Reclassification of *Achromobacter spiritinus* Vandamme *et al.* 2013 as a later heterotypic synonym of *Achromobacter marplatensis* Gomila *et al.* 2011

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A repeat multi-locus sequence analysis (MLSA) of concatenated *nusA, eno, rpoB, gltB, lepA, nuoL* and *nrdA* sequences of strains classified as *Achromobacter marplatensis* was performed. The results revealed that earlier reported sequence data of the proposed type strain were erroneous, and that the corrected concatenated sequence divergence between the *A. marplatensis* LMG 26219T (=CCUG 56371T) sequence type and that of strains of *Achromobacter spiritinus* was well below the 2.1 % threshold value that delineates species of the genus *Achromobacter*. These results therefore demonstrated that strains which were classified as *A. spiritinus* should be reclassified as *A. marplatensis* and that the name *Achromobacter spiritinus* should no longer be used. An emendation of the description of *Achromobacter marplatensis* is warranted.

Multi-locus sequence analysis (MLSA) of concatenated *nusA, eno, rpoB, gltB, lepA, nuoL* and *nrdA* sequences revealed the presence of fourteen unnamed genogroups in the genus *Achromobacter* in a collection of approximately 150 human respiratory isolates (Spilker *et al.*, 2012). In subsequent polyphasic taxonomic studies, the use of 2.1 % concatenated sequence divergence as a threshold value for species delineation in this genus was validated and eight of the genogroups were described and validly named as novel species of the genus *Achromobacter* (Vandamme *et al.*, 2013a, b). The name *Achromobacter spiritinus* was proposed for three isolates obtained from samples of patients in the USA (Vandamme *et al.*, 2013a). However, more recently Gomila *et al.* (2014) performed a study based on a comparative MLSA of four housekeeping genes (*atpD, gyrB, recA* and *rpoB*), revealing discrepancies between the type strains of *A. spiritinus* and *Achromobacter marplatensis*. DNA–DNA hybridization values greater than 80 % between the type strains of *A. spiritinus* and *A. marplatensis* confirmed that the strains represented the same species. The latter organism was first isolated from soil in Argentina (Gomila *et al.*, 2011), but was later also identified among human respiratory isolates (Spilker *et al.*, 2012). The DNA–DNA hybridization results therefore confirmed that *A. spiritinus* should be considered a later heterotypic synonym of *A. marplatensis*. In the present study, we aimed to clarify these contradictory findings.

Preliminary MLSA experiments (data not shown) revealed results for the type strain of *A. marplatensis* were different from those reported originally (Spilker *et al.*, 2012). We therefore repeated the MLSA analysis of *A. marplatensis* LMG 26219T, LMG 26681 and LMG 26682 (Spilker *et al.*, 2012), and performed MLSA on three additional isolates that also were identified as *A. marplatensis*, i.e. strain LMG 3439 (isolated in France from a disinfectant before 1966), strain R-14663 (isolated from sputum of a cystic fibrosis patient in Italy in 2001).

Abbreviation: MLSA, multi-locus sequence analysis.
and strain R-20635 (isolated from sputum of a cystic fibrosis patient in Belgium in 2003). DNA preparation, amplification and sequencing of nusA, eno, rpoB, gltB, lepA, nuoL and nrdA gene fragments was performed as described previously (Spilker et al., 2012). Gene number assignments to each unique allele and assignments of sequence types to each unique allelic profile were done using the http://pubmlst.org/ database tools (Jolley & Maiden, 2010). All sequences and related data are available in the Achromobacter pubMLST database (pubmlst.org/achromobacter).

MLSA results for A. marplatensis LMG 26681 and LMG 26682 confirmed the multi-locus sequence typing (MLST) data reported earlier; i.e., strain LMG 26681 represented sequence type ST-22, while strain LMG 26682 represented ST-191. The novel isolates R-14663 and R-20635 also belonged to ST-22, which now includes isolates from Sweden, Italy and Belgium, and strain LMG 3439 represented a novel sequence type, ST-209. However, the data reported for A. marplatensis LMG 26219T differed from those reported earlier. In the present analysis, A. marplatensis LMG 26219T represented a novel sequence type, ST-208. The mean concatenated nusA, eno, rpoB, gltB, lepA, nuoL and nrdA sequence divergence between ST-208 and A. spiritinus ST-124 (LMG 26692T), A. spiritinus ST-126 (LMG 26693) and A. spiritinus ST-125 (LMG 26694) was 0.8 %, which was well below the 2.1 % threshold value that delineates species of the genus Achromobacter (Vandamme et al., 2013a, b) (Fig. 1). These results demonstrated that strains currently classified as A. spiritinus and A. marplatensis LMG

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**Fig. 1.** Phylogenetic tree based on concatenated sequences (2249 bp) of seven housekeeping gene fragments [nusA (355 bp), rpoB (413 bp), eno (214 bp), gltB (241 bp), lepA (347 bp), nuoL (230 bp) and nrdA (449 bp)] of type strains of members of the genus Achromobacter and reference strains of A. marplatensis. All sequences are available at http://pubmlst.org/achromobacter. Evolutionary history was inferred by using the maximum-likelihood method based on the General Time Reversible model (Nei & Kumar, 2000) in MEGA6 software package (Tamura et al., 2013). The tree with the highest log-likelihood (−7580.5595) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter=0.1720)] and allowed for some sites to be evolutionarily invariable [(+I), 41.3799 % sites]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches if >50 %. Bar, 0.01 substitutions per site.
26219T (ST-208) indeed represent the same species and therefore confirmed the MLSA and DNA–DNA hybridization data reported by Gomila et al. (2014). A formal reclassification of strains of A. spiritinus as A. marplatensis is warranted since the latter name has nomenclatural priority (Lapage et al., 1992). Consequently, the name A. spiritinus should no longer be used and the species description of A. marplatensis should be amended. We previously reported that the distinctness of population ratio (k) parameter between Achromobacter xylosoxidans and the cluster, first but erroneously, identified as A. marplatensis was only 1.68 and therefore these could not be considered separate sequence similarity clusters (Spilker et al., 2012). Consequently we consider the remaining strains which were previously classified as A. marplatensis on the basis of their concatenated sequence divergence towards the erroneous data for the type strain of A. marplatensis [LMG 26681 and LMG 26682 (Vandamme et al., 2013a)] and the isolates LMG 3439, R-14663 and R-20635, as strains of A. xylosoxidans. An emended species description of A. marplatensis is provided based on chemotaxonomic and phenotypic data presented previously (Vandamme et al., 2013a).

**Emended description of Achromobacter marplatensis Gomila et al. 2011**

Achromobacter marplatensis (mar.pla.ten’sis. N.L. masc. adj. marplatensis pertaining to Mar del Plata, the Argentinian city where the type strain was isolated).

Cells are Gram-stain-negative, motile small bacilli (approximatively 0.4–1.1 µm wide and 1.4–2.8 µm long) with rounded ends, and occur as single units or in pairs. After incubation for 48 h on trypticase soy agar at 28 °C, colonies are slightly convex, translucent and non-pigmented with smooth margins and are 1–2 mm in diameter.

Biochemical characteristics are as described previously for all members of the genus Achromobacter (Vandamme et al., 2013a, b). In addition, grows in the presence of 3 % NaCl; reduces nitrate; grows anaerobically with KNO₃ and N₂O production; assimilates D-glucuronate, caprate (classical test), adipate (classical test), L-malate, citrate, phenylacetate, D-lactate and lactate + methionine; exhibits oxidase, C₄-lipase and acid phosphatase activity; and oxidizes the Biolog GEN III substrates L-alanine, L-histidine, L-prolylglutamic acid, d-saccharic acid, l-lactic acid, ß-ketoglutaric acid, d-malic acid, ß-hydroxy-DL-butyric acid and acetic acid. In Biolog GEN III sensitivity tests, oxidation is not inhibited by fusidic acid, D-serine, troleandomycin, niaproof 4, tetrazolium blue, nalidixic acid or sodium butyrate.

Does not grow in O/F medium with D-xylene, on acetamide or in the presence of 6 % NaCl. Negative result in tests for denitrification, liquefaction of gelatin, assimilation of glucose, maltose, D-arabinose and D-mannose, acid production from glucose, and valine arylamidase activity.

Does not oxidize the following Biolog GEN III substrates: α-D-glucose, D-mannose, glycol L-proline, muric acid, γ-amino butyric acid and α-hydroxybutyric acid. In Biolog GEN III sensitivity tests, oxidation is inhibited by 8 % NaCl and sodium bromate. The following activities are strain dependent: growth at 42 °C, on cetrimide agar and in the presence of 4.5 % NaCl; nitrite reduction; assimilation of DL-norleucine, caprate (API 20NE) and adipate (API 20NE); activity of alkaline phosphatase and phosphomidas; oxidation of the Biolog GEN III substrates L-serine, D-gluconic acid, methyl pyruvate, bromosuccinic acid, ß-ketobutyric acid and propionic acid; and inhibition of oxidation by potassium tellurite and minocycline in Biolog GEN III sensitivity tests. Major cellular fatty acids (25–34 %) are C₁₆:0 and summed feature 3 (most likely C₁₆:1ω7c). C₁₈:1ω7c, cyclo-C₁₇:0 and summed feature 2 (most likely C₁₄:0 3-OH) are present in moderate amounts (9–15 %), while C₁₂:0 2-OH and C₁₄:0 are present in minor amounts (3–5 %).

The type strain is LMG 26219ᵀ (=CCUG 56371ᵀ) and was isolated in 2001 from soil in Argentina (Gomila et al., 2014). The DNA G+C content of the type strain is 65.4 mol%. Additional strains were isolated from human respiratory samples in the USA (Vandamme et al., 2013a), Spain, the UK and Belgium (http://pubmlst.org), and from a soil sample in Belgium (unpublished data).

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