Acinetobacter pakistanensis Abbas et al. 2014 is a later heterotypic synonym of Acinetobacter bohemicus Krizova et al. 2014

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Two novel species names, Acinetobacter bohemicus and Acinetobacter pakistanensis, appeared on validation list no. 161 (January 2015) under priority numbers 26 and 28, respectively. As the published data suggested a high similarity of the organisms associated with these names, we aimed to define their taxonomic relationship. The study set included all strains used in the original nomenclatural proposals, i.e. 25 strains of A. bohemicus and one strain of A. pakistanensis. The average nucleotide identity values (95.9 and 96.1 % based on BLAST and MUMmer, respectively) between the whole-genome sequences of A. bohemicus ANC 3994T and A. pakistanensis KCTC 42081T supported the identity of these type strains at the species level. Based on the genus-wide comparative analyses of the rpoB sequences and whole-cell fingerprints generated by matrix-assisted laser desorption/ionization-time-of-flight MS, A. pakistanensis KCTC 42081T fell within the respective clusters formed by the 25 A. bohemicus strains. The same picture was obtained on the basis of comparative analysis of 16S rRNA gene sequences of KCTC 42081T and three A. bohemicus strains. Finally, the metabolic and physiological features of KCTC 42081T were found to be congruent with those of A. bohemicus. Based on these results, we conclude that Acinetobacter pakistanensis is a later heterotypic synonym of Acinetobacter bohemicus.

Validation list no. 161 (Oren & Garrity, 2015) included the names of two novel species of the genus Acinetobacter, Acinetobacter bohemicus and Acinetobacter pakistanensis. The former name was published in the journal Systematic and Applied Microbiology for a phylo-phenetically distinct group of 25 strains isolated from soil and water samples collected in diverse natural ecosystems in the Czech Republic (Krizova et al., 2014). The latter name was proposed afterwards by Abbas et al. (2014) in the Pakistan Journal of Agricultural Sciences for a single strain recovered from a wastewater treatment pond. Comparison of the published sequences of the rpoB and 16S rRNA genes suggested that the organisms for which these two names were proposed were related at the species level and, therefore, that the two names were synonymous. The aim of this study was to define the taxonomic relationship of the organisms named A. bohemicus and A. pakistanensis.

A. pakistanensis strain KCTC 42081T (derived from the original NCCP-644T) was obtained from the Korean Type Culture Collection and deposited in the collection of the Laboratory of Bacterial Genetics under no. ANC 5076T. The properties of this strain were compared with those of the 25 strains of A. bohemicus included in the original nomenclatural proposal of Krizova et al. (2014) using the genus-targeted polyphasic approach applied in our previous nomenclatural studies on the genus Acinetobacter (e.g. Radolfova-Krizova et al., 2016).

The draft whole-genome sequence of the A. pakistanensis KCTC 42081T was determined at the US Department of Energy Joint Genome Institute (JGI) within the Genomic Encyclopedia of Bacteria and Archaea project (Whitman et al., 2015) and is available from the JGI Genome Portal website (http://genome.jgi.doe.gov; JGI project id. 1102390) or under DDBJ/ENA/GenBank accession no. FOZU00000000.1 (size: 3 728 357 bp; number of contigs: 104; DNA G+C content: 39.2 %). The comparison of the sequences of the 16S rRNA, gyrB and rpoB genes (AB916465, AB924048 and AB938199, respectively) of A. pakistanensis NCCP-644T published by Abbas et al. (2014) against the genome sequence of KCTC 42081T revealed complete identity in all cases, which confirms the authenticity of the strain used in the present study.
Later synonym of Acinetobacter bohemicus

Fig. 1. Results of the clustering of the (a) partial nucleotide sequences of the rpoB gene and (b) MALDI-TOF mass spectra of 25 strains of A. bohemicus (Krizova et al., 2014), the A. pakistanensis strain (Abbas et al., 2014), the type strains of all species of the genus Acinetobacter with validly published names and reference strains of some provisional taxa. Analyses of the rpoB sequences were carried out for nucleotide positions 2915–3775 of the coding region of the gene using the BioNumerics 7.5 software (Applied-Maths). Evolutionary distances were computed using Kimura’s two-parameter model, while the tree was reconstructed using the neighbour-joining algorithm with the sequence of Pseudomonas aeruginosa PAO1 (DDBJ/ENA/GenBank accession no. NC002516) as the outgroup. Bootstrap values (>75 %) after 1000 resamplings are indicated at branch points.

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nodes; bar, 5 % of change per nucleotide site. The MALDI-TOF MS analysis was carried out by using the Biotyper MSP Dendrogram Creation Standard Method (Bruker Daltonics) with the correlation distance measure and average linkage algorithm (UPGMA).

study. The comparison of the genome sequence of KCTC 42081\textsuperscript{T} with that of \textit{A. bohemicus} ANC 3994\textsuperscript{T} (DDBJ/ENA/GenBank accession no. APOH00000000; Touchon et al., 2014) was based on average nucleotide identity parameters calculated by using the JSpecies web program (http://www.imedea.uib.es/jspecies; Richter & Rosselló-Móra, 2009) with default settings. The average nucleotide identity values based on BLAST (ANiB) and MUMmer (ANIm) were 95.92 and 96.08 \%, respectively. These values are higher than the 95 \% threshold proposed to distinguish between bacterial species (Richter & Rosselló-Móra, 2009) and, therefore, support the identity of the two type strains at the species level.

To gauge the taxonomic position of the \textit{A. pakistanensis} strain in relation to the 25 strains of \textit{A. bohemicus}, we performed a genus-wide comparative analyses based on the two most widely used markers in the current taxonomy of the genus \textit{Acinetobacter}, a partial sequence of the \textit{rpoB} gene and a whole-cell pattern obtained by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) MS. These analyses were performed according to the methods of Nemec et al. (2009) and Radolfova-Krizova et al. (2016), respectively. As shown in Fig. 1, the \textit{A. pakistanensis} strain fell within a distinct and internally cohesive cluster formed by the 25 \textit{A. bohemicus} strains based on the analysis of each of these markers. The \textit{rpoB} sequence of the \textit{A. pakistanensis} strain showed identities of 98.4–99.1 \% with those of the 25 \textit{A. bohemicus} strains, which are values typically found for members of the same species of the genus \textit{Acinetobacter} (Krizova et al., 2015). Furthermore, the 16S rRNA gene sequences were compared for \textit{A. pakistanensis} KCTC 42081\textsuperscript{T} and three \textit{A. bohemicus} strains (ANC 3994\textsuperscript{T}, ANC 4253 and ANC 4278). As shown in Fig. S1 (available in the online Supplementary Material), these four sequences, again, formed a strongly supported and tight cluster (intra-cluster identities of 99.7 \%) within the context of the whole genus. Thus, the analyses of three independent molecular markers indicated the taxonomic congruence of the \textit{A. bohemicus} population and the \textit{A. pakistanensis} strain at the species level of resolution.

The metabolic and physiological features of the \textit{A. pakistanensis} KCTC 42081\textsuperscript{T} were determined using a genus-targeted set of 43 in-house, strictly standardized, mostly carbon-source assimilation tests as described previously (Nemec et al., 2009). KCTC 42081\textsuperscript{T} grew in brain-heart infusion broth (Oxoid) at 30 °C but not at 35 °C. It neither produced acid from D-glucose nor hydrolysed gelatin. The strain assimilated acetate, 4-aminobutyrate, L-arginine, L-aspartate, benzoate, 2,3-butanediol, ethanol, L-glutamate, 4-hydroxybenzoate, DL-lactate and malonate, with growth visible in 4 days of culture, but showed no growth on trans-aconitate, adipate, \(\beta\)-alanine, L-arabinose, azelate, citraconate, citrate (Simmons), gentisate, D-gluconate, D-glucose, glutarate, histamine, L-histidine, D-malate, phenylacetate, L-phenylalanine, L-leucine, levulinate, L-ornithine, putrescine, D-ribose, L-tartrate, tricarballylate, trigonelline or tryptamine in 10 days. All tests were performed at 25 and 30 °C with the same outcomes. These results are congruent with the description of \textit{A. bohemicus} (Krizova et al., 2014) except for the inability of KCTC 42081\textsuperscript{T} to grow on L-histidine.

Even though Abbas et al. (2014) listed a number of additional phenotypic properties of the \textit{A. pakistanensis} strain, most of them have limited or unknown taxonomic relevance in terms of their ability to discriminate between species of the genus \textit{Acinetobacter} and these have, therefore, not been addressed in the present study. Nevertheless, some of the few basic tests included in both studies yielded different results. First, Abbas et al. (2014) reported that the \textit{A. pakistanensis} strain grew at temperatures of up to 37 °C. However, as stated above, KCTC 42081\textsuperscript{T} showed no growth at 35 °C in brain-heart infusion broth, and this was confirmed by its culture on Columbia agar supplemented with sheep blood (bioMérieux). Moreover, contrary to the data of Abbas et al. (2014), KCTC 42081\textsuperscript{T} yielded negative results for nitrate reduction and Voges–Proskauer tests, using the API 20 NE and API 20 E diagnostic systems (bioMérieux), respectively. Notably, the former test is mostly negative in members of the genus \textit{Acinetobacter} while the latter one is based on the detection of acetoin, a product of fermentative metabolism which is absent in \textit{Acinetobacter}.

Our final remark refers to the fact that even though Abbas et al. (2014) cited the paper describing \textit{A. bohemicus}, they did not include this species in their analyses arguing that the name \textit{A. bohemicus} was not validly published at that time. This argument is rather unconvincing, given that the authors did study a strain representing ‘\textit{Acinetobacter kyonggiensis}’, which was not a validly published name either. It is our belief that any nomenclatural proposal should be the very last step in comprehensive, context-wide taxonomic analysis in which all publicly available data as well as biological material are taken into account. This is a prerequisite for reaching a meaningful and understandable formal classification of bacteria.

In conclusion, all our results consistently indicate that there is no taxonomic support for considering the organisms named \textit{A. bohemicus} and \textit{A. pakistanensis} as separate species. As these names appeared on the same validation list (Oren & Garrity, 2015) under respective priority numbers 26 and 28, we propose \textit{Acinetobacter pakistanensis} Abbas et al. (2014) as a later heterotypic synonym of \textit{Acinetobacter bohemicus} Krizova et al. (2014).
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