Methanoculleus bourgensis, *Methanoculleus olentangyi* and *Methanoculleus oldenburgensis* are subjective synonyms

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Methanoculleus bourgensis, *Methanoculleus olentangyi* and *Methanoculleus oldenburgensis* are subjective synonyms on the basis of phenotypic, genotypic and phylogenetic characteristics. *Methanoculleus bourgensis* must be the name of the united species because it is the type of the genus *Methanoculleus*.

The genus *Methanoculleus* currently includes seven species: *Methanoculleus marisnigri*, *Methanoculleus bourgensis*, *Methanoculleus thermophilus*, *Methanoculleus olentangyi*, *Methanoculleus oldenburgensis*, *Methanoculleus palmolei* and *Methanoculleus chikugoensis* (Maestrojuán *et al.*, 1990; Romesser *et al.*, 1979; Ollivier *et al.*, 1986; Rivard & Smith, 1982; Corder *et al.*, 1983; Blotevogel *et al.*, 1991; Zellner *et al.*, 1998; Dianou *et al.*, 2001). In the course of the identification of *Methanoculleus chikugoensis* MG62^T (Dianou *et al.*, 2001), a DNA–DNA hybridization study revealed that *Methanoculleus bourgensis* MS2^T, *Methanoculleus olentangyi* RC/ER^T and *Methanoculleus oldenburgensis* CB-1^T were genomically closely related (67–104% relatedness to each other), though a similar close relatedness (66%) between *Methanoculleus bourgensis* and *Methanoculleus olentangyi* had already been reported by Xun *et al.* (1989). We supplemented these results by examining some genotypic and phylogenetic features of the type strains and we now confirm the synonymy of these three species on the basis of these features and phenotypic characteristics already reported.

The 16S rDNA sequence of *Methanoculleus oldenburgensis* was determined, since it had not previously been available. The type strain, CB-1^T (=DSM 6216^T), was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. Methods for DNA isolation, PCR amplification and sequencing of 16S rDNA were the same as described previously (Dianou *et al.*,

2001). The sequence similarities of strain CB-1^T to *Methanoculleus bourgensis* MS2^T and *Methanoculleus olentangyi* RC/ER^T and between strains MS2^T and RC/ER^T were respectively 97.1, 98.7 and 97.7%, which was consistent with the DNA–DNA hybridization results mentioned above (Dianou *et al.*, 2001).

These three strains seem to have almost the same phenotypic features: the cells are 1–2 µm in diameter, flagellation and motility are not observed (fimbriae-like structures have been reported in the case of *Methanoculleus oldenburgensis* CB-1^T cells), H₂/CO₂ and formate are used as methanogenic substrates (some secondary alcohols are also used by *Methanoculleus bourgensis* MS2^T and *Methanoculleus olentangyi* RC/ER^T), acetate is required for growth and the optimum temperature, pH and NaCl concentration for growth are respectively about 40 °C (37–45 °C), near neutral (6.7–8.0) and about 0.1 M (0.04–0.17 M) (Blotevogel *et al.*, 1991; Ollivier *et al.*, 1986; Corder *et al.*, 1983; Maestrojuán *et al.*, 1990; Zellner *et al.*, 1990; Chong & Boone, 2001; see also Table 2 of Dianou *et al.*, 2001). Some resemblances of whole-cell protein electrophoretic patterns, S-layer structures and polyamine compositions between *Methanoculleus bourgensis* MS2^T and *Methanoculleus olentangyi* RC/ER^T were also reported (Maestrojuán *et al.*, 1990; Zellner *et al.*, 1990).

The sole differentiating characteristic is the G + C content of DNA. The G + C contents of *Methanoculleus bourgensis* MS2^T, *Methanoculleus olentangyi* RC/ER^T and *Methanoculleus oldenburgensis* CB-1^T were respectively originally reported to be 59, 54.4 and 48.6 mol%, the first two being determined by the buoyant density method and the latter by thermal denaturation. The range is 10 mol%, which seems too large for members of a single species. Therefore, we measured the G + C contents of DNA from these three strains by the same method (HPLC), as described previously (Dianou *et al.*, 2001), using a Hitachi 683-50 HPLC system. The values for *Methanoculleus bourgensis* MS2^T, *Methanoculleus*

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olentangyi RC/ER^T and *Methanoculleus oldenburgensis* CB-1^T were respectively 60.5 ± 0.0 , 61.1 ± 0.3 and 60.2 ± 0.2 mol% (mean $\pm$ SD, $n=3$).

The similarities of phenotypic, genotypic and phylogenetic characteristics described above indicate that *Methanoculleus bourgensis* (Ollivier *et al.* 1986) Maestrojuán *et al.* 1990, *Methanoculleus olentangyi* (Corder *et al.* 1988) Maestrojuán *et al.* 1990 and *Methanoculleus oldenburgensis* Blotevogel *et al.* 1998 are subjective synonyms. Rule 15 of the Bacteriological Code (Lapage *et al.*, 1992) indicates that the type of a taxon is permanently associated with that taxon; therefore, since *Methanoculleus bourgensis* is the type of the genus *Methanoculleus* Maestrojuán *et al.* 1990, the name *Methanoculleus bourgensis* must be retained for the united species.

The physiological variance of strain CB-1, such as its slightly higher temperature for optimum growth, might indicate its affiliation to a subspecies within the united species. However, further information concerning the phenotypic characters of strain CB-1 and the other strains will be needed for the taxonomic description of strain CB-1.

Emended description of *Methanoculleus bourgensis* (Ollivier *et al.* 1986) Maestrojuán *et al.* 1990

The descriptions of the genus *Methanoculleus* and the species *Methanoculleus bourgensis* have been given by Maestrojuán *et al.* (1990). Strains RC/ER and CB-1, previously the type strains of *Methanoculleus olentangyi* and *Methanoculleus oldenburgensis*, respectively, are reference strains of the species *Methanoculleus bourgensis*. The type strain is strain MS2^T (=ATCC 43281^T=DSM 3045^T).

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