Request for an Opinion

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The genus name *Ensifer* Casida 1982 takes priority over *Sinorhizobium* Chen *et al.* 1988, and *Sinorhizobium morelense* Wang *et al.* 2002 is a later synonym of *Ensifer adhaerens* Casida 1982. Is the combination '*Sinorhizobium adhaerens*' (Casida 1982) Willems *et al.* 2003 legitimate? Request for an Opinion

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The synonymy of the genera *Ensifer* and *Sinorhizobium* was recently reported, but it was proposed that the later-named genus, *Sinorhizobium*, take priority in nomenclature. There is no justification in the *International Code of Nomenclature of Bacteria* (Prokaryotes) for this step; *Ensifer* is the correct name of the genus, with *Ensifer adhaerens* as the type species. Species previously allocated to *Sinorhizobium* are here proposed as the new combinations *Ensifer arboris*, *Ensifer fredii*, *Ensifer kostiensis*, *Ensifer kummerowiae*, *Ensifer medicae*, *Ensifer meliloti*, *Ensifer saheli*, *Ensifer terengae* and *Ensifer xinjiangensis*. *Sinorhizobium morelense* was proposed in 2002 [Wang, E. T., Tan, Z. Y., Willems, A., Fernández-López, M., Reinhold-Hurek, B. & Martínez-Romero, E., *Int J Syst Evol Microbiol* **52**, 1687–1693, 2002], but a consideration of all published data indicate that it is a nitrogen-fixing genomovar and later heterotypic synonym of *Ensifer adhaerens*. A Request for an Opinion is made as to whether or not the combination '*Sinorhizobium adhaerens*' (Casida 1982) Willems *et al.* 2003 is legitimate.

Casida (1982) proposed the binomial *Ensifer adhaerens* for a bacterium that multiplied by budding and was predatory on other bacteria. At present, *Ensifer* is recorded as a monotypic genus, with *E. adhaerens* as the type species. Willems *et al.* (2003) compared the type strain and one other strain of *E. adhaerens*, both isolated by Casida, with a selection of strains representing *Sinorhizobium* species. Methods involved comparisons based on the 16S rDNA sequence and part of the *recA* sequence, DNA hybridization of *nod* gene probes, DNA base composition, DNA–DNA reassociation data, SDS-PAGE of whole-cell proteins, auxanographic characteristics, antibiotic sensitivities and nodulation assays. The only datasets in which the type species of *Ensifer* and *Sinorhizobium* were included, enabling the differentiation of the two genera, referred to a large part of the 16S rDNA sequence and a part of the sequence of *recA*. Willems *et al.* (2003) concluded that *Ensifer* Casida 1982 and *Sinorhizobium* Chen *et al.* 1988 were synonyms, acknowledging *Ensifer* as the earlier synonym. Elsewhere, Wang *et al.* (2002) reported a novel species, *Sinorhizobium morelense*, which shared the budding characteristic and

high antibiotic resistance of *E. adhaerens*. Data reported in these papers confirmed the very close relationship of *Ensifer* and *Sinorhizobium* and justify the amalgamation of these two genera.

Willems *et al.* (2003) requested an Opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes, with a proposal that the name *Sinorhizobium* should be preferred to *Ensifer*, and referred to Rule 38 of the *International Code of Nomenclature of Bacteria* (Prokaryotes) [‘the Code’ (Lapage *et al.*, 1992)] because they believed that the union of the two genera with *Ensifer* as the generic name would cause confusion in bacteriology. The reasons given in their request were:

- (i) *Sinorhizobium* is a well-known genus and *Ensifer* is less well known;
- (ii) *Sinorhizobium* contains nine species while *Ensifer* contains one species;
- (iii) the descriptive name derived from ‘*rhizobium*’ implies a distinctive feature of the organisms;
- (iv) *Ensifer* was proposed before comparative 16S rDNA sequence analyses allowed phylogenetic inferences to be

made. Had such analyses been possible, the close relationship of *E. adhaerens* to *Rhizobium* would have been recognized and this species would have been included in *Rhizobium*.

In making their request, Willems *et al.* (2003) do not refer correctly to any rule that supports their proposal. The intention of Rule 38 is to emphasize the importance of the rule of priority, covered in detail in Rule 23, and the status of synonymy, covered in Rule 24. Bacterial names are accorded priority in order of publication, and only the Judicial Commission may make a determination that an earlier name is to be conserved over a later name (Rule 23a; Note 4). The Code does not specify the criteria for conservation of a later over an earlier synonym, but indicates that names may be rejected only if they are ambiguous, doubtful, cause confusion, are perplexing or are perilous. Guidance is offered in Rule 55, which states that a legitimate name may not be replaced merely because it is inappropriate or disagreeable, another name is preferable or is better known, or the name no longer describes the organism. The Request for an Opinion of Willems *et al.* (2003) runs counter to all these criteria.

The argument of Willems *et al.* (2003) that the availability of comparative 16S rDNA sequence analyses when *E. adhaerens* was first characterized (Casida, 1982) would have led to its inclusion in *Rhizobium* is highly doubtful in the absence of the recognition of other *Sinorhizobium* species before the report of Chen *et al.* (1988). The budding, predatory organism *E. adhaerens* might have been recognized as being related to the *Agrobacterium*–*Rhizobium* clade, but on the separate (*Sinorhizobium*) branch. In the absence of demonstrated nitrogen-fixing or gall-forming properties, its allocation to a separate genus was almost certainly assured, following the reasoning of Willems *et al.* (2003).

Central to the proposal of Willems *et al.* (2003) is their view that bacterial names should be familiar and descriptive. However:

(i) if names are to take precedence merely because of their familiarity, the formal organization of nomenclature based primarily on priority will be subverted;

(ii) although the generic name *Sinorhizobium* was published in 1988, the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Agrobacterium* and *Rhizobium* did not endorse the name in 1992 (Martinez-Romero & Jarvis, 1993). The name *Sinorhizobium* came into general use only after 1994 (de Lajudie *et al.*, 1994).

(iii) A recent Request for an Opinion (Logan *et al.*, 1998) that a later synonym be preferred to an earlier synonym merely because it was a more descriptive name was rejected (De Vos & Trüper, 2000; Trüper, 2002). The reasons have also been explained elsewhere (Young, 2000).

Nitrogen-fixing bacteria may represent only a part of the

overall diversity in the genera of the *Rhizobiaceae*. The present record of characterized species is strongly biased in favour of organisms of anthropocentric interest. There is little basis at present for believing even that nitrogen-fixing strains are the predominant populations in species originally identified by their nitrogen-fixing ability. As the catalogue of bacterial diversity is expanded, names based on characteristics such as nitrogen fixation can be expected only to become more confusing. It is misleading to regard names of bacteria as descriptive, and it does no service to bacteriologists when taxonomists support this interpretation.

'*Sinorhizobium adhaerens*' – Request for an Opinion

Willems *et al.* (2003) proposed that *E. adhaerens* be renamed as '*Sinorhizobium adhaerens*'.

On the basis that the discussion above accurately reflects the correct nomenclatural relationships of *Ensifer* and *Sinorhizobium*, it is proposed here that the combination '*Sinorhizobium adhaerens*' (Casida 1982) Willems *et al.* 2003 is illegitimate. *E. adhaerens* is the legitimate heterotypic earlier synonym (the Code; Rule 23). A decision of the Judicial Commission is sought.

Reclassification of *Sinorhizobium* species

The inclusion of *E. adhaerens* and *Sinorhizobium* species in a single genus requires the recombination of *Sinorhizobium* species in the genus *Ensifer*, the name that takes priority. An emended description of the genus *Ensifer*, combining that of Casida (1989) and that of *Sinorhizobium* by Kuykendall *et al.* (2003), is presented. New combinations of *Ensifer* species are proposed.

Emended description of the genus *Ensifer* Casida 1982, 339^{VP}

Rods, 0.5–1.0 × 1.0–3.0 µm. Commonly pleomorphic under adverse growth conditions. Some species multiply by budding. Cells usually contain granules of poly-β-hydroxybutyrate that are refractile under phase-contrast microscopy. Non-spore-forming. Gram-negative. Motile by means of one polar or subpolar flagellum or one to six peritrichous flagella. Fimbriae have been described for a few strains. Aerobic, possessing a respiratory type of metabolism with oxygen as the terminal electron acceptor. The optimum temperature is 25–30 °C, but a wide range of temperatures can be tolerated: most strains can grow at 35 °C, some strains can grow at 10 °C and others grow at temperatures up to 42–44 °C. Tolerate 1.0 % NaCl; some strains grow well on media containing 4.5 % NaCl. The optimum pH is 6–8, but some strains can grow at pH 5.0 and others at pH 10.5. Colonies are circular, convex, semi-translucent, raised and mucilaginous and usually 2–4 mm in diameter within 3–5 days on yeast/mannitol/mineral-salts agar. Generation times are 3–6 h. Chemo-organotrophic, utilizing a wide range of carbohydrates and salts of organic

acids as carbon sources, producing acid without gas formation. Cellulose and starch are not utilized. Produce an acidic reaction in mineral-salts medium containing mannitol. Growth on carbohydrate media is often accompanied by copious extracellular polysaccharide slime production. Do not utilize cellulose or starch. Ammonium salts, nitrate, nitrite and most amino acids can serve as nitrogen sources. All strains require pantothenate and nicotinic acid. Peptone is poorly utilized. Casein and agar are not hydrolysed. Strains produce cytochrome oxidase and catalase. 3-Ketolactose is not produced from lactose. Some species are recorded as resistant to a wide variety of antibiotics. Strains of most species identified so far are characteristically able to invade temperate-zone and tropical-zone leguminous plants (family *Leguminosae*) and to incite the production of root nodules, inside which the bacteria occur as intracellular nitrogen-fixing micro-symbionts. In root nodules, the bacteria occur as endophytes exhibiting pleomorphic forms. Strains of nitrogen-fixing isolates exhibit varying degrees of host specificity. Strains of at least one species, the type species, are capable of the predation of other bacteria. The G + C content of the DNA is 57–66 mol% (T_m). The type species is *Ensifer adhaerens* Casida 1982^{VP}.

Description of *Ensifer arboris* (Nick et al. 1999) comb. nov.

The description is the same as that given for *Sinorhizobium arboris* by Nick et al. (1999). The type strain is ATCC BAA-226^T = DSM 13375^T = HAMBI 1552^T = LMG 14919^T.

Description of *Ensifer fredii* (Scholla and Elkan 1984) comb. nov.

The description is the same as that given for *Sinorhizobium fredii* by Chen et al. (1988). The type strain is ATCC 35423^T = CCUG 27877^T = DSM 5851^T = HAMBI 2075^T = ICMP 11139^T = IFO 14780^T = LMG 6217^T = NRRL B-14594^T = USDA 205^T.

Description of *Ensifer kostiensis* (Nick et al. 1999) comb. nov.

The description is the same as that given for *Sinorhizobium kostense* by Nick et al. (1999). The type strain is ATCC BAA-227^T = DSM 13372^T = HAMBI 1489^T = LMG 19227^T.

Description of *Ensifer kummerowiae* (Wei et al. 2002) comb. nov.

The description is the same as that given for *Sinorhizobium kummerowiae* by Wei et al. (2002). The type strain is CCBAU 71714^T = AS 1.3046^T.

Description of *Ensifer medicae* (Rome et al. 1996) comb. nov.

The description is the same as that given for *Sinorhizobium medicae* by Rome et al. (1996). The type strain is A 321^T = HAMBI 2306^T = ICMP 13798^T = USDA 1037^T.

Description of *Ensifer meliloti* (Dangeard 1926) comb. nov.

The description is the same as that given for *Sinorhizobium meliloti* by de Lajudie et al. (1994). The type strain is ATCC 9930^T = CCUG 27879^T = CFBP 5561^T = DSM 30135^T = HAMBI 2148^T = ICMP 12623^T = IFO14782^T = LMG 6133^T = NCAIM B.01520^T = NRRL L-45^T = USDA 1002^T.

Description of *Ensifer saheli* (de Lajudie et al. 1994) comb. nov.

The description is the same as that given for *Sinorhizobium saheli* by de Lajudie et al. (1994). The type strain is ATCC 51690^T = DSM 11273^T = HAMBI 215^T = ICMP 13648^T = LMG 7837^T = ORS 609^T.

Description of *Ensifer terangae* (de Lajudie et al. 1994) comb. nov.

The description is the same as that given for *Sinorhizobium terangae* by de Lajudie et al. (1994). The type strain is ATCC 51692^T = DSM 11282^T = HAMBI 220^T = ICMP 13649^T = LMG 7834^T = ORS 1009^T.

Description of *Ensifer xinjiangensis* (Chen et al. 1988) comb. nov.

The description is the same as that given for *Sinorhizobium xinjiangense* by Chen et al. (1988). The type strain is ATCC 49357^T = CCBAU 110^T = DSM 5852^T = HAMBI 1673^T = ICMP 11141^T = LMG 17930^T.

Sinorhizobium morelense* is a later heterotypic synonym of *Ensifer adhaerens

The studies of Wang et al. (2002), in which *S. morelense* is proposed as a new species, and the studies of Willems et al. (2003), using the methods described above, can be taken to provide a comprehensive polyphasic comparison between *E. adhaerens* and *S. morelense*. However, the only unequivocal phenotypic difference recorded between the two species is their reaction in the urease test (Wang et al., 2002; Willems et al., 2003). The distinctive feature claimed for *S. morelense*, high levels of antibiotic resistance (Wang et al., 2002), is also recorded for *E. adhaerens* (Willems et al., 2003). The comparative studies of 16S rDNA sequence data show that *E. adhaerens* and *S. morelense* have essentially the same (99–100% rDNA) sequence and that the *recA* sequence of the type strain of *S. morelense* is embedded in the *E. adhaerens* sequence cluster, with the type strain of this species as its nearest neighbour (Willems et al., 2003). A comparative study using SDS-PAGE of total proteins included only *E. adhaerens* and *S. morelense* strains. No other strains of *Sinorhizobium* species were included, there was no numerical analysis of data and inspection gave no indication of differentiation between the two species (Willems et al., 2003). The strains unequivocally characterized as *S. morelense* lacked a nodulating, and hence

a nitrogen-fixing, capacity. DNA–DNA reassociation data (Willems *et al.*, 2003) present a complex picture. *S. morelense* is represented by two strains with less than 70 % (52 and 66 %) mutual similarity. *E. adhaerens* is considered to be represented by three genomic groups by Willems *et al.* (2003). These correspond to genomovars *sensu* Ursing *et al.* (1995) and do not merit species rank. The incompleteness of the reassociation matrix makes it difficult to judge the genotypic relationships with certainty. In the absence of more compelling data, it is proposed that *S. morelense* is a later heterotypic synonym of *E. adhaerens*. *E. adhaerens* therefore takes precedence, with ATCC 33212^T (=LMG 20216^T) as the type strain.

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