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Comparison of 16S rRNA Gene of *Bacillus cereus* with Different Bacterial Species

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The bacterial 16S rRNA gene increasingly used to discover the species and strains of unknown bacteria. These small 16S rRNA sequences are highly conserved regions. This study was designed to amplify the 16S rRNA gene of *Bacillus cereus* and compare it with the Coding DNA Sequences of different bacterial species by phylogenetic analysis. The main objective of this study is to construct a phylogram between *B. cereus* and other bacterial species. The CDS of these bacterial species were taken from NCBI. It was concluded from this study that the phylogram obtained from the sequences reveals that first three species are in group and closely related while other two species are out group, belongs to same ancestor but do not show very close lineage.

Keywords: 16S rRNA gene; B. cereus; PCR; CDS.

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1. INTRODUCTION

The accuracy in identification of microorganisms is very important. The previous methods of phylogenetic analysis were time consuming and difficult. Then they developed methods for genotyping as another alternative to proven phenotypic methods. These genotypic methods are based on sequencing of conserved regions of rRNA [1].

The *B. cereus* is Gram positive and endospore forming bacteria. The bacteria are aerobic and are less phytotoxic. These species have many applications in industries and resolving environmental issues e.g. degradation of organic waste [2]. Application of molecular methods to identify bacterial species is widely used nowadays. These molecular methods are based on 16S ribotyping. The 16S rRNA gene is amplified by PCR reaction and gel electrophoresis and cloned in a vector [3]. These molecular methods reduced the use of plate count method to notice and count the bacterium subsets. There is diverse range of quantitative methods have been developed for the identification of functional genes. The phylogenetically informative genes information can be also obtained by using some bioinformatics software. The 16S ribosomal RNA gene has been proven to be used as a powerful tool for identification of species of bacteria [4]. The universal primers of different bacterial species have been made to amplify the 16S rRNA gene. The phylogenetic analysis of the nucleotides can be done.

2. MATERIALS AND METHODS

2.1 Amplification of 16S rRNA Gene

The *B. cereus* was isolated from compost which was prepared by using municipal solid waste. This bacterium takes active part in degradation of organic waste. The DNA from this bacterium was extracted by using Phenol Chloroform DNA extraction method. The 16S rRNA genes were amplified by using Uni500 primers designed for *Bacillus* Species. The sequence of primers is given below:

	Primers
Forward sequence	5'-AAACTYAAAKGAATTGACGG-3'
Reverse sequence	5'-ACGGGCGGTGTGTRC-3'

The 16S rRNA gene of *B. Cereus* was amplified by using PCR reaction with the final volume of 10 µl [5]. The PCR components were mixed properly and the amplification was performed in Mycycler^{TM} [6].

Table 1. Shows the components of PCR reaction

Sr. no	Reagents	Required solution	Final concentration
1	PCR buffer	1X	2 µl
2	dNTPs	2.5 mM	1.5 µl
3	$MgCl_2$	2-2.5 mM	1 µl
4	Forward primer	0.24 µM	1 µl
5	Reverse primer	0.24 µM	1 µl
6	Taq polymerase	0.15 units	0.06 µl
7	Genomic DNA	25 ng	10 µl
8	dH_2O	2.4 4µl	Final volume. 10 µl

The sequences of 16S rRNA were analysed by using PCR reaction. The *B. cereus* was isolated from the soil and its 16S rRNA gene was amplified by using a pair of universal primers for *Bacillus*-species. The nucleotide sequences were evaluated by using different bioinformatics tools. The peaks of nucleotide sequences were observed by using Chromaslite version 2.1.1, these nucleotide sequences were copied in FASTA format and pasted in NCBI BLAST for identifying the maximum paralogs of sequence. The BLAST sequences which showed maximum homology were compared by using multiple sequence alignment tool. The CDS sequences of *Bacillus thuringiensis* strain, *Salmonella enterica* subsp. enterica serovar, *Shigella flexneri* 2a str. 301, *Escherichia coli* str. K-12 substr were copied in FASTA format from NCBI and compared with the 16S rRNA sequence of *Bacillus cereus* by using multiple sequence alignment tool and their phylogenetic tree was developed.

3. RESULTS AND DISCUSSION

The results of PCR were analyzed by using gel electrophoresis. The Fig. 1 shows the band of 16S rRNA gene of *B. cereus* on 1.5% gel electrophoresis when observed under transilluminator. The size of PCR product was 850 bp. 1 kb plus ladder was used to determine the band size.

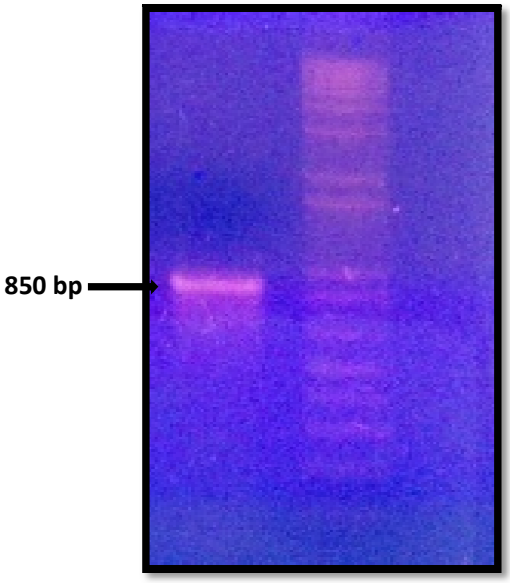

Fig. 1. Gel electrophoresis of PCR product

3.1 PCR product: 1Kb Plus Ladder (Invitrogen, cat. 10787-018)

The PCR product was sent to 1st base Sequencing Malaysia. The sequence of *B. cereus* AB1 was compared with other species of bacteria to check the similarity of 16S rRNA gene and their phylogenetic lineage. The CDS of *Bacillus thuringiensis* strain, accession: KR002672.1 *Salmonella enterica* subsp. *enterica serovar*, accession: 16763390, *Shigella flexneri* 2a str. 301, accession: 344915202, *Escherichia coli* str. K-12 with accession 556503834 in NCBI were compared with the forward sequence of 16S rRNA gene of *B. cereus*. The phylogenetic results showed that the first two species in phylogram belongs to in group and the last three species belongs to out group (Fig. 6). The *B. cereus* bacteria showed direct lineage with *B. thuringiensis* because both belongs to same species, both are Gram positive and spore forming bacteria. The other three species of bacteria included *E. coli, Shigella* and *salmonella* do not showed direct lineage. These are outgroup species, having very less linkage with *Bacillus*. The phylogram showed that the ancestor could be same for all bacterial species included *Shigella, Salmonella, E. coli, B. cereus* and *B. thuringiensis*. B-1 is B. Cereus and B. is *B. thuringiensis* in phylogram (Fig. 6). 16S rRNA can discover the species sub species and strain of bacteria [7]. These 16S rRNA housekeeping genes are widely used to identify bacteria because these regions are conserved [6].

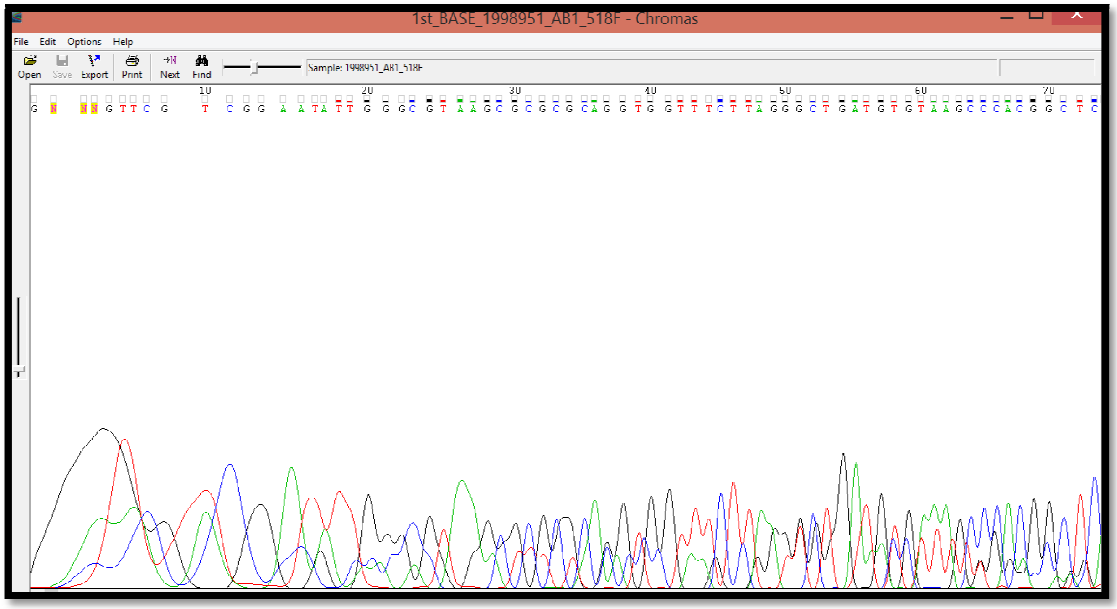

Fig. 2. Analysis by using chromaslite

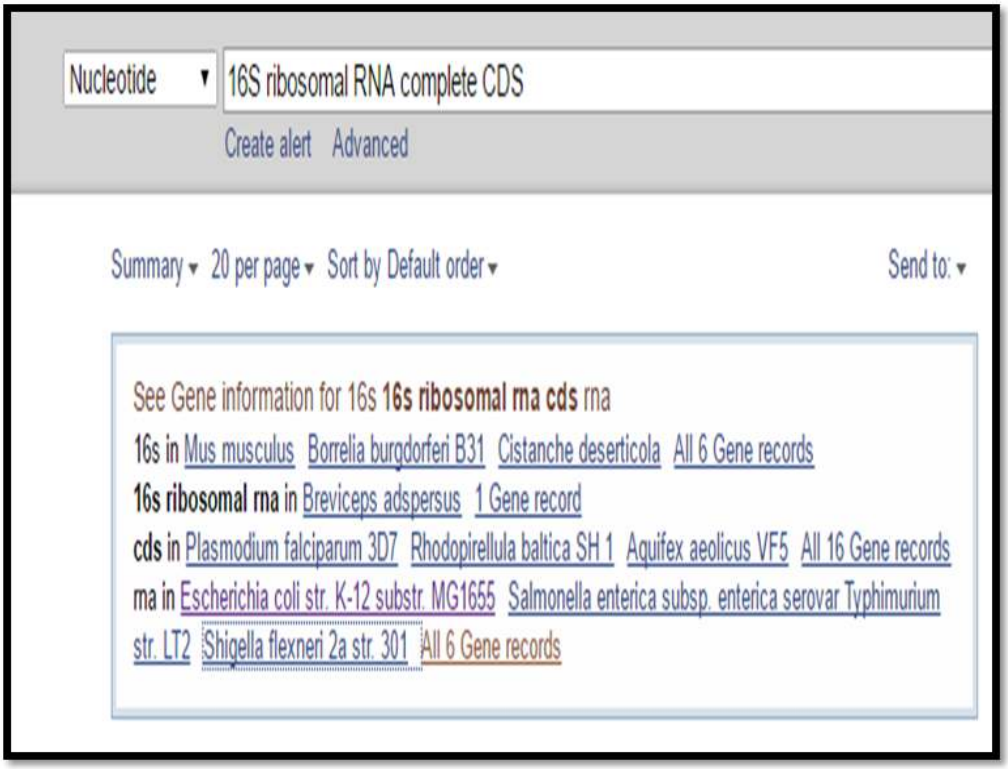

Fig. 3. Coding DNA Sequence of different organisms by using NCBI (nucleotide)

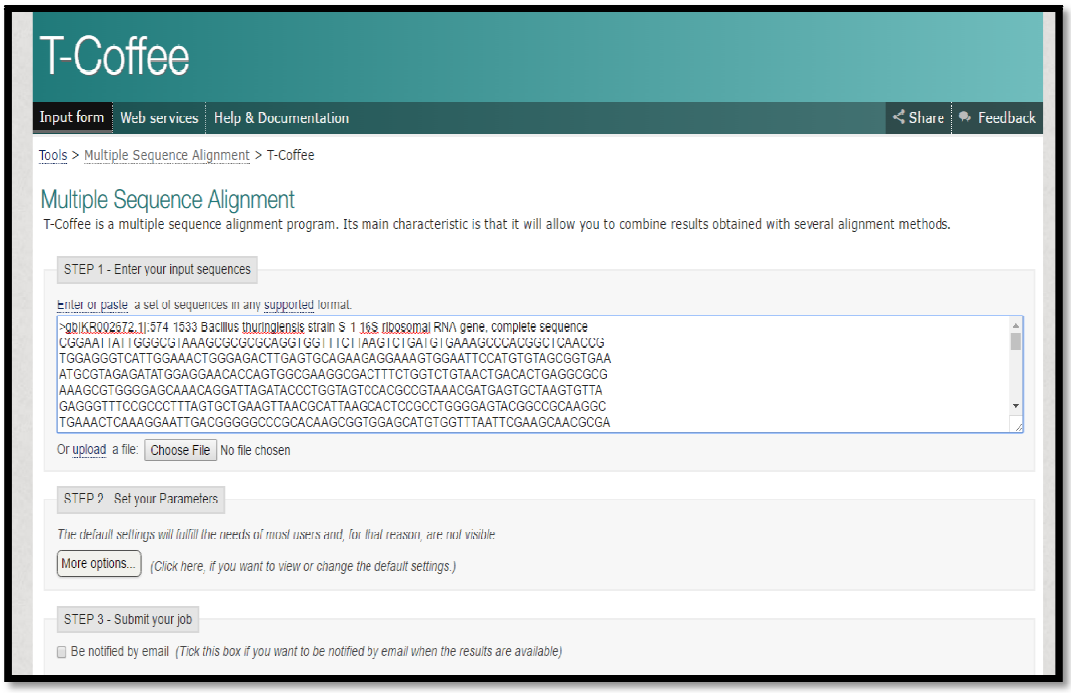

Fig. 4. Comparison of *Bacillus cereus* 16S rRNA gene forward sequence with other bacterial species Coding DNA Sequences by using T-coffee

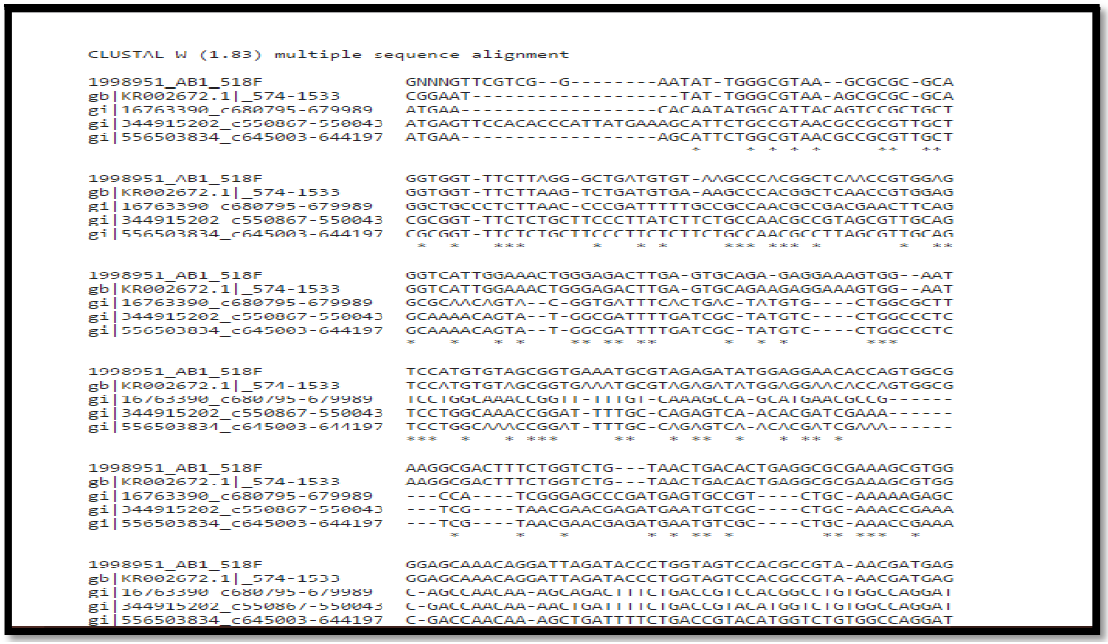

Fig. 5. Alignments of CDS and *Bacillus cereus* 16S rRNA gene forward sequence (AB1)

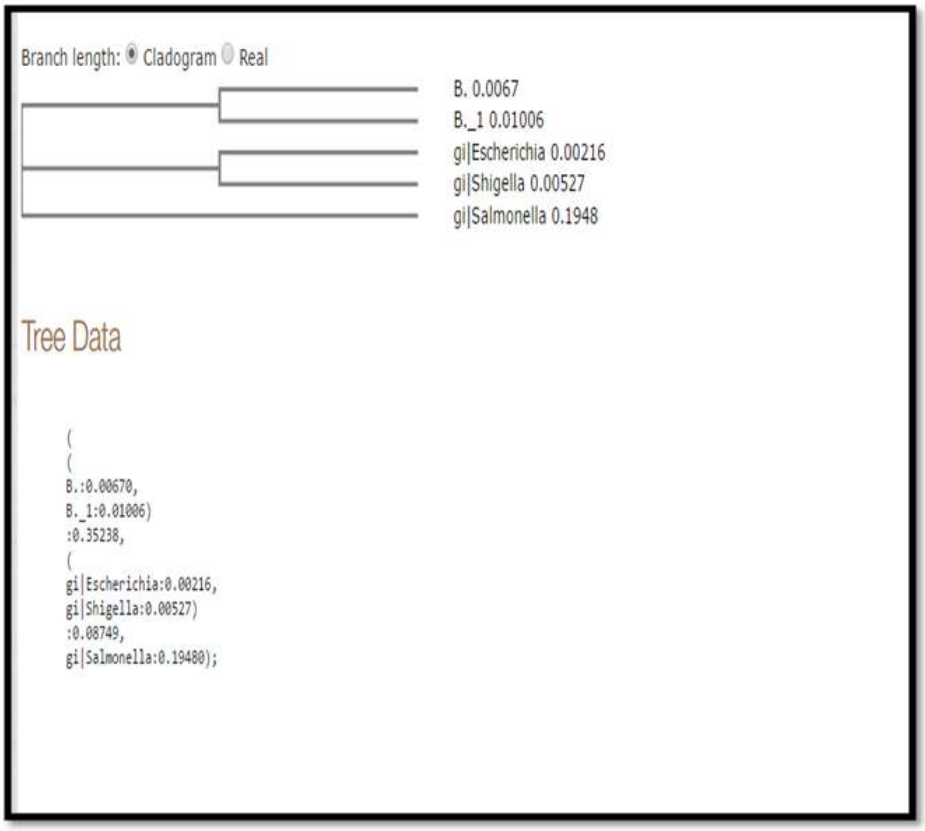

Fig. 6. The phylogenetic tree analysis of 16s rRNA gene with different bacterial species coding DNA sequence

4. CONCLUSION

16S rRNA gene has many conserved regions. These conserved regions are used to identify the bacterial species and strains. It was concluded from this study that the *Bacillus cereus* directly belongs to the first three species in phylogram. These species are closely related to *B. cereus* and the two others belong to same ancestor but are out group and do not show direct lineage with *B. cereus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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