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Genomic characterisation of multidrug-resistant *Bacillus toyonensis* strain 4HC1 isolated from marine plastic in Norway

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ABSTRACT

Objectives: *Bacillus toyonensis* is widespread in nature. Multidrug-resistant *B. toyonensis* strain 4HC1 was isolated from polyethylene submerged in the water column near a beach in Øygarden, Norway. We analysed the whole genome sequence of strain 4HC1 in order to understand the genetic basis of the observed phenotypic antibiotic resistance.

Methods: Whole-genome sequencing of *B. toyonensis* strain 4HC1 was performed on Illumina MiSeq platform using 2 × 300 bp chemistry. The genome sequence was assembled using SPAdes v.3.13.0 and was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP).

Results: The draft genome of strain 4HC1 is 6 156 259 bp (133 contigs) in size with a GC content of 34.95%. The genome comprises 6089 protein-coding genes, 86 tRNAs and 24 rRNAs. Strain 4HC1 is resistant to cefotaxime, trimethoprim and ampicillin and carries various antibiotic resistance genes (ARGs), including several β-lactamases, aminoglycoside 6-adenylyltransferase, a TetM family tetracycline resistance gene, two different tetracycline efflux pumps, and a bleomycin resistance gene. Several virulence genes including genes involved in immune evasion, iron acquisition and toxins were also detected in strain 4HC1.

Conclusion: The draft genome sequence of *B. toyonensis* strain 4HC1 released here shows the presence of various ARGs and virulence genes in a multidrug-resistant strain isolated from marine plastic.

1. Introduction

Plastic use and associated plastic pollution have become a global problem, especially in the marine environment [1,2]. Recently, the toxic effects of plastics on aquatic life have been extensively reported [2]. Plastics in the marine environment provide a surface for attachment of bacteria and the formation of biofilms. Marine plastics can thus function as a vector for transport of microbes in marine environments [1,3]. Hence, studying the microbiota associated with marine plastics is important for understanding the secondary effects of plastic pollution. The aim of this study was to perform genomic characterisation of multidrug-resistant *Bacillus toyonensis* strain 4HC1 isolated from marine plastic in order to understand the resistome and to provide insights into the total metabolic potential of this strain.

2. Materials and methods

Strain 4HC1 was isolated from plastic-associated biofilms obtained from marine plastic/polyethylene submerged in the water column near a beach (60°29′54.5″ N 4°54′52.6″ E) in Øygarden, Norway, collected on 26 August 2019. The methods used in this paper have been described previously [1]. Briefly, serial dilutions of suspensions were made from the biofilms and were spread onto Mueller–Hinton agar plates containing 10 μg/mL cefotaxime and incubated aerobically at 25°C for 48 h. The strain was identified using a Bruker MALDI Biotyper at the Institute of Marine Research (Bergen, Norway). Minimum inhibitory concentrations (MICs) were determined for freshly grown culture (at 30°C) using Etest® strips (bioMérieux, Paris, France) for cefotaxime, tetracycline, ciprofloxacin, ampicillin, meropenem, streptomycin, trimethoprim, gentamicin, imipenem and chloramphenicol, whereas the broth dilution method was used for vancomycin. Genomic DNA was extracted from freshly grown culture, grown aerobically overnight at 30°C, using a QIAamp® Fast DNA Stool Mini

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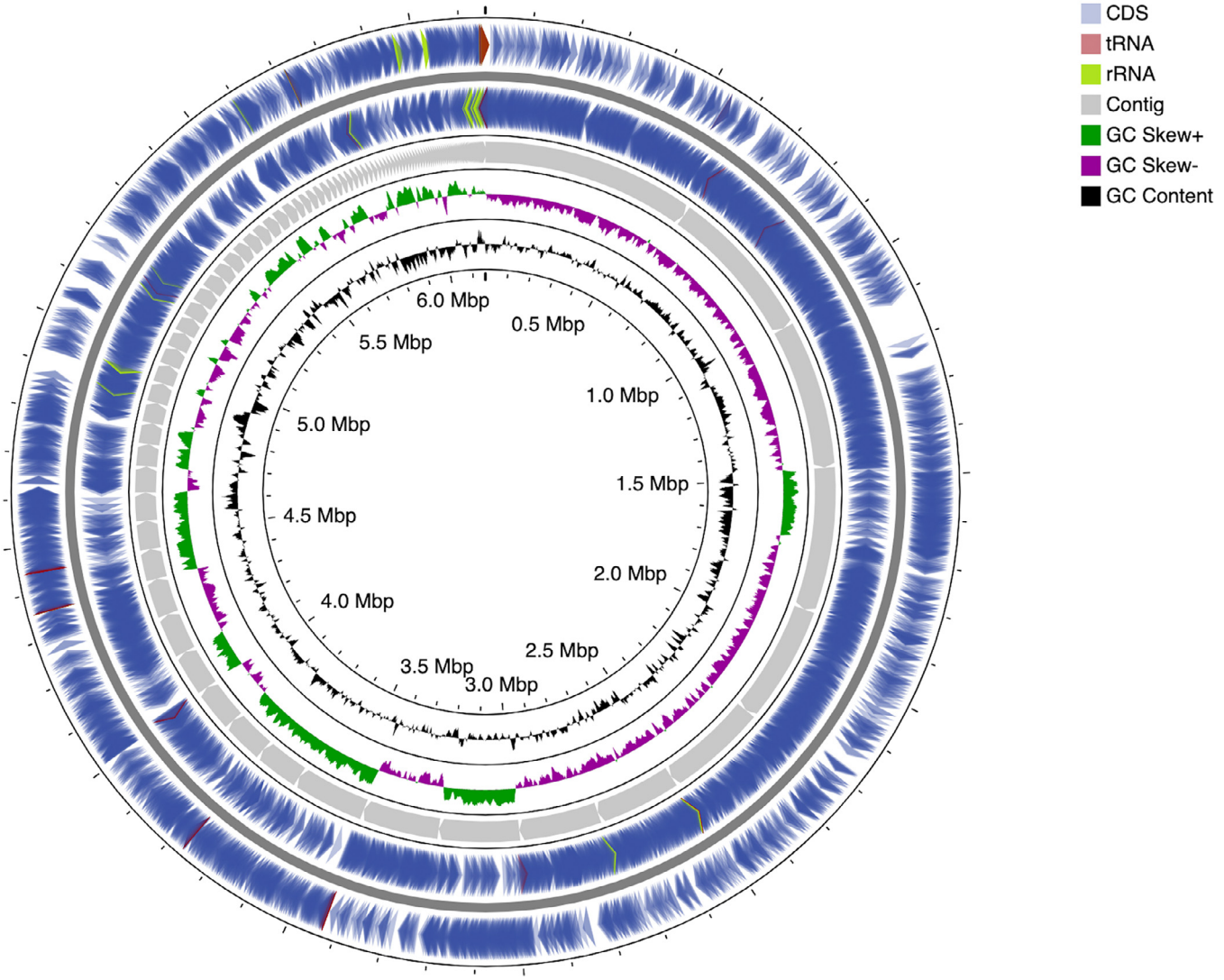

Fig. 1. Circular genome map of *Bacillus toyonensis* strain 4HC1 (GenBank accession no. **JADDKN000000000**). The map was created using CGView software (http://wishart.biology.ualberta.ca/cgview/) with default settings. The contigs are ordered according to size.

Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Extracted genomic DNA was quantified using NanoDrop and Qubit™ dsDNA BR Assay Kit (Thermo Scientific, Waltham, MA, USA) and was sent for sequencing to the Norwegian Sequencing Centre (Oslo University Hospital, Ullevål, Oslo, Norway) at 4°C. Sequencing libraries were prepared using a SWIFT 2S Turbo DNA Library Kit (Swift Biosciences, Ann Arbor, MI, USA). Sequencing was performed using an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) with 2 × 300 bp chemistry. Adapters were removed from the raw reads, followed by quality trimming using BBDuk v.38.75. Sequences were assembled using SPAdes v.3.13.0 [4]. The National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) was used for genome annotation. Antibiotic resistance genes (ARGs) were screened using ResFinder v.3.2 and CARD v.3.0.7 databases. Virulence genes were identified using VFanalyzer and Virulence Factor Database (VFDB). PlasmidFinder v.2.0 was used for identification of plasmids.

3. Results and discussion

The draft genome of strain 4HC1 comprises 133 contigs (>500 bp) with a total sequence length of 6 156 259 bp (largest contig, 609 862 bp) and a GC content of 34.95%. The genome contains 6089 protein-coding genes, 86 tRNAs and 24 rRNAs. Sequencing coverage was approximately 121-fold and the N_{50} value was 232 291 bp. The circular genome map of this strain is presented in Fig. 1.

Strain 4HC1 was identified as *Bacillus cereus* by Bruker MALDI Biotyper. The average nucleotide identity based on BLAST (ANIb), calculated using JSpeciesWS (http://jspecies.ribohost.com/jspeciesws/), confirmed that this strain belonged to *B. toyonensis* (ANIb value, 98.3%) and not *B. cereus* (ANIb value, 90.6%) using cut-off values >95% as suggested by Chun and Rainey [5]. MICs suggested that strain 4HC1 was susceptible to tetracycline (0.25 μg/mL), ciprofloxacin (0.125 μg/mL), meropenem (0.064 μg/mL), streptomycin (1 μg/mL), gentamicin (0.38 μg/mL), imipenem (0.094 μg/mL), chloramphenicol (1.5 μg/mL) and vancomycin (<1 μg/mL) but was resistant to cefotaxime (>32 μg/mL), trimethoprim (>32 μg/mL) and ampicillin (>256 μg/mL).

The strain carries several ARGs, including two class A β-lactamases (*bla* and *bla1*) and a BcII family subclass B1 metallo-β-lactamase (*bla2*) sharing 100% homology (amino acid) with ARGs detected in *B. toyonensis* type strain BCT-7112^T (Supplementary Table 1). These β-lactamases have also been detected in other *Bacillus* spp. belonging to the *B. cereus* group [6]. We further identified aminoglycoside 6-adenylyltransferase, tetracycline resistance

gene *tet*(M), two different tetracycline efflux pumps, bacitracin resistance undecaprenyl-diphosphatase and two bleomycin resistance genes. The strain also carried two different copies of dihydrofolate reductases, thus explaining its resistance to trimethoprim. Strain 4HC1 showed the presence of two rep3 plasmid replicons with 86.7% and 81.2% nucleotide identity, respectively, to the known replicons.

Virulence genes involved in immune evasion, iron acquisition, regulation and toxins were identified in strain 4HC1 (Supplementary Table 2). VFanalyzer suggested the presence of the *pagA* gene, which is one of three genes required for the formation of the anthrax toxin found in *Bacillus anthracis* [7]. On further analysis, this putative PagA protein (identified as hypothetical protein, locus tag-INP75_25825) was 46.2% identical to PagA from *B. anthracis* (accession no. **QHD25216**). The PagA protein and the putative PagA protein from strain 4HC1 both shared the same four functional domains (pfam17475, pfam17476, pfam03495 and pfam07691), suggesting that the protein present in strain 4HC1 may represent a potentially new variant of the PagA toxin. This putative *pagA* gene was located on contig 26 between positions 51 431–53 905 bp and was flanked by another hypothetical protein (locus tag-INP75_25820) that has a functional domain for *N*-terminal protective antigen-binding domain of anthrax toxin lethal factor and edema factor and a recombinase family protein. The two hypothetical proteins were flanked by a truncated IS*4* family transposase and a truncated IS*6* family transposase on one side and a recombinase family protein on the other, suggesting that these are a part of a transposon and thus may be mobile. Comparative genome analysis showed that this putative *pagA* gene was absent in the whole-genome sequencing of *B. toyonensis* type strain BCT-7112^T (accession no. **CP006863**). Although virulence genes were detected in this study, their expression is not guaranteed. Further studies are therefore needed to confirm the functionality of these genes.

4. Conclusion

Here we report the draft genome sequence of multidrug-resistant *B. toyonensis* strain 4HC1 isolated from marine plastic collected from Norway. We show the presence of several ARGs as well as some clinically important virulence genes. This genome may serve as a reference while studying the genome sequences of marine plastic-associated bacteria.

Nucleotide sequence accession no

The draft genome sequence of *B. toyonensis* 4HC1 has been deposited in DDBJ/ENA/GenBank under the accession no. **JADDKN000000000**.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.07.002.

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