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Genome Note

Draft genome sequence of multidrug-resistant β-lactamase-producing *Bacillus cereus* S66 isolated from China

Zhe Song[a,b], Qianru Zhao[a], Liying Zhu[c,*], Zhidong Zhang[d], Ling Jiang[b,*], He Huang[e]

[a] *College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 210009, People's Republic of China*
[b] *College of Food Science and Light Industry, Nanjing Tech University, Nanjing 210009, People's Republic of China*
[c] *College of Chemical and Molecular Engineering, Nanjing Tech University, Nanjing 210009, People's Republic of China*
[d] *Institute of Microbiology, Xinjiang Academy of Agricultural Sciences, Xinjiang Uygur Autonomous Region, Urumqi, People's Republic of China*
[e] *College of Pharmaceutical Sciences, Nanjing Tech University, Nanjing 210009, People's Republic of China*

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ABSTRACT

Objectives: *Bacillus cereus* is associated with a variety of human diseases such as haematological malignancies, tetanus, gas gangrene, food poisoning and pseudomembranous colitis. Our team found and isolated a strain, named *B. cereus* S66, from radiation-contaminated soil in Xinjiang Uygur Autonomous Region. In this study, the drug resistance and toxicity of *B. cereus* S66 was systematically studied and analysed.

Methods: Genomic DNA of *B. cereus* S66 was sequenced using an Illumina HiSeq 2000 sequencing system. The generated reads were assembled using SOAPdenovo, and GapCloser was used to fill in the gaps. The draft genome was annotated, and virulence genes and drug resistance genes were identified by comparison with multiclass databases.

Results: The genome of *B. cereus* strain S66 is 5 628 370 bp with a GC content of 34.95% and contains various genes related to toxicity and antimicrobial resistance. The number of toxicity genes identified was 496, some of which are associated with gastrointestinal infection, eye infection and neutrophil-activating protein NapA. Antimicrobial resistance-related genes comprised 3.23% of the total genes and conferred resistance to lincomycin, tetracycline, fosfomycin, aminoglycosides, linezolid, chloramphenicol, rifampicin, macrolides, daunorubicin and mupirocin. Three different β-lactamases were also found in isolate S66.

Conclusions: The genome sequence of *B. cereus* S66 provides a valuable reference to further research on multidrug resistance characteristics and pathogenic system analysis of *Bacillus* sp. in the clinic.

Bacillus cereus is ubiquitous in nature and is frequently isolated from soil and water samples, the rhizosphere and the intestinal tract of animals [1]. It has the ability to withstand harsh environments owing to its highly adherent endospores that are resistant to dehydration, heat and other physical stresses [2]. As an opportunistic pathogen, *B. cereus* is becoming one of the most important causes of food-poisoning in modern society. *B. cereus* produces a range of virulence factors that may cause gastroenteritis manifested by diarrhoeal or emetic syndromes as well as severe local or systemic infections such as endophthalmitis and septicaemia [3]. *B. cereus* isolated from clinical conditions is usually ecologically enduring, surviving growing in a wide variety of different environments and temperatures, but is susceptible to most antibiotics except penicillin [4]. We recently isolated a new strain from soil samples contaminated by a nuclear explosion in Xinjiang Uygur Autonomous Region, China, identified as *B. cereus* on the basis of 16S DNA sequence analysis, and finally named *B. cereus* S66. Antimicrobial susceptibility testing performed by Etest showed that *B. cereus* S66 was resistant to at least 12 antibiotics, including lincomycin, tetracycline, fosfomycin, aminoglycosides, linezolid, chloramphenicol, rifampicin, macrolides, daunorubicin, mupirocin, cephalosporins, carbapenems, etc. In the present study, the genome sequence of *B. cereus* S66 was determined in order to reveal details of the genetic functional basis associated with its multidrug resistance.

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* Corresponding authors.
E-mail addresses: zlyhappy@njtech.edu.cn (L. Zhu), jiangling@njtech.edu.cn (L. Jiang).

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Genomic DNA was extracted with an E.Z.N.A.® Bacterial DNA Kit (Omega Bio-Tek Inc., Norcross, GA) and was sequenced using an Illumina HiSeq 2000 sequencing system (Shanghai Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China) using a paired-end 2× 150-bp format. Before assembling, the *k*-mer frequency distribution analysis method was used to estimate the genome size. Clean sequencing data were subsequently assembled using SOAPdenovo and were optimised using GapCloser. Based on the comparison results, the results were basically annotated from six databases, namely NR, Swiss-Prot, Pfam, eggNOG, GO and KEGG.

The whole genome size of *B. cereus* S66 was calculated as 5 628 370 bp with a GC content of 34.95%, which was assembled into 79 contigs and 67 scaffolds (>500 bp length). The number of candidate protein-coding sequences was 5590 using GeneMarkS and Glimmer software analysis. Identification by tRNA-scan-SE 2.0 and barrnap 0.8, the genome possessed 48 tRNA genes regions and five rRNA regions, including three 5S rRNA regions, one 23S rRNA region and one 16S rRNA region. In addition, 8 genomic islands and 25 genes associated with CRISPR-Cas were discovered from the genome. A circular genome map of *B. cereus* S66 was drawn using Circos (http://www.circos.ca/) (Supplementary Fig. S1).

The Virulence Factors Database (VFDB) integrates the information of pathogenic bacterial virulence factors, and the virulence gene information contained in *B. cereus* S66 was obtained through database annotation. It was found that there were 496 virulence genes annotated in *B. cereus* S66. Among them, the main virulence genes producing cytotoxins responsible for vomiting and diarrhoea are haemolysin BL (*hbl*), non-haemolytic enterotoxin (*nhe*) and cytotoxin K (*cytK*). One toxic gene sequence one, and the listeria adhesion protein Lap (*lap*) gene alignment score up to 3381 suggests that this protein could cause intestinal epithelial barrier dysfunction and accelerate the invasion of *B. cereus* S66 into the intestinal tract. Fibronectin-binding protein (FbpA) was found in *B. cereus* S66, which is recognised as a matrix molecule that adheres to host cells and induces inflammatory cytokine production. To successfully invade the host, *B. cereus* S66 encodes a protease called immune inhibitor A metalloprotease (InhA), which specifically degrades antibacterial proteins produced by the host [5].

Information on the antimicrobial resistance genes contained in the genome of strain S66 was obtained through Comprehensive Antibiotic Resistance Database (CARD) annotation. *B. cereus* S66 has a wide range of antimicrobial resistance genes, consistent with the results of antimicrobial susceptibility testing, such as genes encoding resistance to lincomycin (*clbC*), tetracycline (*tetA*), fosfomycin (*fosB*), aminoglycosides [*aac(6′)*-Ib11], linezolid (*zmaR*), chloramphenicol (*catB*), rifampicin, macrolides (*macB*), daunorubicin (*drrA*) and mupirocin (*ileS*). In addition, other resistance genes such as to aminocoumarins (*alaS*), fluoroquinolones (*mfd*), trimethoprim (*dfrG*), glycopeptides (*vanRF*), fusidic acid (*fusB*) and polymyxin (*arnA*) were found in the genome. *B. cereus* S66 also encodes the major facilitator superfamily (MFS) transporter EfpA, the MFS efflux pump Blt, and an ATP-binding cassette (ABC)-type transport system that confers multidrug resistance. It is worth noting that strain S66 was capable of secreting three different β-lactamases (I–III) responsible for multidrug resistance, which is unique among β-lactamase-producing bacteria [6]. They fall into two classes, i.e. β-lactamases I and III in class A and β-lactamase II in class B, both of which were regulated by a proteolytic-dependent signalling event between the transcriptional repressor BlaI and the antirepressor BlaR.

Intensive use of antimicrobial agents for the prevention and treatment of human diseases may promote the fixation of multidrug resistance genes in bacteria. Given that foodborne pathogenic *B. cereus* is commonly present in food-production environments, it may be able to transfer its multidrug resistance genes through horizontal transfer to human-adapted pathogens or to human gut microbiota [7]. Whole-genome sequencing represents an important tool to monitor *B. cereus* S66 and can result in a deeper understanding of its role as a reservoir for mechanisms of multidrug resistance in other pathogenic *Bacillus* sp.

Nucleotide sequence accession no

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession **RBAJ00000000**. The version described in this paper is version **RBAJ01000000**. The BioProject ID in GenBank is **PRJNA493557**.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.02.019.

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