (Kg K $hec.^{-1}$ soil)	148.0 ± 0.49

(Qiagen, Hilden, Germany), after seakem GTG (FMC) agarose gel electrophoresis (1× Trisacetate EDTA or 1× tris borate EDTA running buffer). Forward and reverse DNA sequencing of PCR amplicon was carried out by using BDT v3.1 cycle sequencing kit on ABI 3730× 1 genetic analyzer and consensus sequence was generated by Aligner Software. Comparative and bioinformatic analysis of sample were carried out online (http://www.ncbi.nlm.nih.gov). The 16S rDNA gene sequence was used to carry out BLAST with nrdatabase of NCBI gene bank database [8, 9]. Based on maximum identity score first ten sequence were selected and aligned using multiple alignment software program Clustal W.

RESULTS AND DISCUSSION

The bacterium was identified as *Bacillus cereus* strain Probio-32 (Genebank Accession Number: GU471752.1) based on nucleotide homology, which is a Gram-positive aerobic or facultatively anaerobic spore forming rod. It is a probably ubiquitous soil bacterium and opportunistic pathogen causing food poisoning manifested by diarrhoeal or emetic syndromes [10-12].

A single discrete PCR amplicon band of 1500 bp was observed when amplified fragment of 16s rDNA was resolved on agarose gel (Figure 1). Result of forward and reverse DNA sequencing reaction of PCR amplicon were 833 and 848bp when sequencing is carried out with 8F and 1492R primer (Tables 2 and 3). Consensus sequence of 1427bp rDNA gene was generated from forward and reverse sequence data using aligner software (Table 4).

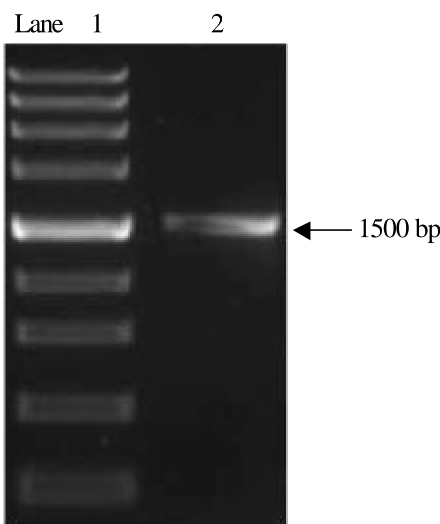

Fig. 1: Gel image of 16S rDNA amplicon
Lane 1: DNA marker
Lane 2: 16S rDNA amplicon band

Bacillus cereus is closely related to the animal and human pathogen *Bacillus anthracis* and the insects pathogen *Bacillus thuringiensis*. The former being used as a biological weapon and the latter as a pesticides. *Bacillus anthracis* and *Bacillus thuringiensis* are readily distinguished from *Bacillus cereus* by the presence of plasmid borne specific toxins (*Bacillus anthracis* and *Bacillus thuringiensis*) and capsule (*Bacillus anthracis*) [12]. But, phylogenetic studies based on the analysis of chromosomal genes being controversial results and it is unclear whether, *Bacillus cereus, Bacillus anthracis* and *Bacillus thuringiensis* are variants of the same species or different species. *Bacillus anthracis* contains several chromosomally encoded proteins that may contribute to pathogenicity including haemolysis, phospholipases and identified numerous surface proteins that might be important targets for vaccines and drugs [13].

The analysis of the structural reinforced concrete building caused by a BLAST load is presented in this paper in figure 2.

Table 2: 8F_SON1014_079_A09 (833 bp) of the bacterium

GCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACT
GGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTA
TGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC
CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTA
CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG
CGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAA
GAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACT
GACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGG
GTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTC

Fig. 2: BLAST DATA: (Alignment view using combination of NCBI GeneBank); Sequence Producing Significant Alignments

Table 3: 1492R_S1014_009_D01 (848 bp) of the bacterium

CACCTTAGGCGGCTGGCTCCAAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGC
CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACT
GAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAA
GGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTA
AGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTC
CCGAAGGAGAAGCCCTATCTCTAGGGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTC
AGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCAC
GCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACAC
ATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCC

Table 4: Consensus Sequence (1427bp) of the bacterium

GCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACT
GGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTA
TGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC
CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTA
CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG
CGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAA
GAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACT
GACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGG
GTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGA
GATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA
CGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTA
ATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCC
GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTTG
GAGCCAGCCGCCTAAGGTG

Table 5; Sequence producing significant Alignments of the bacterium

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
GU812900.1	*Bacillus cereus* strain JBS10	2636	2636	100%	0.0	100%
GU826154.1	*Bacillus cereus* strain Q34	2636	2636	100%	0.0	100%
GU566345.1	*Bacillus sp.* R5(2010)	2636	2636	100%	0.0	100%
GU471752.1	*Bacillus cereus* strain Probio-32	2636	2636	100%	0.0	100%
AB542372.1	*Bacillus sp.* TSA4w	2636	2636	100%	0.0	100%
GU125426.1	*Bacillus cereus* strain IMAU80004	2636	2636	100%	0.0	100%
GU125425.1	*Bacillus cereus* strain IMAU80003	2636	2636	100%	0.0	100%
GQ383905.1	*Bacillus sp.* 4CCS8	2636	2636	100%	0.0	100%
FJ188297.1	*Bacillus cereus* strain BU040901-022	2636	2636	100%	0.0	100%
FJ803926.1	*Bacillus cereus* strain 0-9	2636	2636	100%	0.0	100%

Triggering the extensions of the 161 Blast hits combined with a new heuristic for generating gapped alignments yields a gapped BLAST programme and its variants check each entry in the databank independently against a query sequence of amino acids. BLAST programme was carried out to generate significant alignment and the close matches to the query sequence of *Bacillus cereus* strain Probio-32. On the query sequence of 1250bp, 161 blast hits matched the alignments scores of > = 200. The 10 different strains of *Bacillus* were also found (Table 5). All of them scored maximum score 2636, which is equal to total score. This shows the 100% sequence similarity in query coverage of amino acids (Table 5). The Expectation Value (E) of all these *Bacillus* strain was 0.0, which depicts that all the sequence of 10 different strains is homogenous in comparison to *Bacillus cereus* strain Probio-32. A good criterion for homogeneity is taken as E<= 0.001 or 0.01 for proteins when searched against a protein database. Thus, the E value of *Bacillus cereus* strain Probio-32 is more significant to the match.

Analysis of *Bacillus cereus* strains isolated from soil demonstrated a very high diversity in multilocus genotypes, indicating that *Bacillus cereus* exhibits a low degree of clonality and that exchange of genetic material occurs frequently in the natural environments [14]. By performing comparative genome hybridization 19 *Bacillus cereus* and *Bacillus thuringiensis* strains againsed a *Bacillus anthracis* DNA microarray confirmed the general similarity of chromosomal genes among this group of close relatives [13, 15, 16].

Collectively, *Bacillus cereus, Bacillus anthracis* and *Bacillus thuringiensis* represent microbes of high economic, medical and biodefence importance. Given this significance, this group contains the highest number of closely related fully sequenced genomes [17-19].

Results were most informative for closely related organisms with small subunits 16S rDNA sequence similarities. At this level of relatedness, the similarity between all the strains of *Bacillus* was strongly correlated with sequence.

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