Study on isolation and Molecular characterization of *Bacillus cereus* isolated from hot burning tawa

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Abstract

B. cereus is ubiquitous in the environment, unable to link clinical cases to their environmental sources. *Bacillus* is widespread in foods causing food spoilage and poisoning. This study was designed to isolate and characterise the thermo tolerant bacteria. A gram positive rod shaped facultative anaerobic accommodate kitchen tawa was isolated. Isolate identified as *Bacillus cereus* in a hot burning tawa temperature at 212^0 F. Generally these groups were recorded in improper refrigeration or stored food particles, germinated in-between 50^0 F to 122^0 F and are as the survivability status. Optimum temperature was 40° C and pH was 6 for maximum growth. Growth was attained significantly at 60 ° C and moderately at 70-100°c. In this context the newly isolated strain were undergone for traditional biochemical test followed by 16S rRNA molecular technique were adopted to confirm the strain comes under the bacillus group and finalized. The analysis of AP-PCR fingerprints revealed isolate is heterogeneity in nature.

Keywords: endospore, Thermophillic, Bacillus, Growth, DNA

Introduction

Bacillus cereus is ubiquitous in nature and a common cause of emetic and variety of infections, including endophthalmitis, bacteremia, septicemia, endocarditis, salpingitis, cutaneous infections, pneumonia, and meningitis (Hoffmaster et al., 2004). Most B. cereus isolates appear to be harmless, but some are considered opportunistic pathogens. This bacterial community present and grows with soil, water by warmth nearing 0°C. Meanwhile several species typical thrive in water at boiling temperature at 100°C or even more. Archaea's prefer extreme temperature recess and the foremost division of Archael hierarchy consisting thermopiles. The contamination of foods with *Bacillus cereus* group bacteria may lead to food poisoning that usually occurs under two types of syndromes, the emetic, and/or the diarrheal syndromes. Selective solid media such as MYP (mannitol-egg yolk-phenol red-polymyxin-agar) and PEMBA (polymyxin-pyruvate-egg yolk-mannitol-bromthymol blue-agar) were frequently used for the isolation of *B. cereus* from food(Jensen et al., 2003). The selectivity of these media is based on the hydrolysis of egg yolk lecithin and the absence of the use of mannitol by *B. cereus* (Hendriksen and Hansen, 2011). Several molecular typing methods that rely on DNA sequence differences have been used to reveal the genetic relationship of B. cereus group strains (Hill et al., 2015). Certain types of *Bacilli* are thermophiles which can survive the enzymes of these thermophiles are necessary for functioning majority of the *Bacilli* are facultative thermophilic that can thrive at high temperature, but also at a low temperature below 50°C. The *Bacillus* was not reported with hyper-thermophiles which have the optimal temperature growth above 80°C. Gram-positive floras have been affected by the changes in the growing conditions (Ellwood and Tempest, 1972).

Techniques such as the Polymerase Chain Reaction (PCR) and 16S rDNA sequencing have made it possible to specifically detect and identify the microorganisms with a high level of precision. Phenotypic and molecular identification of a novel thermophilic lipase-producing bacterium was isolated from a Malaysian hot spring (Olusesan *et al.,* 2009).The discovery of an even deeper environment for life was reported by Parkes (2014), a geologist at the University of Cardiff in Wales. Two species of heat-loving, single-celled microbes were found living at temperatures up to the boiling point in mud cores extracted by a drill ship from 111 million-year-old sediments a mile beneath the North Atlantic seafloor off the Newfoundland coast. Thermophilic microorganisms have received a great interest in recent years because they are not usually denatured by high temperature, even active at elevated temperature (Beg *et al.,* 2000). The enzymes from these microorganisms are resistant to chemical reagents and extreme pH values in comparison to their mesophilic homologs. More recently, the study of the molecular

and physiological properties of extremophiles became of interest, therefore many studies devoted to the comprehension of the molecular basis of the adaptation to high temperature. At present, most of the scientists work to offer new hyperthermostable enzymes obtained from thermophilic microorganisms which catalyse the polymerization of DNA. Isolation and identification of the thermophilic microorganisms from hot spring has paved way for achieving this goal. In the present study explore the extremophiles of *Bacillus* group from the locality of Vivekananda College, Thiruvedakam (West), Madurai district, Tamil Nadu, India.

Materials and Method

Sampling site

The samples were collected from burning tawa of kitchen Vivekananda College, Thiruvedakam (west) Madurai. The sample is obtained and later transferred phosphate buffer gradually to determine the adaptation and growth of the organisms.

Isolation of *B.cereus*(Liu et al., 2012)

one grams of sample was homogenized 100 ml of buffered peptone water under magnetic stirrer. Serial dilutions were prepared, and 0.1 ml of each 10^6diluted sample was streaked in MYP agar medium. Plates were incubated for 24 h at 50 °C. Furthermore, from each sample, a typical colony presumed to belong to the B. cereus group was subcultured on double strength Nutrient agar and incubated for 24 h at 30°C

Biochemical characters

For taxonomic identification, the isolates were subjected to a series of biochemical tests, which included IMViC, nitrate reduction, anaerobic growth, gas production from glucose, growth at different NaCl, temperature (50,60,70,100° C) and pH ranges, degradation of casein, urea and gelatin.

Protocol for DNA isolation, PCR, and Sequencing

The sources for DNA extraction were pure cultures of bacteria isolated from burning bakery furnace, burning kitchen furnace, burning rotti tawa and vermicompost. The standard molecular biology methods were used according to Asubel *et al.,* (1994). All centrifugations were performed at 4°C unless otherwise mentioned.

Sequencing, Sequence Analysis and Amplification

All fragments were amplified from genomic DNA. Genomic DNA was prepared as described by Helgason et al(2004). Initial PCR contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 20 pmol of each primer, 2.5 U of AmpliTaq DNA

polymerase, and approximately 1 ng template DNA in a 100-µl total reaction volume. PCR amplicons were analyzed by electrophoresis and subjected to sanger's sequencing.

Result and Discussion

Bacillus cereus strain VP11 *was* isolated from the bottom of the burning tawa of the kitchen in Vivekananda College, Thiruvedagam (west), which has the growth temperature of 85ºC. Typical colonies are pink-orange and uniform and they are surrounded by a zone of precipitation indicating lecithinase production. This organism is Gram-positive, straight rod, motile with peritrichous flagella, produce white-grey shiny with raised margin, circular, convex, translucent to a semi-opaque colony of 2 to 3mm. Isolate is positive on MR, vp, nitrate, catalase, oxidase, gelatin, starch and citrate and negative on indole, urease, H_2S (Table 1). In general, the *Bacillus cereus* is a straight or curved slender *Bacilli* with square ends, singly or in short chains. *Bacillus cereus* groups are facultative anaerobes and produce endospores. They possess peritrichous flagella and exhibit two types of motility, swimming and swarming. These colonies were presumptively identified to be *B. cereus* shows an optimum growth at 40ºC with irreugular colony morphology and ellipsoidal, endospore cells. The growth was achieved at 75 ºC and optimum growth at 40ºC with pH ranges from 7 to 9 (Jung *et al.*, 2011). This organism agrees with the present project organism except for the temperature for growth because of *Bacillus cereus* strain VP11 has shown growth of the colonies at 80ºC-85ºC. The plates have shown to the tawa facing side of the hot flames with a red-hot portion for the isolation. Liu *et al.*, (2012) the morphological features of the study organism are similar to the *Bacillus* strain *FJAT*13831 except the growth temperature (Rasko et al., 2007).

Molecular characterization

The phylogenetic tree construction for the species *Bacillus cereus* strain VP11 with the Genbank accession number JX025736 with the other related species of the genus *Bacillus*. In this tree construction, the organism *Bacillus enclensis* with the Genbank accession number KF265350 was used as outgroup.In the constructed tree, the study organism is closely related to *Bacillus sp. FJAT*13831 with Genbank number JN885201. The other closely related organisms are *Bacillus manliponensis* and *Bacillus gaemokensis* at the bootstrap level of 333.. The present project organism also has all the characters as explained earlier and closely related to *Bacillus* sp. strain FJAT13831, which was isolated from the no1. pit soil of Emperor Qin's terracotta warriors, Xiancity, China. Another organism related to *Bacillus cereus* strain VP11 is *Bacillus manliponensis* with Genbank number FJ416490. The general characters of the *Bacillus cereus* group are except for prophages region of the genome, the other region shows major similarity among them (Okstad and Kolsto, 2012). It is further reported that many genome features have been identified as common for the *Bacillus cereus* groups. The factors that are identified as common for the genome are generally in the same size and range with core gene sets (Ivanova *et al.,* 2003) (Lapidus *et al.,* 2008). *Bacillus cereus* group has genes, potentially involved in the selection of environment and niche determination. A phylogenetic study of *Bacillus cereus* group

shows that they are closely related to *Bacillus anthracis and Bacillus thuringiensis*. Though much of the genomic study of gene and whole genome sequencing were not done with the organism *Bacillus cereus* strain VP11, the organism as a strain of *Bacillus cereus* must also possess similar genomic group and the phylogenetic tree also shows it clades connected with *Bacillus cereus, Bacillus thuringiensis, Bacillus weihenstephanesis and Bacillus mycoides.* Thus we can ascertain with these facts that the present project organism belongs to the group of *Bacillus cereus*.

CLC Bio main workbench is a basic and an advanced sequence analysis tool. This software supports a large number of DNA, RNA, and protein sequence analyses. It helps to visualize the metadata contest as the alignment of sequences, reconstruction of phylogenetic trees, grouping of nodes and labeling of sub-trees (Allison *et al.,* 2006). The nucleotide sequence statistics of *Bacillus cereus* strain VP11, it has the total of 1000 base pairs and single-strand DNA molecular weight as 311.185kDa. The frequencies of the nucleotide for *Bacillus cereus* strain VP11 and the other aligned species were worked in CLC workbench and it gives the frequency ratio for the base Adenine, Cytosine, Guanine and Thymine for *Bacillus cereus* strain VP11 as 0.256: 0.219: 0.318: 0.207, *Bacillus anthracis* (0.257: 0.224: 0.309: 0.209), *Bacillus cereus* (0.257: 0.225: 0.308: 0.210), *Bacillus* FJAT-13831 (0.254: 0.227: 0.309: 0.209), *Bacillus anthracis* (0.256: 0.255: 0.308: 0.211), *Bacillus pseudomycoides* (0.255: 0.228: 0.309: 0.209), *Bacillus thuringieneis* (0.257: 0.225: 0.309: 0.209), *Bacillus toyonensis* (0.258: 0.225: 0.309: 0.209), *Bacillus gaemokensis* (0.258: 0.229: 0.306: 0.208), *Bacillus mycoides* (0.259: 0.223: 0.307: 0.211), *Bacillus manliponensis* (0.257: 0.23: 0.303: 0.209), *Bacillus weihenstephanensis* (0.259: 0.223: 0.307: 0.211), *Bacillus cytotoxicus* (0.252: 0.231: 0.313: 0.203), *Bacillus oryzaecorticis* (0.253: 0.235: 0.302: 0.209), *Bacillustianshenii* (0.251: 0.234: 0.310: 0.205), *Bacillus shackletonii* (0.253: 0.234: 0.311: 0.201). These results indicate that although *Bacillus cereus* group is phylogenetically heterogeneous, strains of the same species, as well as different species, may be very closely related and phylogenetically intermixed. As the nature of this species to grow in the unusual environment that is in the hot tawa, the one such species variation is once again established in the organism *Bacillus cereus* strain VP11.

Conclusion

The study organism, isolated from extreme temperature, the phenotypical and biochemical similarities suggest that it is as a different strain of *Bacillus cereus* due to its habitat, and molecular modifications. A phylogenetic study of *Bacillus cereus* strain VP11 reveals that it is related to *Bacillus FJAT*-13831,

Table 1 .Biochemical characteristics of Isolated bacterial strain

Spl charecters	***Bacillus cereus* strain Vp12**
Colony	Irregular rhizoidal, opaque
Gram stain	+
Shape	Rod
Motility	+
Spore	+
Catalase	+
Oxidase	+
Methyl Red	+
Voges Proskauer	+
Indole	-
Citrate	+
Urease	-
Nitrate	+
H_2S	-
Starch Hydrolysis	+
Gelatin Hydrolysis	+

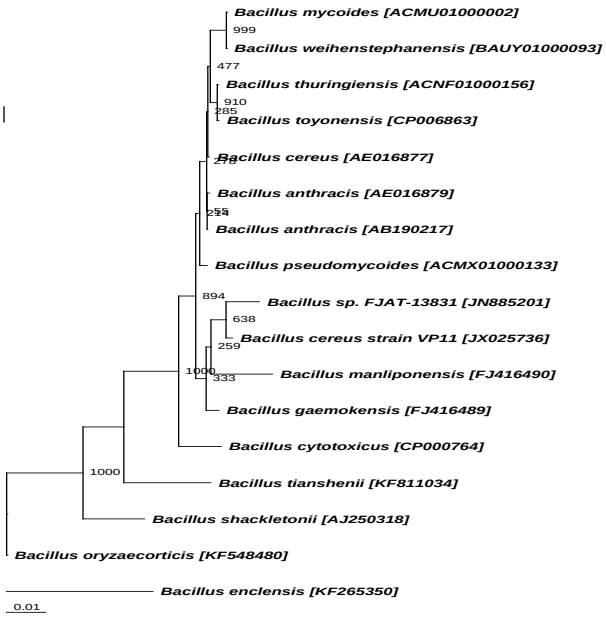

Fig 1: Phylogenetic Neighbour-Joining tree based on 16S rDNA sequences .

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