Genome-based taxonomic reclassification of Acinetobacter species using type and reference strains

Masato Suzuki (✉ suzuki-m@nih.go.jp)  
National Institute of Infectious Diseases  
https://orcid.org/0000-0001-8975-2193

Shotaro Maehana  
Kitasato University

Hidero Kitasato  
Kitasato University

Short Report

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Abstract

*Acinetobacter* species are widely distributed in the environment and clinical settings worldwide and serve as natural reservoirs of antimicrobial resistance genes and occasional human pathogens responsible for nosocomial infections. In this study, we performed genomic analysis of *Acinetobacter seohaensis* DSM 16313, a type strain of the proposed *Acinetobacter* species. This species was estimated to be evolutionary close to *Acinetobacter towneri* but the genome sequence of *A. seohaensis* was not publicly available. Pangenome analysis of the genome sequence of *A. seohaensis* along with those of genome-available type and reference strains of 82 *Acinetobacter* species including *A. towneri* suggested that three groups of *Acinetobacter* species, *A. seohaensis* and *A. towneri*, *Acinetobacter pullorum* and *Acinetobacter portensis*; and *Acinetobacter idrijaensis*, *Acinetobacter mesopotamicus*, and *Acinetobacter lwoi*, were phylogenetically very similar to each other. Genome comparisons based on *in silico* DNA-DNA hybridization and the average nucleotide identity confirmed that these three groups of *Acinetobacter* species are conspecific. Based on the rules of priority, *A. seohaensis*, *A. pullorum*, and *A. idrijaensis/A. mesopotamicus* should be reclassified as later heterotypic synonyms of *A. towneri*, *A. portensis*, and *A. lwoi*, respectively.

Main Text

*Acinetobacter towneri*, belonging to the genus *Acinetobacter* whose members are gram-negative aerobic coccobacilli, is often isolated from water environments worldwide (1). This species has become increasingly important in recent years as a natural reservoir of antimicrobial resistance (AMR) genes (2–6). *Acinetobacter baumannii*, a cause of opportunistic infections in humans, has acquired resistance mechanisms to various antimicrobials, including clinically important carbapenems, making antimicrobial therapy difficult (7). AMR genes, such as those for carbapenem-hydrolyzing enzymes (carbapenemases), have spread among environmental and clinical *Acinetobacter* species via mobile gene elements such as plasmids (2–4). Tigecycline is a last-resort antimicrobial with promising activity against carbapenemase-producing gram-negative bacteria, including *Acinetobacter* species; however, mobile genes for tigecycline-inactivating enzymes, *tet*(X), have also emerged in *A. towneri* (5, 6) and *A. baumannii* (8–10). Accumulation of such clinically relevant AMR genes in environmental bacteria such as *A. towneri* and their transmission to human pathogenic bacteria such as *A. baumannii* poses a global public health threat.

As of July 1, 2021, the List of Prokaryotic names with Standing in Nomenclature (LSPN) listed 92 species of the genus *Acinetobacter* (https://lpsn.dsmz.de/genus/acinetobacter). Of these, *Acinetobacter venetianus* and *Acinetobacter refrigeratoris* (formerly *Acinetobacter refrigeratorenensis*) were listed in duplicate. Additionally, *Acinetobacter grimontii*, *Acinetobacter guangdongensis*, *Acinetobacter pakistanensis*, and *Acinetobacter dijkshoorniae* were later identified as different species (*Acinetobacter junii*, *Acinetobacter indicus*, *Acinetobacter bohemicus*, and *Acinetobacter lactucae*, respectively) (11–14); therefore, the LSPN lists 86 unique species of *Acinetobacter* (Table S1). Of these, 68 species were validly published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM), whereas the
remaining 18 species were not validly published (Table S1). To date, the genome sequences of type and reference strains of 79 species are available in the NCBI database, with all 68 species published in the IJSEM (68/68, 100%) and 11 species not validly published (11/18, 61.1%) (Table S1). Although not listed in the LSPN, one novel species of *Acinetobacter*, *Acinetobacter kanungonis*, has been validly published in the IJSEM (15), and two novel species of *Acinetobacter*, *Acinetobacter rongchengensis* and *Acinetobacter tianfuensis*, have been proposed from large-scale reanalysis on 3,956 genomes of *Acinetobacter* species in public databases and published in another journal (16) (Table S1).

During molecular epidemiological analysis of carbapenem-resistant *A. towneri* isolates from hospital sewage in Japan, we performed genomic analysis of *Acinetobacter seohaensis* DSM 16313, a proposed type strain whose genome sequence was estimated to be similar to that of *A. towneri* but the genome sequence of *A. seohaensis* was not publicly available (17). The 16S rRNA gene sequence of *A. seohaensis* DSM 16313 (accession no. AY633608) (17) was shown to be nearly identical (99.8%) to that of *A. towneri* DSM 14962T (type strain, accession no. EF611416). The Illumina sequencing library (paired-end, insert size 500–900 bp) was prepared using the Nextera XT DNA Library Prep Kit (Illumina). Whole-genome sequencing using the HiSeq X system (Illumina) was performed, followed by de novo assembly of Illumina reads using Shovill v1.1.0 (https://github.com/tseemann/shovill) with default parameters. The resulting draft genome sequence of *A. seohaensis* DSM 16313 (accession no. BPEQ00000000) consisted of 298 contigs with a genome size of 2,99 Mbp and GC content of 41.3%.

We further performed pangenome analysis of the draft genome sequence of *A. seohaensis* DSM 16313 along with those of genome-available type and reference strains of the aforementioned 82 species of *Acinetobacter*, including *A. towneri* DSM 14962T, using Roary v3.13.0 (https://github.com/sanger-pathogens/Roary) with the parameter of minimum percentage identity for blastp = 60%. Phylogenetic analysis with their core genome using RAxML v8.2.4 (https://github.com/stamatak/standard-RAxML) with default parameters suggested three groups of *Acinetobacter* species, *A. seohaensis* and *A. towneri*, *Acinetobacter pullorum* and *Acinetobacter portensis*, and *Acinetobacter idrijaensis*, *Acinetobacter mesopotamicus* and *Acinetobacter lwofii*, to be phylogenetically very similar to each other (Fig. 1). Of these, two pairs of *Acinetobacter* species, *A. pullorum* and *A. portensis* as well as *A. mesopotamicus* and *A. lwofii*, have been suggested to be phylogenetically identical to each other in journals other than the IJSEM published in May 2021 and January 2021, respectively (16, 18). We confirmed that the 16S rRNA gene sequences of *A. pullorum* B301 (proposed type strain, accession no. MN909715) and *A. portensis* 877T (type strain, accession no. KX870877) are nearly identical (99.7%), and that those of *A. idrijaensis* MII (proposed type strain, GS19_03400 in accession no. JQCU01000127, *A. mesopotamicus* GC2 (proposed type strain, accession no. KJ867435), and *A. lwofii* NCTC 5866T (type strain, accession no. AB626125) are nearly identical (99.7% for *A. idrijaensis* compared with *A. lwofii* and 99.7% for *A. mesopotamicus* compared with *A. lwofii*), respectively.

The results of in silico DNA–DNA hybridization (DDH) analysis using the Type Strain Genome Server (https://tygs.dsmz.de/) and average nucleotide identity (ANI) analysis using FastANI v1.3 (https://github.com/ParBLiSS/FastANI) confirmed that *A. seohaensis* DSM 16313 (accession no.
BPEQ00000000) and *A. towneri* DSM 14962\(^T\) (accession no. JHZH00000000) (17) are conspecific with 77.5% of DDH and 97.7% of ANI according to their proposed minimal standards (≥ 70% of DDH or ≥ 95% of ANI) (19) (Fig. 1). Moreover, our results confirmed that *A. pullorum* B301 (accession no. JAAARQ000000000) (20) and *A. portensis* AC 877\(^T\) (accession no. LWRV0000000000) (21) are conspecific with 82.6% of DDH and 98.6% of ANI, and that *A. idrijaensis* MII (accession no. JQCU00000000) (22), *A. mesopotamicus* GC2 (accession no. JAALFF0000000000) (23), and *A. Iwoffii* NCTC 5866\(^T\) (accession no. CAADHN0000000000) (24) are conspecific with 76.8% of DDH and 96.1% of ANI (*A. idrijaensis* compared with *A. Iwoffii*) and 68.5% of DDH and 96.0% of ANI (*A. mesopotamicus* compared with *A. Iwoffii*), respectively (Fig. 1). Thus, comparative genomic analysis demonstrated that the aforementioned three groups of *Acinetobacter* species are conspecific and suggested that genome-level comparisons are essential for proposing novel bacterial species among highly similar species.

The priority of prokaryotic names is governed by the International Code of Nomenclature of Prokaryotes (25). Rule 23a of the code states that, “In a given position, a species can bear only one correct epithet, that is, the earliest that is in accordance with the Rules of this Code”. Rules 23b, 24a, and 24b establish the priority of names based on their dates of valid publication in the IJSEM. In our case, *A. towneri* was validly published in the IJSEM in July 2003 (1) and *A. seohaensis* was published in another journal in November 2007 (17); *A. portensis* was validly published in the IJSEM in August 2020 (21) and *A. pullorum* was published in another journal in April 2020 (20); *A. Iwoffii* was validly published in the International Journal of Systematic and Evolutionary Bacteriology (predecessor journal of the IJSEM) in April 1986 (24), and *A. idrijaensis* and *A. mesopotamicus* were published in other journals in November 2014 and October 2020, respectively (22, 23). Based on the rules of priority, *A. seohaensis*, *A. pullorum*, and *A. idrijaensis/A. mesopotamicus* are later heterotypic synonyms of *A. towneri*, *A. portensis*, and *A. Iwoffii*, respectively.

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Competing Interests
The authors declare no competing interests.

Figures
Figure 1

Core genome phylogeny of publicly available genomes of type and reference strains of 83 species of Acinetobacter. Bar lengths represent the number of substitutions per site in the core genome. Names of type and reference strains of Acinetobacter species, accession nos. of the genome sequences, years when each strain was validly published in the International Journal of Systematic and Evolutionary Microbiology (or years when each strain was published in another journal), and average nucleotide
identity (ANI) values to reference genomes of A. seohaensis DSM 16313, A. pullorum B301, A. idrijaensis M11, and A. mesopotamicus GC2, respectively, are shown. Three groups of Acinetobacter species, A. seohaensis and A. towneri; Acinetobacter pullorum and Acinetobacter portensis; and Acinetobacter idrijaensis, Acinetobacter mesopotamicus, and Acinetobacter Iwoffii are highlighted in red, blue, and green, respectively.

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