Acinetobacter guangdongensis Feng et al. 2014 is a junior heterotypic synonym of Acinetobacter indicus Malhotra et al. 2012

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Abstract
A draft whole-genome sequence was obtained for Acinetobacter guangdongensis strain KCTC 42012T and compared against those of the type strains of all Acinetobacter species with validly published names. High similarity was found to Acinetobacter indicus CCM 7832T (average nucleotide identity based on BLAST and digital DNA–DNA hybridization values of 96.3 and 70.4 %, respectively). In addition, the metabolic, physiological and chemotaxonomic features of KCTC 42012T were shown to be congruent with those of A. indicus. We conclude that Acinetobacter guangdongensis Feng et al. 2014 is a later heterotypic synonym of Acinetobacter indicus Malhotra et al. 2012.

In July 2017, the formal classification of the genus Acinetobacter included 56 validly published species names (http://apps.szu.cz/anemec/Classification.pdf). The majority of these names were proposed on the basis of polyphasic characterization of multiple strains per species using genotypic and phenotypic methods that were validated by comprehensive sets of reference strains. Several names, however, were based on studies of single strains characterized by a limited number of taxonomically relevant tests and some of them later appeared to be synonymous with other validly published names. This is the case with Acinetobacter grimonitii and Acinetobacter pakanistanensis, which were shown to be junior synonyms of Acinetobacter junii [1] and Acinetobacter bohemicus [2], respectively. Another possible pair of synonyms encompasses the recently validly published names Acinetobacter dijkshoorniae and Acinetobacter lactucae, as indicated by high similarity of the whole-genome sequences of the respective type strains (A. Nemec, unpublished). In the present study, we provide evidence for another case of synonymy of validly published names, showing that Acinetobacter guangdongensis proposed by Feng et al. [3] for a single environmental isolate is a later heterotypic synonym of Acinetobacter indicus Malhotra et al. 2012 [4].

A. guangdongensis strain KCTC 42012T (derived from the original isolate 1NM-4T [3]) was purchased from the Korean Type Culture Collection (KCTC). The phenotypic features of KCTC 42012T obtained in our laboratory agreed with those reported by Feng et al. [3]. However, the 861 nt rpoB sequence determined anew by us for KCTC 42012T (DDBJ/ENA/GenBank accession no. KR611818.1) differed from that published originally for 1NM-4T (accession no. KJ701021.1). To clarify this discrepancy, we tried to obtain the culture of 1NM-4T directly from the authors of the nomenclatural proposal. As this attempt was unsuccessful, we discussed the problem with the scientific staff of KCTC. Based on this communication and the results of our analyses (see below), we concluded that KCTC 42012T was the authentic organism described by Feng et al. [3] while there was a problem with the quality of the originally published rpoB sequence.

The raw sequencing data for the genome of A. guangdongensis KCTC 42012T were generated on an Illumina MiSeq platform in the Genomics Core Facility (EMBL) and assembled de novo using the Geneious 9.1.4 software (Biомatters). The resulting genome sequence (accession no. NEXW00000000.1; size: 3 012 884 Mb; number of contigs: 33; and DNA G+C content: 45.6 mol%) was compared with those of the type strains of all Acinetobacter species with validly published names. The list of these sequences published by Nemec et al. [5] was completed with those of Acinetobacter populi PBJ7T (accession no. NEXX00000000.1; this
study) and Acinetobacter larvae BRTC-1T (accession no. CP016895.1). The average nucleotide identity based on BLAST (ANiB) and digital DNA–DNA hybridization (dDDH) parameters were calculated, respectively, using the JSpecies (www.imedea.uib.es/jspecies) and GGDC 2.1 (http://ggdc.dsmz.de) programs, with the recommended parameters and/or default settings. The highest ANiB (96.3 %) and dDDH (70.4 %) values were found for the genome of A. indicus DSM 4215T (derived from CCM 7832T; accession no. ATGH00000000.1, DNA G+C content: 45.4 mol%). Similar values (ANiB of 96.2 % and dDDH of 69.7 %) were obtained for the genomes of A. guangdongensis KCTC 42012T and A. indicus CIP 53.82 (accession no. APRK0000000.1). In light of the recommended threshold values for species circumscription (ANiB of 95–96 % [6] and dDDH of 70 % [7]), these data indicate the genomic congruence of A. guangdongensis KCTC 42012T with A. indicus at the species level of resolution. Notably, the DNA–DNA relatedness value found by Feng et al. for A. guangdongensis 1NM-4T and A. indicus DSM 25388T was only 53.7 % [3]. However, significant differences between the outcomes of dDDH and conventional DDH may occur [8], possibly stemming from the known high experimental error and limited inter-laboratory reproducibility of the latter approach [1].

The availability of whole-genome sequences also allowed us to unravel the problem of the 861 nt rpoB sequence of A. guangdongensis 1NM-4T reported by Feng et al. [3]. The comparison of this sequence with that derived from the whole-genome sequence of A. guangdongensis KCTC 42012T revealed that these sequences were completely identical in the 5′ region (nucleotide positions 1–376), whereas their 3′ regions (positions 377–861) shared only 88.2 % identical bases. Genus-wide comparative analysis showed that while the 5′ region of A. guangdongensis 1NM-4T corresponded to those of A. indicus strains ANC 4215T and CIP 53.82 (identity of 97.9 and 98.9 %, respectively), its 3′ region was congruent with the rpoB sequences of Acinetobacter variabilis (identity of 96.9–100 %) published by Krizova et al. [9]. Important in this context is that the authors of the A. guangdongensis paper proposed, in the same year, the name ‘Acinetobacter refrigeratoris’ for another single strain (KCTC 42011). The whole-genome sequence of KCTC 42011 (=ANC 5078) is now available under JGI project ID no. 1102394 from the JGI Genome Portal website (http://genome.jgi.doe.gov). The comparison of the 861 nt rpoB sequence of A. guangdongensis 1NM-4T with that derived from the genome sequence of ‘Acinetobacter refrigeratoris’ KCTC 42011 showed their complete identity in the 3′ region but only 87.8 % identity of their 5′ regions. Consistent with these findings is the ANiB value of 96.03 % found for the genome sequences of A. refrigeratoris KCTC 42011 and A. variabilis ANC 2171T (accession no. APRS0000000.1), which indicates that the former strain belongs to A. variabilis. Based on these data and the fact that the two regions of the original rpoB sequence of 1NM-4T were obtained using independent sequencing reactions [3], it is very likely that the chimeric nature of this rpoB sequence is a result of a laboratory error (i.e. the confusion of the 3′ end sequence of 1NM-4T with that of KCTC 42011).

The metabolic and physiological features of A. guangdongensis KCTC 42012T were assessed using a genus-targeted set of 43 in-house, strictly standardized, mostly carbon-source assimilation tests as described previously [10]. KCTC 42012T grew well in brain–heart infusion broth (Oxoid) at up to 41 °C, whereas its growth at 44 °C was weak. The strain did not produce acid from D-glucose, haemolysis on sheep blood agar or gelatinase. It grew well within 4 days of culture in a defined mineral medium containing the following substrates as single carbon and energy sources: acetate, benzoate, ethanol, L-glutamate, glutarate, DL-lactate, D-malate and phenylacetate. No growth was seen in 10 days of culture on trans-aconitate, adipate, β-alanine, 4-amino-butyrate, L-arabinose, L-arginine, L-aspartate, azelate, 2,3-butanediol, citraconate, citrate (Simmons), gentisate, D-glucuronate, D-glucose, histamine, L-histidine, 4-hydroxybenzoate, L-leucine, levulinate, malonate, L-ornithine, L-phenylalanine, putrescine, D-ribose, L-tartrate, tricarballylate, trigonelline and tryptamine. These data are congruent with the properties of A. indicus (strains CCM 7832T and CIP 53.82) as published by Krizova et al. [11] except for the ability of KCTC 42012T to grow on glutarate and D-malate.

The data published for A. guangdongensis KCTC 42012T [3] and A. indicus CCM 7832T [4] also showed their high similarity in chemotaxonomic characteristics. The major fatty acids profile of both strains included C18:1ω9c, summed feature 3, C16:0 and C12:0, while their major polyamine was 1,3-diaminopropane, and the predominant polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. This further supports the overall resemblance of the two strains even though the genus-wide assessment of the capacity of chemotaxonomic markers to differentiate between Acinetobacter species is still lacking.

Overall, all the aforementioned data indicate that the organisms named A. guangdongensis and A. indicus should not be considered as separate species. We conclude that the original proposal for A. guangdongensis was based on incorrect genotypic data and that this name is a junior heterotypic synonym of Acinetobacter indicus Mal hotra et al. 2012.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The authors declare that the article does not proclaim any work which requires approval by an ethics committee.
References


