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Taxonomy of the genus *Cupriavidus*: a tale of lost and found

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DNA–DNA hybridization experiments and an evaluation of phenotypic characteristics, DNA base ratios and 16S rRNA gene sequences demonstrated that *Wautersia eutropha* (Davies 1969) Vaneechoutte *et al.* 2004, the type species of the genus *Wautersia*, is a later synonym of *Cupriavidus necator* Makkar and Casida 1987, the type species of the genus *Cupriavidus*. In conformity with Rules 15, 17, 23a and 37a(1) of the International Code of Nomenclature of Bacteria, the genus name *Cupriavidus* has priority over the genus name *Wautersia*, and all other members of the genus *Wautersia* are reclassified into *Cupriavidus* as *Cupriavidus basilensis* comb. nov. (type strain LMG 18990^T = DSM 11853^T), *Cupriavidus campinensis* comb. nov. (type strain LMG 19282^T = CCUG 44526^T), *Cupriavidus gilardii* comb. nov. (type strain LMG 5886^T = CCUG 38401^T), *Cupriavidus metallidurans* comb. nov. (type strain LMG 1195^T = DSM 2839^T), *Cupriavidus oxalaticus* comb. nov. (type strain LMG 2235^T = CCUG 2086^T = DSM 1105^T), *Cupriavidus pauculus* comb. nov. (type strain LMG 3244^T = CCUG 12507^T), *Cupriavidus respiraculi* comb. nov. (type strain LMG 21510^T = CCUG 46809^T) and *Cupriavidus taiwanensis* comb. nov. (type strain LMG 19424^T = CCUG 44338^T).

Cupriavidus necator was described by Makkar & Casida (1987) to accommodate a non-obligate bacterial predator of various Gram-negative and Gram-positive soil bacteria and fungi (Byrd *et al.*, 1985; Sillman & Casida, 1986; Zeph & Casida, 1986). The single known isolate, strain N-1^T (=LMG 8453^T), was obtained from soil in the vicinity of University Park, PA, USA. When confronted with *Agromyces ramosus* mycelia during the so-called 'attack–counter-attack' predation process, this strain produces several chemical signals, one of which chelates copper. *C. necator* is highly resistant to copper and its growth initiation is strongly stimulated by copper (Makkar & Casida, 1987).

Makkar & Casida (1987) reported the DNA base ratio and a wide range of morphological, biochemical and nutritional properties of this organism but did not examine its phylogenetic position through 16S rRNA studies as is currently standard procedure in prokaryotic taxonomy. They noticed several characteristics their organism shared with members of the genus *Alcaligenes*, which, at that time, comprised multiple species, including *Alcaligenes faecalis* (the type species), *Alcaligenes xylosoxidans* and allied species (now all classified in the genus *Achromobacter*; Yabuuchi *et al.*, 1998) and *Alcaligenes eutrophus* [first reclassified in the genus *Ralstonia* (Yabuuchi *et al.*, 1995) and recently transferred again, to the novel genus *Wautersia* (Vaneechoutte *et al.*, 2004)]. However, a few unique biochemical characteristics and the spectacular predatory activity convinced Makkar & Casida (1987) to classify their strain into a novel genus and species.

In the course of a long-term study of the biodiversity of various *Burkholderia cepacia*-like bacteria, we discovered a nearly complete 16S rRNA gene sequence that was deposited for *C. necator* in the public database under the accession number AF191737. This sequence was very similar to that of *Wautersia eutropha* isolates. Fig. 1 shows the result of the comparison of the 16S rRNA gene sequence of *C. necator* LMG 8453^T with those of strains representing *Wautersia*, *Ralstonia* and other *β-Proteobacteria*. The phylogenetic analysis, including a bootstrap analysis with 1000 replicates, was performed using the BioNumerics 3.5 software package (Applied Maths) and was based on the neighbour-joining method using nearly complete sequences and excluding undetermined base positions from the calculations. The similarity level between the 16S rRNA gene sequences of strains *C. necator* LMG 8453^T and *W. eutropha* LMG 1199^T was 99·7 %.

We subsequently compared the whole-cell protein electrophoretic profiles of *C. necator* and *W. eutropha* isolates. SDS-PAGE of whole-cell proteins was performed as described previously (Pot *et al.*, 1994), after growth of the isolates for 48 h at 37 °C on trypticase soy agar (BBL). Densitometric analysis, normalization and interpolation of the protein profiles were performed using the GelCompar 4.2 software

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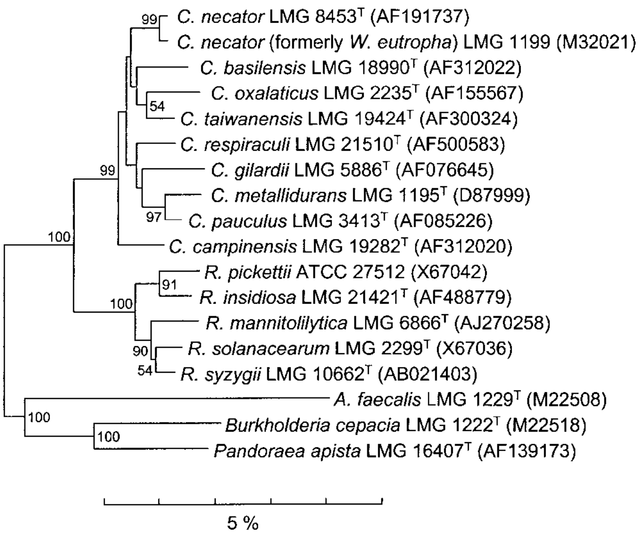

Fig. 1. Rooted phylogenetic tree based upon 16S rRNA gene sequence similarity of the genera *Cupriavidus* and *Ralstonia* and representative species of the *β-Proteobacteria*. Cluster analysis was based upon the neighbour-joining method with *Alcaligenes faecalis* LMG 1229^{T} as the outgroup root. The scale bar represents 5 % sequence divergence. Numbers at branch-points are percentages of 1000 bootstrap resamplings that support the topology of the tree (only values above 50 % are presented).

package (Applied Maths). The whole-cell protein profiles of *C. necator* LMG 8453^{T} and of *W. eutropha* LMG 1199^{T} (=TF93^{T}) and LMG 1201 (=H16), two established *W. eutropha* reference strains (Jenni *et al.*, 1988), were very similar (Fig. 2).

DNA–DNA hybridization experiments were subsequently performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader for the fluorescence measurements. The hybridization temperature was 50 °C. DNA was prepared as described by Pitcher *et al.* (1989). The DNA–DNA binding values obtained were 100 % between *W. eutropha* LMG 1199^{T} and LMG 1201 (which is in perfect agreement with a previously published value; Jenni *et al.*, 1988), 79 % between *W. eutropha* LMG 1199^{T} and *C. necator* LMG 8453^{T} and 92 % between *W. eutropha* LMG 1201 and *C. necator* LMG 8453^{T}. These data indicate unambiguously that the three isolates represent the same genospecies and confirm that, in the genera *Ralstonia* and *Wautersia*, high protein electrophoretic similarity correlates with a high level of DNA–DNA hybridization (Coenye *et al.*, 1999, 2003a; Vandamme *et al.*, 1999; Goris *et al.*, 2001).

Given the reported difference in DNA base ratio for the two taxa [67 mol% G+C for *W. eutropha* (Goris *et al.*, 2001) versus 57 mol% for *C. necator* (Makkar & Casida, 1987)], we determined the DNA base ratio of *C. necator* LMG 8453^{T} by two approaches. The DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The obtained nucleoside mixture was then separated by HPLC using a Waters SymmetryShield C8 column thermostatted at 37 °C. The solvent was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5 % acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference. In addition, the DNA base ratio was also determined by thermal denaturation and calculated as described by De Ley (1970). The former method yielded a G+C content of 65 mol%, the latter 66 mol%. These values are similar to values previously determined for *W. eutropha* (Goris *et al.*, 2001; Jenni *et al.*, 1988) and clearly different from the value for *C. necator* determined by Makkar & Casida (1987). We believe that this difference is due to experimental error in the original study.

The results of the extensive biochemical characterization of *C. necator* LMG 8453^{T} generally correlate well with those provided by Yabuuchi *et al.* (1995) and De Baere *et al.* (2001) for *W. eutropha*. Both organisms are reported as Gram-negative, peritrichously flagellated bacteria with an oxidative metabolism. They produce catalase and oxidase and reduce nitrate to nitrite but exhibit no DNase activity. They hydrolyse Tween 80, but not urea, gelatin or aesculin. The remarkable resistance to (and growth stimulation by) copper was one of the key arguments for excluding strain LMG 8453^{T} from the genus *Alcaligenes* (Makkar & Casida, 1987). However, resistance to copper and a range of other metals is well-documented for species now classified in *Wautersia* and is often plasmid-borne (Mergeay *et al.*, 2003).

The results of the present study indicate that, in the 1980s, the isolate described by Makkar & Casida (1987) should have been classified as *Alcaligenes eutrophus* Davis 1969. *Alcaligenes eutrophus* was reclassified in the novel genus *Ralstonia*, together with two former *Burkholderia* species, *Burkholderia solanacearum* and *Burkholderia pickettii* (Yabuuchi *et al.*, 1995). Subsequently, the genus *Ralstonia* was divided into *Ralstonia sensu stricto* and the novel genus

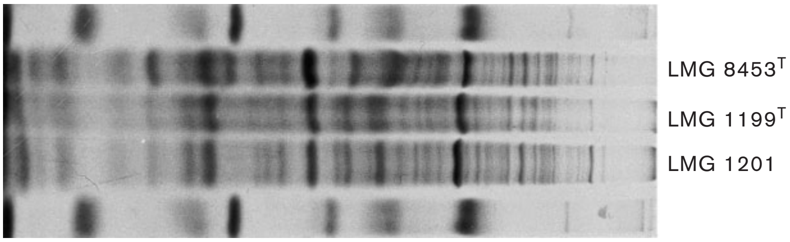

Fig. 2. Whole-cell protein profiles of *C. necator* LMG 8453^{T} and of *W. eutropha* strains LMG 1199^{T} and LMG 1201.

Wautersia, with *W. eutropha* as the type species. As outlined above, the name *C. necator* was validly published in 1987 and the names *Ralstonia* and *Wautersia* were only published much later. Rule 23a of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992) specifies that each taxon above a species can bear only one correct name, that is, the earliest that is in accordance with the Rules of the Code.

In addition, the nomenclatural type of a taxon is that element of the taxon with which it is permanently associated. Rule 42 specifies that, in the case of subspecies, species, subgenera and genera, if two or more taxa of the same rank are united, the oldest legitimate name or epithet is retained. Therefore, the genus name *Wautersia* is a later synonym of the genus *Cupriavidus*, for which the type species is *C. necator* (Rule 15 of the Code). Furthermore, the Code stipulates that the type determines the application of the name of a taxon if the taxon is subsequently divided or united with another taxon (Rule 17). While renaming and subsequent further renaming of bacterial species causes confusion and, not the least, irritation in the wider microbiological community, adhering to the rules of nomenclature is essential for establishing a truly systematic taxonomy. Therefore, while it may be inconvenient to deal with two name changes for a bacterium within one year (i.e. *Ralstonia* to *Wautersia* to *Cupriavidus*), in the long run, such reorganizations of the taxonomy of organisms are warranted as new data come to light.

Conforming to Rule 37a(1), that the name of a taxon must be changed if the nomenclatural type is excluded, we propose that the name *Wautersia* be replaced by *Cupriavidus* and that all species of the genus *Wautersia* be considered species of the genus *Cupriavidus*. We consequently propose to reclassify the remaining *Wautersia* species, i.e. *Wautersia basilensis* (Steinle *et al.* 1999) Vaneechoutte *et al.* 2004, *Wautersia campinensis* (Goris *et al.* 2001) Vaneechoutte *et al.* 2004, *Wautersia gilardii* (Coenye *et al.* 1999) Vaneechoutte *et al.* 2004, *Wautersia metallidurans* (Goris *et al.* 2001) Vaneechoutte *et al.* 2004, *Wautersia oxalatica* (Sahin *et al.* 2000) Vaneechoutte *et al.* 2004, *Wautersia paucula* (Vandamme *et al.* 1999) Vaneechoutte *et al.* 2004, *Wautersia respiraculi* (Coenye *et al.* 2003b) Vaneechoutte *et al.* 2004 and *Wautersia taiwanensis* (Chen *et al.* 2001) Vaneechoutte *et al.* 2004, into the genus *Cupriavidus*.

In contrast with the etymology presented by Makkar & Casida (1987), the gender of the genus name *Cupriavidus* is masculine [Rule 65(2)]. We therefore propose to reclassify the former *Wautersia* species as follows.

Emended description of the genus *Cupriavidus*

The description of the emended genus *Cupriavidus* is based on that presented by Makkar & Casida (1987) with some modifications. Cells are Gram-negative, peritrichously flagellated rods. Chemoheterotrophic or chemolithotrophic. The metabolism is oxidative. Several amino acids are used as sole carbon and nitrogen sources. Catalase and oxidase activity is produced. Resistance to various metals is widespread. The respiratory quinone Q8 has been reported in *W. eutropha* (Yabuuchi *et al.*, 1995). The DNA G+C content is between 63 and 69 mol%. Species occur in soil and human clinical specimens, particularly in samples from debilitated patients. The type species is *Cupriavidus necator*.

Description of *Cupriavidus basilensis* comb. nov.

Cupriavidus basilensis [N.L. masc. adj. *basilensis* from *Basilea* (Basel), where the type strain was isolated].

Basonym: *Ralstonia basilensis* Steinle *et al.* 1999.

The description is identical to that given for *Wautersia basilensis* by Vaneechoutte *et al.* (2004). The type strain is LMG 18990^T (=DSM 11853^T).

Description of *Cupriavidus campinensis* comb. nov.

Cupriavidus campinensis (N.L. masc. adj. *campinensis* from the Kempen or Campine, the geographical region of north-east Belgium where this bacterium was initially isolated).

Basonym: *Ralstonia campinensis* Goris *et al.* 2001.

The description is identical to that given for *Wautersia campinensis* by Vaneechoutte *et al.* (2004). The type strain is LMG 19282^T (=CCUG 44526^T).

Description of *Cupriavidus gilardii* comb. nov.

Cupriavidus gilardii (N.L. gen. n. *gilardii* of Gilardi, named in honour of G. L. Gilardi, an American microbiologist).

Basonym: *Ralstonia gilardii* Coenye *et al.* 1999.

The description is identical to that given for *Wautersia gilardii* by Vaneechoutte *et al.* (2004). The type strain is LMG 5886^T (=CCUG 38401^T).

Description of *Cupriavidus metallidurans* comb. nov.

Cupriavidus metallidurans (N.L. masc. part. adj. *metallidurans* enduring metal, to indicate that these bacteria are able to survive high heavy-metal concentrations).

Basonym: *Ralstonia metallidurans* Goris *et al.* 2001.

The description is identical to that given for *Wautersia metallidurans* by Vaneechoutte *et al.* (2004). The type strain is LMG 1195^T (=DSM 2839^T).

Description of *Cupriavidus oxalaticus* comb. nov.

Cupriavidus oxalaticus (N.L. masc. adj. *oxalaticus* pertaining to oxalate).

Basonym: *Ralstonia oxalatica* (*ex* Khambata and Bhat 1953) Sahin *et al.* 2000.

The description is identical to that given for *Wautersia oxalatica* by Vaneechoutte *et al.* (2004). The type strain is LMG 2235^T (=CCUG 2086^T=DSM 1105^T).

Description of *Cupriavidus pauculus* comb. nov.

Cupriavidus pauculus (L. masc. adj. *pauculus* rare, few, to indicate that these strains only sporadically cause human infections).

Basonym: *Ralstonia paucula* Vandamme *et al.* 1999.

The description is identical to that given for *Wautersia paucula* by Vaneechoutte *et al.* (2004). The type strain is LMG 3244^T (=CCUG 12507^T).

Description of *Cupriavidus respiraculi* comb. nov.

Cupriavidus respiraculi (L. gen. n. *respiraculi* of the respiratory system).

Basonym: *Ralstonia respiraculi* Coenye *et al.* 2003.

The description is identical to that given for *Wautersia respiraculi* by Vaneechoutte *et al.* (2004). The type strain is LMG 21510^T (=CCUG 46809^T).

Description of *Cupriavidus taiwanensis* comb. nov.

Cupriavidus taiwanensis (N.L. masc. adj. *taiwanensis* from Taiwan, where root-nodulating strains were isolated).

Basonym: *Ralstonia taiwanensis* Chen *et al.* 2001.

The description is identical to that given for *Wautersia taiwanensis* by Vaneechoutte *et al.* (2004). The type strain is LMG 19424^T (=CCUG 44338^T).

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