Kerstersia similis sp. nov., isolated from human clinical samples

Peter Vandamme,1 Evie De Brandt,1 Kurt Houf2 and Thierry De Baere3

1Laboratorium voor Microbiologie, Vakgroep Biochemie en Microbiologie, Faculteit Wetenschappen, Universiteit Gent, Gent, Belgium
2Vakgroep Veterinaire Volksgezondheid en Voedselveiligheid, Faculteit Diergeneeskunde, Universiteit Gent, Gent, Belgium
3Laboratorium Moleculaire Diagnostiek Ardolab, H.-Hartziekenhuis Roeselare-Menen VZW, Roeselare, Belgium

Analysis of gyrB gene sequences, (GTG)5-primed PCR fingerprinting and biochemical characteristics determined in the Biolog GEN III microtest system were used to differentiate an unnamed Kerstersia species from Kerstersia gyiorum, the type and only named species in this genus. The inability to oxidize D-galacturonic and D-glucuronic acids and the ability to oxidize D-serine, along with gyrB gene sequence analysis and (GTG)5-PCR fingerprints, readily differentiated the unnamed taxon from the type species. Therefore, we propose to formally classify this unnamed taxon as Kerstersia similis sp. nov. with strain LMG 5890T (=CCUG 46999T), isolated from a leg wound in the USA in 1983, as the type strain.

At the time of writing, the genus Kerstersia comprised Kerstersia gyiorum, the type species, and an unnamed genospecies, which proved to be biochemically indistinguishable in a polyphasic taxonomic study consisting of 16S rRNA gene sequencing, DNA–DNA hybridization experiments, analysis of DNA G+C content, whole-cell protein and fatty acid profiling, and extensive phenotypic characterisation by means of the API 20NE, API 50CH, API 50AO and API 50AA microtest systems, various classical phenotypic tests and antimicrobial susceptibility testing (Coenye et al., 2003).

The present study was prompted by the isolation of a novel clinical strain, R-47068, from a neck abscess of a 54-year-old male in Belgium, which was tentatively assigned to the unnamed Kerstersia species based on 16S rRNA gene sequence analysis. To re-examine diagnostic procedures for this novel species we used gyrB gene sequence analysis and (GTG)5-primed PCR fingerprinting and analysed biochemical characteristics available in the Biolog GEN III microtest system.

All strains were grown aerobically on trypticase soy agar (TSA; BBL) at 28 °C unless otherwise indicated. DNA was prepared by heating one or two colonies at 95 °C for 15 min in 20 μl lysis buffer containing 0.25 % (w/v) SDS and 0.05 M NaOH. Following lysis, 180 μl distilled water was added to the lysate.

The 16S rRNA gene sequences (Coenye et al., 2003) of K. gyiorum LMG 5906T and Kerstersia sp. LMG 5890 showed 99.3 % similarity as determined with the EzTaxon server 2.1 database (Chun et al., 2007). To obtain a more discriminatory sequence-based species differentiation, we amplified gyrB gene sequences of K. gyiorum strains LMG 5906T, R-2516 and LMG 15979 and Kerstersia sp. strains LMG 5890, LMG 5891, LMG 5892 and R-47068 (Tayeb et al., 2008). Amplification products of about 355 bp were obtained and sequenced. Sequences were aligned by using CLUSTAL X and imported into the BioNumerics version 6.5 software (Applied Maths) for phylogenetic analyses and bootstrap analysis (500 replicates). Unknown bases were discarded from the analysis and phylogenetic trees were constructed via the neighbour-joining, maximum-likelihood and maximum-parsimony methods. Levels of gyrB gene sequence similarity among K. gyiorum and Kerstersia sp. strains were above 98.9 and 99.2 %, respectively. Levels of gyrB gene sequence similarity between K. gyiorum and Kerstersia sp. strains were between 97.2 and 98 %. Fig. 1 shows a neighbour-joining phylogenetic tree based on these gyrB gene sequences; maximum-likelihood and maximum-parsimony methods revealed the same clustering of strains (data not shown).

(GTG)5-primed PCR fingerprinting of the same strains and of an additional four K. gyiorum strains (Coenye et al., 2003) was performed as described by Gevers et al. (2001).
The resulting fingerprints were analysed by using the BioNumerics version 6.5 software package. Similarity among the digitized profiles was calculated with the Pearson correlation and an average linkage (UPGMA) dendrogram (Fig. 2) was derived from the profiles. From Fig. 2 it is clear that (GTG)5-primed PCR fingerprinting allows a straightforward differentiation among the two species.

Oxidation of 71 carbon sources and 23 growth susceptibility tests were examined by using Biolog GEN III microplates according to the manufacturer’s instructions. The strains examined were *K. gyiorum* LMG 5906T, R-2516 and LMG 15979 and *Kerstersia* sp. LMG 5890, LMG 5891, LMG 5892 and R-47068. Taxon- or strain-dependent characteristics are shown in Table S1 available in IJSEM Online. Most notably, *K. gyiorum* was positive for oxidation of D-galacturonic acid and D-glucuronic acid whereas *Kerstersia* sp. was negative, and *K. gyiorum* was negative for oxidation of D-serine whereas *Kerstersia* sp. was positive. Other characteristics of the novel species are listed in the description below.

For electron microscope analysis, strain LMG 5890 was grown on TSA plates for 48 h at 28°C under aerobic conditions and then harvested and fixed in HEPES buffer with 2.5% glutaraldehyde for 24 h. Subsequently, the sample was post-fixed in 1% osmium tetroxide for 2 h at room temperature on a 0.2 μm pore size polycarbonate filter. The fixed sample was then dehydrated through ascending grades of ethanol/acetone and transferred to a critical-point drier (CPD 030; Bal-tec). The dried filter was mounted on a metal stub and sputter-coated with platinum (JEOL JFC-1300 auto fine coater). Examination of the sample was performed on a JEOL JSM 5600 LV scanning electron microscope. Cells of strain LMG 5890 were small coccobacilli (about 0.3–0.5 μm wide and 0.85–1.3 μm long) with rounded ends that occurred singly or in pairs (Fig. 3; Fig. S1). Flagella were not observed.

The present study provides new phenotypic and genotypic data to identify the unnamed *Kerstersia* species represented by strains LMG 5890, LMG 5891, LMG 5892 and R-47068, and therefore we propose to formally classify it as *Kerstersia similis* sp. nov. with strain LMG 5890T as the type strain.

**Description of Kerstersia similis sp. nov.**

*Kerstersia similis* (si’mi.lis. L. fem. adj. similis similar, resembling, as strains of the species biochemically resemble strains of *K. gyiorum*).

Data are taken from Coenye et al. (2003) and the present study. Cells are Gram-negative, non-motile small coccobacilli (about 0.3–0.5 μm wide and 0.85–1.3 μm long) with
rounded ends that occur singly or in pairs. On nutrient agar, colonies are flat or slightly convex with smooth margins, are white to light brown and have a swarming appearance (Coenye et al., 2003). Positive for catalase activity but negative for oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, activity but negative for oxidase, arginine dihydrolase, l-lysine decarboxylase, ornithine decarboxylase, β-galactosidase, gelatinase, amylase, urease and DNase activity. No reduction of nitrate or nitrite, hydroylsis of aesculin, or production of acid or H₂S from triple-sugar-iron agar was observed. Grows at 28–42 °C and at NaCl concentrations of 0–4.5 % (Coenye et al., 2003). Assimilates (API microtest systems) acetate, propionate, butyrate, n-valerate, n-caproate, heptanoate, pelargonate, caprate, succinate, fumarate, 2-ketoglutarate (weakly), D-lactate, DL-lactate plus methionine, DL-3-hydroxybutyrate, D-malate, L-malate, pyruvate, citrate, phenylacetate, p-hydroxybenzoate, α-D-alanine, α-D-l-alanine, L-norleucine, L-phenylalanine, L-tyrosine, L-aspartate, l-glutamate, L-ornithine, L-tryptophan, L-proline, DL-γ-aminobutyrate, 2-aminobenzoate, 4-aminobenzoate, L-alanine, L-norleucine, L-arginine, L-pyroglutamic acid, L-serine, L-lysine, L-phenylalanine, D-phenylalanine, D-leucine, L-leucine, L-tyrosine, L-methionine, D-tryptophan, tryptophan, L-lysine, L-citrulline, L-arginine, histidine, L-valine, D-lysine, D-serine, L-threonine, L-lysine, L-cysteine, L-methionine, D-tryptophan, trigonelline, L-lysine, L-citrulline, L-arginine, betaine, creatine, DL-γ-aminobutyrate, DL-γ-aminobutyrate, 3-aminobenzoate, urea, acetaldehyde, sarcosine, ethylamine, ethanolamine, benzylamine, diaminobutane, spermine, histamine or glucosamine (Coenye et al., 2003). Assimilation of glycerol, glyconate, isobutyrate, isovalerate, caprylate, glutarate, adipate, pimelate, suberate, azelate, sebacate, levulinate, citraconate, itaconate, mesaconate, aconitate, L-leucine, L-isoleucine, L-valine, DL-norvaline, DL-2-aminovalerate, β-alanine and butylamine is strain dependent (Coenye et al., 2003). Oxidizes (Biolog GEN III microtest system) L-serine, gluconuramide (weakly), quinic acid, p-hydroxyphenylacetic acid, L-lactic acid, L-malic acid, propionic acid and acetic acid, but not dextrin, maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, α-D-lactose, melibiose, methyl β-D-glucoside, D-salicylic acid, D-acetylglucosamine, N-acetyl-β-D-mannosamine, N-acetyl-β-D-galactosamine, N-acetyl-neuraminic acid, α-D-glucose, D-mannose, L-fructose, D-galactose, 3-methyl glucose, D-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabinol, myo-inositol, glycerol, D-glucose 6-phosphate, D-fructose 6-phosphate, D-aspartic acid, gelatin, glycyl-L-proline, L-arginine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, D-glucuronic acid, L-galactonic acid lactone, D-gluconic acid, muric acid, D-saccharic acid, D-lactic acid methyl ester, α-ketoglutaric acid, bromosuccinic acid, Tween 40, acetoacetic acid or formic acid. In sensitivity tests, the tetrazolium redox dye is reduced at pH 6 but not at pH 5; the dye is reduced in the presence of 1 % NaCl, 1 % sodium lactate, rifampicin 5V, lincomycin, guanidine HCl, niaproof 4, vancomycin, tetracyclazole violet, tetracyclazole blue, nalidixic acid and sodium butyrate, but not in the presence of 8 % NaCl or minocycline. Contains the following fatty acids: C₁₄:0, C₁₀:0, C₁₃:0 3-OH, C₁₅:0 2-OH, C₁₈:2, C₁₈:1ω7c, C₁₉:0 cyclo ω8c, summed feature 2 (C₁₅:0 3-OH, iso-C₁₇:0 3-OH), C₁₇:0 cyclo ω9c, ω7c, summed feature 3 (C₁₉:0 17c and/ or C₁₁:0 17c), C₁₇:0 ω7c, C₁₈:0 13c, C₁₃:0 2-OH (Coenye et al., 2003). The DNA G+C content of the type strain is 61.5 mol% (Coenye et al., 2003).

The type strain, LMG 5890T (＝CCUG 46999T), was isolated from a leg wound in the USA in 1983. Two additional strains (LMG 5891 and LMG 5892) were also isolated from leg wounds (Coenye et al., 2003), and a fourth strain (R-47068) was isolated from a neck abscess. Characteristics for the type strain are the same as described above for the species. In addition, the type strain oxidizes L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, D-malic acid, γ-aminobutyric acid and β-hydroxy-DL-butyric acid; in Biolog GN III susceptibility tests, the tetrazolium redox dye is reduced in the presence of fusidic acid, D-serine, troleandomycin, lithium chloride and aztreonam but not with potassium tellurite or sodium bromate. The type strain also assimilates butyrylamine but not glycerol, gluconate, isobutyrate, isovalerate, caprylate, glutarate, adipate, pimelate, suberate, azelate, sebacate, levulinate, citraconate, itaconate, mesaconate, aconitate, L-leucine, L-isoleucine, L-valine, DL-norvaline, DL-2-aminovalerate or β-alanine.

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References


