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PCR Study of *Bacillus Species* Isolated From Food Samples

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Abstract:

A study has been conducted to analyze the occurrence and diversity of *Bacillus* species with antibacterial activity from various food sources. *Bacillus* species were selectively screened from different fermented foods, vegetables and dairy products based on the antagonistic activity. Genetic diversity of these isolates was analyzed using Random Amplified Polymorphic DNA (RAPD). Some of the isolates produced cell wall stress causing antibacterial substance.16S rRNA and *gyr B* gene sequence homology in combination with morphological, physiological and biochemical characteristics were used as the tools for identification of selected isolates. The isolates were identified as *Bacillus Cereus LB1*

Key words:
Cultivation method, chromogenic medium, genera specific PCR, species-specific PCR.

Introduction:

Genera *Bacillus* are formed by Gram-positive rods able to produce endospores resistant to unfavorable external conditions[1] that can be distinguished from other spore-formers (*Sporolacto bacillus*, *Clostridium*, *Desulfoto maculum*, *Sporosarcina*or *Thermo actinomycetes*) due to their aerobic nature (strict or facultative), rod-shaped cells and catalase synthesis[2]

Genera *Bacillus* are divided into 11 groups. Thiswork is focused on *B. cereus* (group of *B. anthracis*, *B. cereus*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, and *B. weihanstephanensis*) and *B. licheniformis* (group of *B. subtilis*, *B. amyloliquefaciens*, *B. atrophaeus*. *B. mojavensis*, *B. licheniformis*, *B. sonorensis*, and *B. vallismortis*)[3] Group of *B. cereus* (so-called *B. cereussensulato*) can be distinguished by the formation of intracellular crystallic toxin in *B. thuringiensis*, presence of rhizoids and loss of motility in *B. mycoides*, or sensitivity to penicillin in *B. anthracis*[4]. In 1998, psychrotolerant *B. cereus*strains, able to grow at 4–7°C but not at 40–43°C.[5]*B. cereus* as well as *B. subtilis, B. licheniformis* and *B. pumilus*is an important food-borne pathogen.[6]

Bacillus sp. is detected and isolated by methods based on the resistance of spores to heating or ethanol. However, direct isolation of particular species requires a selective medium or other selective conditions that are available only for a few species. The cultivation methods for the determination of *B.*

cereus – as tested in this work – are typical examples. These methods detect the failure to utilize mannitol, lecithinase activity or β-glucosidase activity, while other bacteria including some bacilli are inhibited by polymyxin B sulphate or trimethoprim. Other methods for the detection and identification of *B. cereus* are e.g. serotyping, pyrolytic gas chromatography, pyrolytic mass spectrometry, ribotyping, phage typing, plasmid profiles, electrophoresis in pulse electric field and polymerase chain reaction (PCR) using genera-specific and species- specific primers.[7] The main task of this work is to compare the cultivation methods for the determination of *B. cereus* and PCR methods for *Bacillus* sp., *B. cereus*.

Material and methods:

Antibiotic, such as ampicillin and hydrogen peroxide was obtained from Himedia (Mumbai, India). Reagents like ethidium bromide, calcium chloride, sodium chloride, gelatin starch, casein and agarose were obtained from SRL chemicals (Mumbai, India). X-Gal, and other reagents and chemicals used for molecular analysis were procured from Sigma (St. Louis, USA). A 10 Kb and/or 1Kb DNA ladder was used as a molecular size standard, procured from Fermentas (Germany). The primers used in this study were obtained from Sigma (St. Louis, USA). The QIAquick Gel extraction kit and pGEM-T Easy Vectors was purchased from Qiagen (India) and Promega Corporation (Germany), respectively. The media used throughout the work, such as Luria-Bertani (LB) broth and agar, Nutrient broth (NB) and Nutrient agar (NA), Brain heart infusion (BHI) broth, Simmons citrate agar and Baird Parker agar were purchased from Hi-media (Mumbai, India).

PCR for detection of B. Cereus

The strains were tested for the presence of enterotoxin genes. Each amplification process was performed in a 50 μL reaction mixture containing 100 ng of genomic DNA as the template, 5 μL of10x reaction buffer (100 mMTris-HCl (pH 8.8), 500mMKCl,0.8% (vv−1) Nonidet P-40, and 1.5mM MgCl2), 10 μM ofeach of the primers, 0.2mM of each of the four dNTPs (Fermentas), and 2U Taq DNA polymerase (Fermentas). Reactions were initiated at 95◦C for 5min, followed by 30cycles of 95◦C for 1min, 58◦C for 1min, 72◦C for 1min, and afinal elongation step at 72◦Cfor 10min,withafinal holdat 4◦Cin a DNA thermal cycler (Mastercycler Gradiant, Eppendorf, Germany). The diarrheagenic strain of B. cereus (ATCC14579) was used as a positive control and the sterile water was used as a negative control. PCR products were analyzed in1.5% (wv−1) TAE agarose gels and all PCR experiments wereper formed twice for each strain.

PCR Amplification

Reagents and the optimal PCR reaction mixture

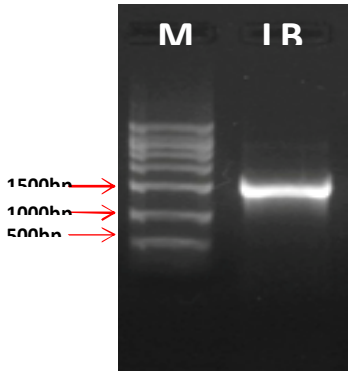
PCR amplification of ITS region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq*DNA polymerase, 0.05 U; primer, 1 pmol and template DNA, 50 ng. Sterile nuclease free water is used as negative control. Oligonucleotide primers of 16S FP sequences (5'-3') is AGAGTTTGATCCTGGCTCAG and 16S RP is AAGGAGGTGATCCAGCCGCA were used and the product size is 1500 bp. PCR temp. of initial denaturation is 94^0 C for 2 min. and final extension is 72^0Cfor 6 min.

Statistical Analysis.

Statistical analysis was performed using SPSS/18.0 software for significant relationship between hot and cold seasons for occurrence of bacteria in water. Chisquare test was performed and differences were considered significant at *P*value < 0.05.

Result and discussion:

Screening of *Bacillus* spp. with potential antibacterial activity is essential, as it helps to develop novel bacteriocins which can be used in food industry. Through this study, we reported several new potent *Bacillus* spp. from acidic to alkaline food sources suggesting a novel source for isolation. These *Bacillus* isolates exhibited inhibitory activity against a broad spectrum of food-borne pathogens/spoilage-causing microorganisms, which is of crucial important from bio preservative application point of view. The use of a range of different morphological, physiological and biochemical parameters, as well as genetic approaches, such as 16S rRNA and *gyr*B gene sequence analysis allowed identification of the selected isolates to the species level. Furthermore, biochemical tests and molecular approaches, such as RAPD served as a useful tool for claiming diversity of the *Bacillus* spp. in food systems. The *Bacillus* isolates showing antibacterial activity obtained in this study can find potential application in food industry. Hence these cultures need to be further characterized in terms of the technological properties of the antibacterial substance, safety of the culture, and its suitability for application in food system either as bio preservation or as probiotic.



Strain identificationGenetic analysis

References:

1. **Logan N. A. and de Vos P. (2009):** Genus I. *Bacillus*. In: De Vos P., Garrity G.M., Jones D., Krieg N.R., Ludwig W., Rainley F.A., Schleifer K.H., Whitman W.B. (eds): Bergey's Manual of Systematic Bacteriology. Springer Science + Business Media, New York: 21–108.

2. **Slepecky R. A. and Hemphill H. E. (2006):** The genus *Bacillus* – nonmedical. In: Dworkin M., Falkow S., Rosenberg E., Schleifer K. (eds): The Prokaryotes. A Handbook of the Biology of Bacteria. Springer Science + Business Media, New York: 530–555.

3. **Ludwig W., Schleifer K.H. and Whitman W.B. (2009):** Revised road map to the phylum *Firmicutes*. In: de Vos P., Garrity G.M., Jones D., Krieg N.R., Ludwig W., Rainley F.A., Schleifer K.H., Whitman W.B. (eds): Bergey's Manual of Systematic Bacteriology. Springer Science + Business Media, New York: 1–8.

4. **Standhouders J. (1992):** Taxonomy of *Bacillus cereus*. Bulletin IDF, **275**: 4–8.

5. **Lechner S., Mayer R., Francis K.P., Prűs M., Kaplan T., Wiessner-Gunkel E., Steward G.S.A.B. and Scherer S. (1998):** *Bacillus Weihen stephanensis* spp. Is a new psychrotolerant species of the *Bacillus cereus* group. International Journal of Systematic Bacteriology, **48**: 1373–1382.

6. **Kotiranta A., Lounatmaa K. and Haapasalo M. (2000):** Epidemiology and pathogenesis of *Bacillus cereus*. Microbes Infection, **2**: 189–198.

7. **TeGiffel M., Beumer R., Christianson A. and Griffiths M. (2000):** *Bacillus cereus* in milk and milk products. Advances in detection, typing and epidemiology. Bulletin IDF, **357**: 47–54.