

Journal of Advances in Biology & Biotechnology 7(2): 1-6, 2016; Article no.JABB.26524 ISSN: 2394-1081



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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.

Article Information

DOI: 10.9734/JABB/2016/26524 <u>Editor(s):</u> (1) Joana Chiang, Department of medical laboratory Science and Biotechnology, China Medical University, Taiwan. <u>Reviewers:</u> (1) A. I. Raji, University of Ilorin, Nigeria. (2) Anonymous, AI-Azhar University, Egypt. (3) Hassan Awad Ahmed Mohamed, Saratov State University, Russia. (4) Triveni Krishnan, National Institute of Cholera and Enteric Diseases, Kolkata, India. (5) Essam Hussein Abdelshakour, AI-Azhar University, Cairo, Egypt. Complete Peer review History: <u>http://sciencedomain.org/review-history/15202</u>

Original Research Article

Received 22nd April 2016 Accepted 20th June 2016 Published 28th June 2016

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 ael 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 genes. identification of functional 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	5'-AAACTYAAAKGAATTGACGG-			
sequence	3'			
Reverse	5'-ACGGGCGGTGTGTRC-3'			
sequence				

The 16S rRNA gene of *B. Cereus* was amplified by using PCR reaction with the final volume of 10 μ I [5]. The PCR components were mixed properly and the amplification was performed in MycyclerTM [6].

Table 1. Shows the components of PCR	
reaction	

Sr.	Reagents	Required	Final
no		solution	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	0.24 µM	1 µl
	primer		
5	Reverse	0.24 µM	1 µl
	primer		
6	Taq	0.15 units	0.06 µl
	polymerase		
7	Genomic	25 ng	10 µl
	DNA		
8	dH ₂ O	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.

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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

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5	ee Gene information for 16s 16s ribosomal rna cds rna	
1	is in Mus musculus Borrelia burgdorferi B31 Cistanche deserticola All 6 Gene records	
1	is ribosomal rna in Breviceps adspersus 1 Gene record	
C	Is in Plasmodium falciparum 3D7 Rhodopirellula baltica SH 1 Aquifex aeolicus VF5 All 16 Ge	ne recor
	a in Escherichia coli str. K.12 substr. MG1655, Salmonella enterica subsn. enterica serovar Tun	himuriur
ſ	a in Eschenenia con su. Nº12 subsu. Nº1055 Oaimonena entenca subsp. entenca serovar ryp	minunu

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

T-Coffee	
Input form Web services Help & Documentation	are 🗣 Feedback
Tools > Multiple Sequence Alignment > T-Coffee	
Multiple Sequence Alignment T-Coffee is a multiple sequence alignment program. Its main characteristic is that it will allow you to combine results obtained with several alignment methods. STEP 1 - Enter your input sequences	ods.
Enter or paste a set of sequences in any supported format.	
SDIKR002572.11:574 1533 Bacillus thuringlensis strain S 1 165 tibosomal RNA gene, complete sequence CIGGAA I AI I I GIGGGI AAAGCGCCCCCAGGI GGI I I CI I AAGI I CI GAI GI GAAAGCCCACCGGI CI CAACCG TGGAAGGGT CATTGGAAACTGGAGACTGAGACAGAAAGGGAAACTGGAATTCACTGTTGACCGGTGAA ATGCCTAGAGATATGGAGGAACACCAGTGGCCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCG AAACCGTGGGGAGACAACAGGATTAGATACCCTGGTAGTCCACGGCGTAACCGATGCATGC	•
Or upload a file: Choose File No file chosen	
STEP 2 Set your Parameters	
The default settings will fulfill the needs of most users and, for that reason, are not visible. More options (Click here, if you want to view or change the default settings.)	
STEP 3 - Submit your Job	
Be notified by email (Tick this box if you want to be notified by email when the results are available)	

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

1998951 AB1 518F	GNNNGTTCGTCGGAATAT-TGGGCGTAAGCGCGC-GCA
eb KR002672.1 574-1533	CGGAATTAT - TGGGCGTAA - AGCGCGC - GCA
ri 16763390 c680795-679989	ATGAACACAATATGGCATTACAGTCCGCTGCT
ei 344915202 c550867-550043	ATGAGTTCCACACCATTATGAAAGCATTCTGCCGTAACGCCGCGTTGCT
zi 556503834 c645003-644197	ATGAAAGCATTCTGGCGTAACGCCGCGTTGCT
-	* * * * * **
1998951 ∧B1 518F	GGTGGT-TTCTTAGG-GCTGATGTGT-AAGCCCACGGCTCAACCGTGGAG
gb KR002672.1 574-1533	GGTGGT-TTCTTAAG-TCTGATGTGA-AAGCCCACGGCTCAACCGTGGAG
g1 16763390 c680795-679989	GGCTGCCCTCTTAAC-CCCGATTTTTGCCGCCAACGCCGACGAACTTCAG
gi 344915202 c550867-550043	CGCGGT-TTCTCTGCTTCCCTTATCTTCTGCCAACGCCGTAGCGTTGCAG
gi 556503834_c645003-644197	CGCGGT-TTCTCTGCTTCCCTTCTCTGCCAACGCCTTAGCGTTGCAG
	* * *** * * * *** * * **
1998951_AB1_518F	GGTCATTGGAAACTGGGAGACTTGA-GTGCAGA-GAGGAAAGTGGAAT
gb KR002672.1 _574-1533	GGTCATTGGAAACTGGGAGACTTGA-GTGCAGAAGAGGAAAGTGGAAT
gi 16763390_c680795-679989	GCGCAACAGTAC-GGTGATTTCACTGAC-TATGTGCTGGCGCTT
gi 344915202_c550867-550043	GCAAAACAGTAT-GGCGATTTTGATCGC-TATGTCCTGGCCCTC
gi 556503834_c645003-644197	GCAAAACAGTAT-GGCGATTTTGATCGC-TATGTCCTGGCCCTC * * * * * ** ** * * * * * * * *
1998951_AB1_518F	TCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCG
gb KR002672.1 _574-1533	TCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCG
gi 16/63390_c680/95-6/9989	ICCIGGCAAACCGGII-IIIGI-CAAAGCCA-GCAIGAACGCCG
gi 344915202_c550867-550043	TCCTGGCAAACCGGAT-TTTGC-CAGAGTCA-ACACGATCGAAA
gi 556503831_c615003-611197	TCCTGGC////CCGG/T-TTTGC-C/G/GTC/-/C/CG/TCG/// *** * * *** ** * * * * * * * *
1998951 AB1 518F	AAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG
gb KR002672.1 574-1533	AAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG
gi 16763390_c680795-679989	CCATCGGGAGCCCGATGAGTGCCGTCTGC-AAAAAGAGC
gi 344915202_c550867-550043	TCGTAACGAACGAGATGAATGTCGCCTGC-AAACCGAAA
g1 556503834_c645003-644197	TCGTAACGAACGAGATGAATGTCGCCTGC-AAACCGAAA * * * * * * * * * * * ** *
1998951 AB1 518E	
b KB002672.1 574-1533	GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA-AACGATGAG
ei 16763390 c680795-679989	C-AGCCAACAA-AGCAGACTTTCTGACCGTCCACGGCCTGTGGCCAGGAT
ei 344915202 c550867-550043	

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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> Peer-review history: The peer review history for this paper can be accessed here: http://sciencedomain.org/review-history/15202