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Phylogenetic Analysis of *Bacillus* Bacteria from Compost

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Authors' contributions

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

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ABSTRACT

Many activated regions of gene can be used to identify the particular organism, this is easier by using the technique of 16S ribotyping of rRNA gene. These regions are present on rRNA gene and are conserved, that is the reason to use them for identification purpose. This study was mainly focused on the phylogenetic analysis of reverse sequence of *bacillus* strains for 16S rRNA gene. It was concluded that the AB1 and AB2 were closely related and showed direct lineage while the *E.coli* strain and *salmonella* showed outgroup relation.

Keywords: PCR; gel electrophoresis; 16S reverse sequence; AB1 and AB2.

1. INTRODUCTION

The most developed genetic analysis methods include 16S rRNA gene analysis of different organisms [1]. The old methods of genetic analysis were time consuming and expensive. The ribotyping of 16S rRNA gene is used to identify a particular individual species, sub species and strains [2].

Bacillus is a Gram positive bacterium. It produces terminal spore. This includes many

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strains which are pathogenic and nonpathogenic. This bacterium is widely present in environment, especially in soil and air. The *bacillus* bacteria shows excellent degradation ability, it can be used as an inoculum with some other species to degrade the organic waste [3]. The majority of the strains are not harmful for human beings. The conventional methods that were used to identify the bacterial species include colony morphology, microscopy and growth pattern of colonies. The most developed ribotyping methods used PCR reaction, gel electrophoresis and sequencing of 16S and 23S ribosomal RNA genes [4].

Bio informatics tool can be used to analyze the sequencing results and for multiple alignment of sequences. These tools can also be used for the phylogenetic analysis or contrasting phylogenetic trees to relate the ancestors and find out the lineage between species [5].

2. MATERIALS AND METHODS

Bacillus cereus bacteria and sub species were collected from compost sample. The two isolated *Bacillus* bacterium were named as AB1 and AB2. The genomic DNA was extracted from these isolates and PCR reaction was optimized by using 10µl of total volume for each sample. The bands were visualized under UV trans illuminator and extracted from gel by gene clean method. The 16S rRNA gene was amplified by using universal primers [6].

Table 1. PCR reaction mixture

Reagents	Required Solution	Final concentration
PCR buffer	1 X	2 µl
Forward primer	0.25 µM	1 µl
Reverse primer	0.25 µM	1 µl
dNTPs	2.8 mM	1.5 µl
Mgcl2	3-2.5 mM	1 µl
Taq polymerase	0.15 units	0.06 µl
Genomic DNA	27 ng	10 µl
dH2O	2.44 µl	Final volume.10 µl

The sequences of two isolated were analyzed by using chromaslite. The Complete DNA Sequence of *E. coli* UMN026, *Sallmonela enterica* sub species were taken and copied in FASTA format from NCBI nucleotide and compared with the *bacillus cereus* and *bacillus cereus* sub species reverse sequence of 800 base pairs by using multiple sequence alignment tool T-coffee. There are two DNA sequences, one is forward DNA sequence or the other is reversed. The phylogenetic tree was extracted by using T-coffee.

3. RESULTS AND DISCUSSION

The genomic DNA was analyzed by using 1% agarose gel electrophoresis. The PCR results were visualized by running samples on 1.5% gel electrophoresis and visualized under UV trans illuminator [7]. The sharp bands of PCR product were observed (Williams, 1989). The DNA ladder was run with samples to determine the band size. The size of PCR product was 500 bp.

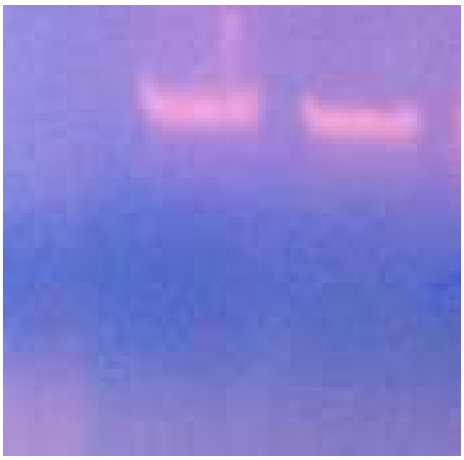

Fig. 1. 1% gel electrophoresis

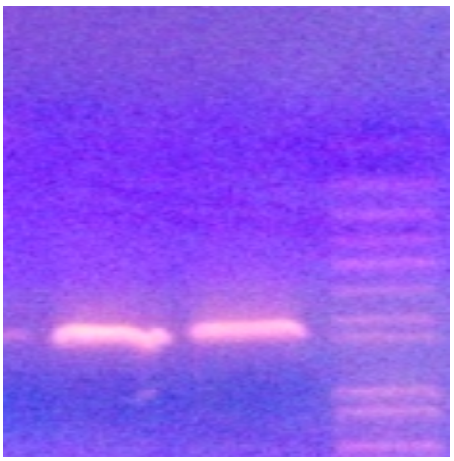

Fig. 2. 1.5% gel electrophoresis for PCR

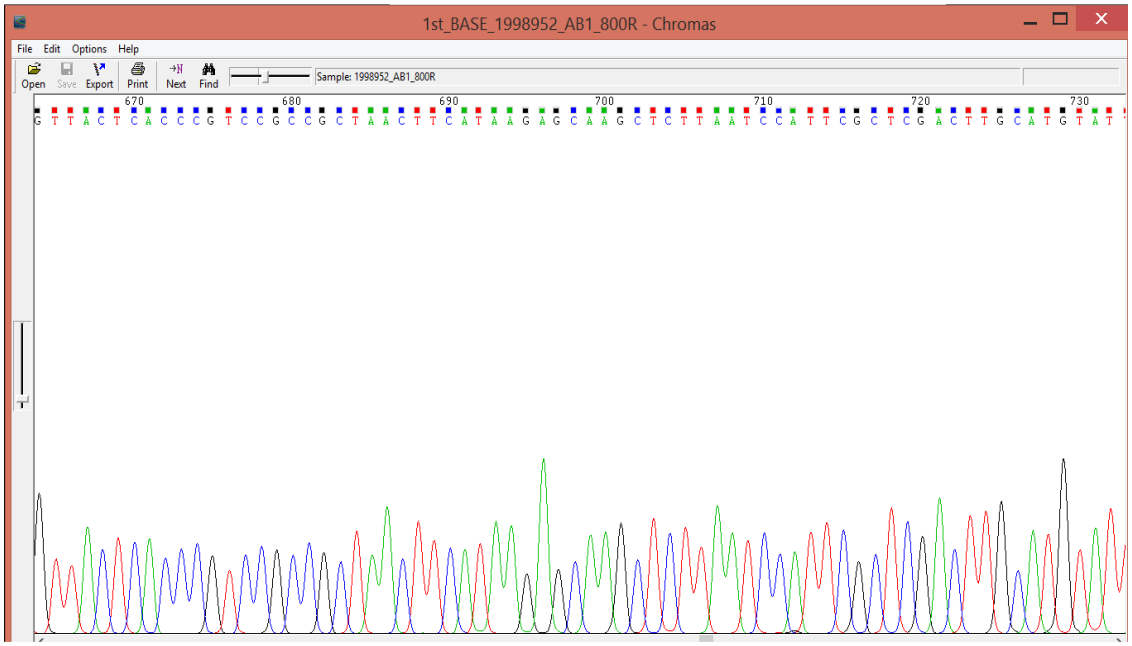

Fig. 3. Sequence analysis of *Bacillus cereus* subspecies and *Bacillus cereus*

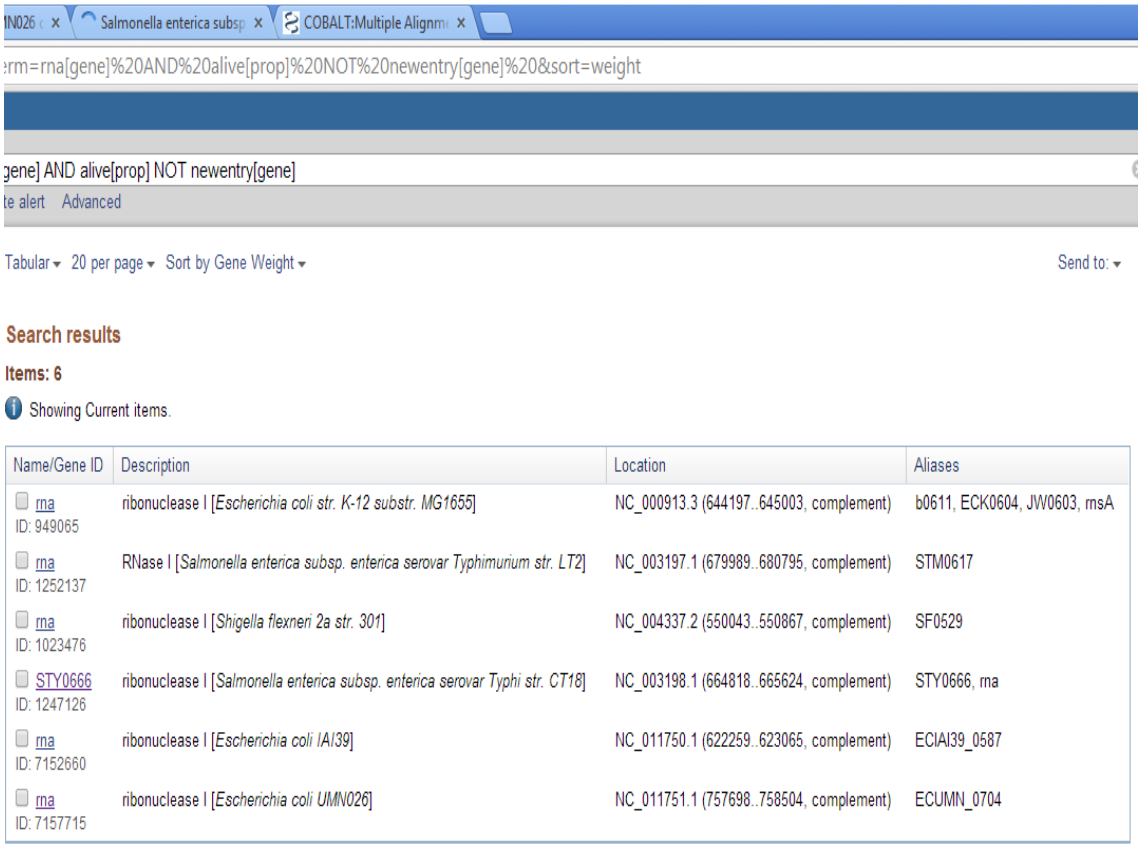

Fig. 4. CDS collection from NCBI nucleotide

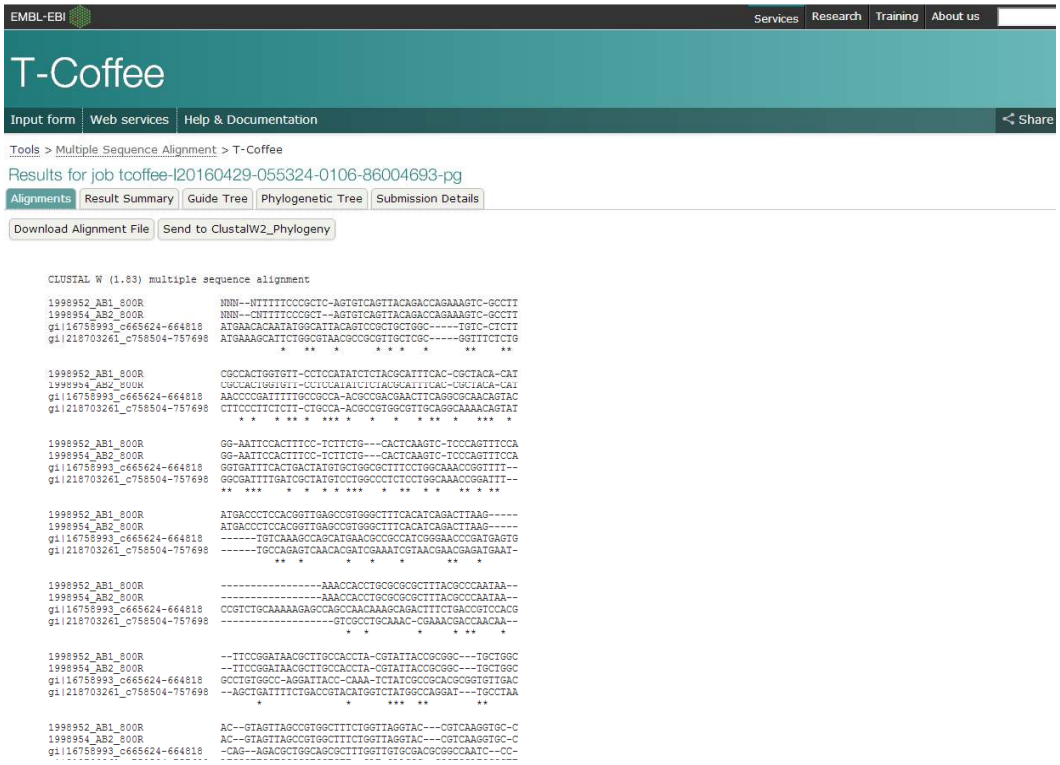

Fig. 5. Multiple alignment sequence

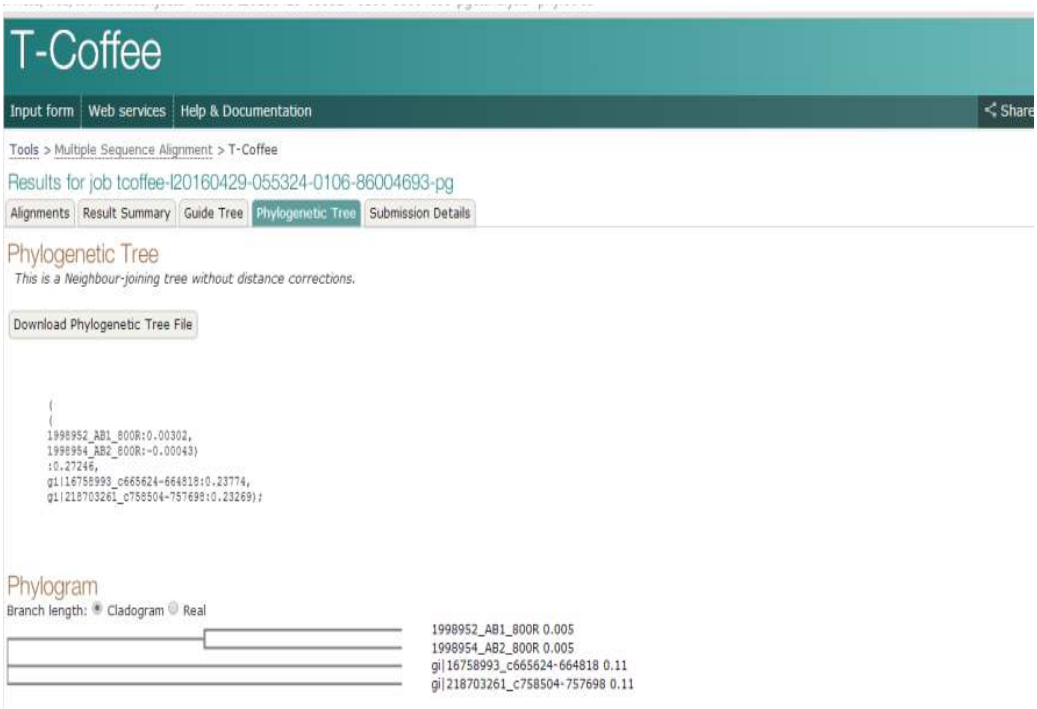

Fig. 6. Phylogenetic tree

The 16S rRNA gene contains conserved sequences. These can be similar in many species. This technique is very useful to molecular characterize the bacteria. The

sequences of AB1 and AB2 were sent to 1st base sequencing Malaysia. The phylogram showed the direct lineage of isolates with each other. The AB1 and AB2 sequences show in group relationship and other two species showed out group relation, their ancestors could be same. The comparison of 16S rRNA reveals six possible matches with other similar gene variants of 16S rRNA gene. All resulted through process of speciation. To study the evolutionary relationships among all related genes, the predicted phylogram can better infer the lineages [8]. The Phylogram of AB1 and AB2 gene shows the distance covered is approximately 0.005 nucleotides. Therefore, they emerged from same ancestor showing maximum possible similarity. The identical peaks obtained from chromaslite for both AB1 and AB2 shown much variation and high GC content. Both of the genes were found to be showing high nucleotide content with adenine (A) starting at position number 655. The variations among peaks off all nucleotides shown different conserved peaks. However, to study the genetic diversity, genetic variation and genetic similarities all ways of inferring phylogenetic relationships, taxonomy and branching patterns can be used. The distance based methods of phylogenetic prediction can be used to infer the natural occurrence of genes from their evolutionary pathways. The distance among genes can be further calculated by scoring matrices. The BLOSUM 61 matrix showed high scores among both AB1 and AB2 genes. Hence, this was proved that higher the similarity, higher will be the score calculated by evolutionary distances. Therefore, the analysis of 16S rRNA gene performed can be used for further identification of more related genes. It can also be useful to overcome many gene silencing problems.

4. CONCLUSION

Hence it is conclude from this study that the identified bacteria was bacillus cereus and its sub specie. The AB1 and AB2 showe direct lineage when phylogram analysis was performed. The AB1 and AB2 showed outgroup relationship with *E. coli* and *Salmonella* species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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