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Detection and sequencing of plasmid encoded tetracycline resistance determinants (*tetA* and *tetB*) from food–borne *Bacillus cereus* isolates

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ABSTRACT

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Objective: To investigate the detection and sequencing of plasmid encoded tetracycline resistance genes (*tetA* and *tetB*) from food–borne and standard strains of *Bacillus cereus* (*B. cereus*). **Methods:** A PCR was carried out to detect the tetracycline resistance genes (*tetA* and *tetB*) in food–borne *B. cereus* strains and the amplified products were sequenced. **Results:** The phenotypic resistance against tetracycline was observed in 39 of the 118 food–borne isolates and two reference strains (MTCC 430 and MTCC 1307) of *B. cereus*. Among the phenotypically resistant isolates, *tetA* was detected in 36 food–borne isolates and two reference strains (MTCC 430 and MTCC 1307), whereas, *tetB* was detected in 12 food–borne isolates and MTCC 1307 strain. **Conclusions:** A close association was therefore found between phenotypic resistance against tetracycline and presence of tetracycline resistance genes. The *tetA* and *tetB* gene fragments were amplified, purified and sequenced. The gene sequences of the isolates studied herein were found similar to *tetA* and *tetB* gene sequences of other bacteria available in NCBI. The occurrence of *tetA* and *tetB* genes in *B. cereus* indicate the horizontal transfer of antibiotic resistance determinants from other bacteria into *B. cereus*. The transfer of these resistant determinants to other potentially pathogenic bacteria may be a matter of great concern.

1. Introduction

The infectious diarrheal diseases are responsible for considerable morbidity and mortality, especially in developing countries[1]. In India, one third of total pediatric admissions in hospitals are due to diarrheal diseases and 17% of all deaths in indoor pediatric patients are diarrhea related[2]. *Bacillus cereus* (*B. cereus*) is one of the common enteropathogen involved in number of gastrointestinal disorders and a variety of local and systemic infections like necrotic enteritis, liver failure, bacteraemia, meningitis

etc[3].

Enteropathogens have developed high level of resistance to antibiotic agents used for empiric treatment of diarrhea[4]. The antibiotic resistance has been studied extensively and genetic basis elucidated. Among the major mechanisms which account for the evolution of bacterial resistance to antibiotics, spread of old resistance genes (already known) into new bacterial hosts (genera or species that were previously uniformly susceptible) is known since the early findings[5]. Antibiotic resistance commonly spreads by transfer of antibiotic resistant genes, once the resistant genes get conveyed by plasmids, transposons or integrons dissemination is rapid and horizontal gene transfer is common among bacteria[6]. Until recently, it was known that this type of genetic transfer only occurred between closely related bacterial[7]. However, it has been established now that the transfer of antibiotic resistance genes in natural

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environments can occur between phylogenetically distant bacterial genera, in particular between gram-positive and gram-negative bacteria[6].

There is no data available regarding the occurrence of tetracycline resistance genes (*tetA* and *tetB*) in *B. cereus*. There is every chance that the resistance genes can spread from *B. cereus* to other potentially pathogenic microorganisms. Keeping in view the above facts the isolates were screened for tetracycline resistance genes as a high level of resistance was noted against tetracycline and also sequencing of *tetA* and *tetB* fragments was carried out.

2. Materials and methods

2.1. Bacterial strains

Standard strain of *B. cereus* (ATCC 14579) was procured from Hi-media (India) and *B. cereus* (NCTC 11143) was kindly provided by Dr. M. M. Willayat, Professor and Head, Division of Veterinary Public Health (SKUAST-K, India). The MTCC 430 and MTCC 1307 strains were procured from Institute of Microbial Technology–Chandigarh, India (IMTECH). Besides, there were 118 food-borne *B. cereus* isolates available with the Department of Veterinary Public Health, Guru Angad Dev Veterinary and Animal Sciences University–Ludhiana (India).

2.2. Antibiotic sensitivity

Antibiotic susceptibility against tetracycline was determined by disc diffusion technique in accordance with the protocol recommended by antibiotic disc supplier (Hi-Media, India).

2.3. Detection of tetracycline resistant genes (*tetA* and *tetB*) in *B. cereus*

A PCR of isolates was carried out to detect the tetracycline resistance genes, *tetA* and *tetB*. The primers used in the current study to amplify *tetA* (TetAF–5'GGCGGTCTTCTTCATCATGC3' and TetAR–5'CGGCAGGCAGAGCAAGTAGA3', forward and reverse, respectively) and *tetB* genes (TetBF–5'CATTAATAGGCGCATCGCTG3' and TetBR–5'TGAAGGTCATCGATAGCAGG3', forward and reverse, respectively) are described elsewhere[8], designed for the detection of tetracycline resistance genes (*tetA* and *tetB*) in *Escherichia coli*.

2.4. Template DNA preparation

The individual colony of the *B. cereus* was inoculated in 5 mL nutrient broth and incubated in shaker incubator

(150 rpm) for 5 h at 32 °C. Cells were harvested from 1 mL of culture broth by centrifugation (5 000 *g* for 3 min), washed once with 500 µL of sterile MilliQ water. Plasmid and chromosomal DNAs were prepared as per the method described elsewhere[9]. The concentration and purity of the harvested DNA was checked spectrophotometrically (Nanodrop–Applied Biosystems, Singapore).

2.5. Screening tetracycline resistance genes

The DNA amplification was set up in a final reaction volume of 25 µL, which contained 1× PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl and 15 mM MgCl₂), 200 µM dNTP's (MBI–Fermentas), 0.28 µM each of forward and reverse primers (Operon, India), 1 U of *Taq* DNA polymerase (MBI–Fermentas), 3 µL of template and volume was made 25 µL with sterile MilliQ water. PCR assay was performed in a thermocycler (Biometra, Germany) with heated lid. The PCR cycling conditions were as per the previous procedure[8] with change in annealing temperature. The amplification was carried out with the following cycling temperature: initial denaturation of 4 min at 95 °C followed by 35 cycles each consisting of 1 min at 95 °C, 1 min at 58 °C, 1 min at 72 °C, and one cycle of final elongation at 72 °C for 7 min. Amplified PCR products were subjected to gel electrophoresis and were separated on agarose gel (1.5%) stained with ethidium bromide (5 µg/mL) and applying 5–6 volts/cm. The sizes were estimated using 100 bp DNA ladder (MBI–Fermentas, India).

2.6. Sequencing of *tetA* and *tetB* genes

The *tetA* (502 bp) and *tetB* (930 bp) genes of *B. cereus* isolates were amplified in bulk and the PCR products were analyzed on low melting agarose gel (1.5%) containing ethidium bromide (SRL–India). The DNA band from gel was excised and DNA was extracted by using AuPreP gel extraction kit (Life Technologies India Pvt. Ltd.). The PCR amplicons after purification were sequenced using automated dye-terminator cycle sequencing method with Ampli *Taq* DNA polymerase in ABI PRIZM 377 DNA sequencer (Perkin–Elmer). The fragments were sequenced at least twice with both forward and reverse primers to reduce possibility of sequencing artifacts. Sequences were analyzed on-line by using the BLAST (Basic Local Alignment Search Tool) family of programs of GenBank.

3. Results

The phenotypic resistance against tetracycline was noted against 39 (33.05%) of the 118 food-borne isolates and two reference strains (MTCC 430 and MTCC 1307) of *B. cereus*. However, the reference strains ATCC 14579 and

NCTC 11143 were susceptible to tetracycline. Among the 39 phenotypically resistant isolates, *tetA* and *tetB* genes were detected in 36 (Figure 1) and 12 (Figure 2) isolates, respectively. The three isolates although phenotypically resistant against tetracycline did not carry *tetA* or *tetB* genes and one of the intermediately susceptible isolate showed presence of *tetA* gene. The *tetA* and *tetB* genes were sequenced and fragments of 502 and 930 bp length for *tetA* and *tetB*, were obtained, respectively. The nucleotide sequences of *tetA* and *tetB* were submitted to NCBI, GenBank and the Accession numbers GQ184724 and GU324768 were allotted, respectively.

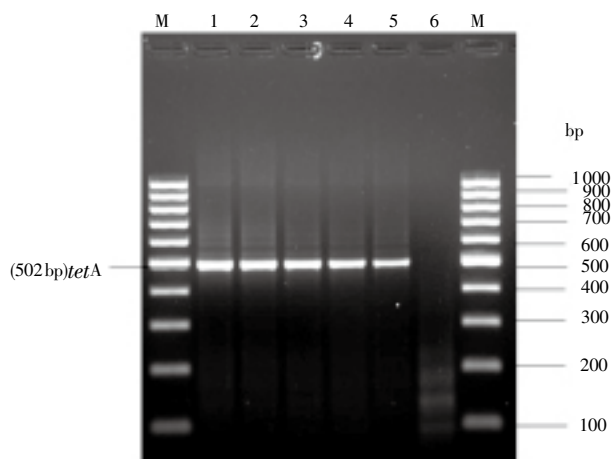


Figure 1. Agarose gel (1.5%) electrophoresis showing *tetA* gene in different *B. cereus* isolates.

M presents 100 bp DNA marker; Lane 1 presents standard strain of *B. cereus* (MTCC 1307); Lane 2 to Lane 5 presents *B. cereus* isolates from foods samples; Lane 6 presents NCTC 11145 strain of *B. cereus* devoid of *tetA*.

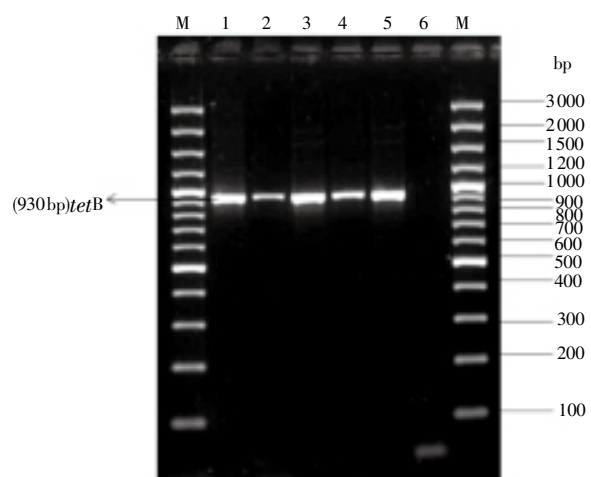


Figure 2. Agarose gel (1.5%) electrophoresis showing *tetB* gene in different *B. cereus* isolates.

M presents 100 bp DNA marker plus; Lane 1 presents standard strain of *B. cereus* (MTCC 430); Lane 2 to Lane 5 presents *B. cereus* isolates from foods samples; Lane 6 presents ATCC 14579 strain of *B. cereus* devoid of *tetB*.

4. Discussion

A relatively higher phenotypic resistance against tetracycline (33.05%) was noted compared to the studies of Schlegelova *et al*[10,11] and Whong and Kwaga[12], who found 3.03% and 6.7% of *B. cereus* isolates resistance against tetracycline, respectively, therefore the isolates were screened for presence of tetracycline resistance genes. Among the phenotypically resistant isolates 36 and 12 isolates showed the presence of *tetA* and *tetB* genes, respectively. Three isolates were phenotypically resistant against tetracycline, but did not carry *tetA* or *tetB* gene, which is perhaps due to presence of other tetracycline resistance genes *eg. tetM, tetL*[13]. One of the strain carried *tetA* but was intermediately susceptible to tetracycline, possibly *tetA* was not expressed in that particular isolate or the mutation in the gene may have occurred. The results indicate a close association between tetracycline resistance genes (*tetA* and *tetB*) and phenotypic resistance against tetracycline. Plasmid characterization was carried in order to determine origin of tetracycline resistant genes. The amplification was observed when the plasmid DNA was used as template, whereas, no amplification was there when chromosomal DNA was used as template, indicating that tetracycline resistant determinants occur in plasmid rather than in chromosome.

Antibiotic resistance commonly spreads due to transfer of antibiotic resistant genes, once the resistant genes get conveyed by plasmids, transposons or integrons dissemination is rapid and horizontal gene transfer is common among bacteria even distantly related ones[6]. This process is thought to be a significant cause of increased drug resistance when one bacterial cell acquires resistance, it can quickly transfer the resistance genes to many species[14]. There have been reports of transfer of antibiotic resistance genes between different bacterial species, transfer of tetracycline resistant genes *tet(M)* from *Enterococcus*, *Streptococcus*, *Staphylococcus* to *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus* spp.[15] and gentamicin, tetracycline and erythromycin resistant genes from *Pseudomonas aeruginosa* to *Escherichia coli*[16].

When the gene transfer between gram-positive and gram-negative bacteria is suspected, strong cases can be made because of the availability of sequence data from a variety of microorganisms[6]. Online similarity of sequence analysis of *tetA* fragment in *B. cereus* strains isolated during the current study were identical to the sequences found in *Klebsiella pneumonia* (HM371195.1), *Pseudomonas aeruginosa* (HM804085), *Escherichia coli* (FN554766.1), *Acinetobacter baumannii* (FJ172370.3) *etc.* The sequence of *tetB* was similar to *Escherichia coli* (FJ917423.1), *Haemophilus*

parasuis (HQ622101.1), *Pasteurella multocida* (EU252517.1), *Salmonella typhi* (AY150213.1), *Shigella flexneri* (AF162223.1) etc. This suggests that there may be transfer of tetracycline resistant genes from these isolates to *B. cereus* or vice versa. The present report is first of its kind wherein the presence of *tetA* and *tetB* genes is being reported from *B. cereus*.

The presence of tetracycline resistance genes in *B. cereus* is responsible for high level of resistance against tetracycline and induction of this tetracycline resistance is most probably a case of horizontal transfer of antibiotic resistance genes in *B. cereus* from some other bacterial species, as there is no report of presence of these resistant genes in *B. cereus* till date.

Conflict of interest statement

We declare that we have no conflict of interest.

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