Request for an Opinion

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Bacillus aeolius DSM 15084^T (=CIP 107628^T) is a strain of *Bacillus licheniformis*.

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Based on phenotypic properties, 16S rRNA gene sequence and MALDI-TOF analysis, strains DSM 15084^T and CIP 107628^T, deposited as the type strain of *Bacillus aeolius*, do not represent the original type strain, strain 4-1^T. It is therefore proposed that the Judicial Commission of the International Committee on Systematics of Prokaryotes places the name *Bacillus aeolius* on the list of rejected specific and subspecific epithets in names of species and subspecies of bacteria if a suitable replacement for the type strain or a neotype cannot be found within 2 years of publication of this Request.

In 2003, strain 4-1^T (=DSM 15084^T =CIP 107628^T) was proposed to represent a novel species within the genus *Bacillus*, *Bacillus aeolius*, on the basis of differences between its chemotaxonomic and phenotypic properties and those of related moderately thermophilic species, e.g. *Bacillus smithii*, *B. fumarioli*, *B. oleronis*, *B. sporothermodurans* and *B. infernus* (Gugliandolo *et al.*, 2003a, b). The G+C content of the DNA of strain 4-1^T was 40.8 mol%, falling within range of values determined for species of the genus *Bacillus* rRNA group 1 as defined by Ash *et al.* (1991). Phylogenetic analysis based on analysis of the nearly complete 16S rRNA gene sequence placed strain 4-1^T close to the type strain of *B. smithii* (96.7%), and binary similarity values determined with other type strains ranged between 95 and 96% (*Bacillus methanolicus*, 96.7%; *B. fumarioli*, 95.9%; *B. infernus*, 95.3%).

Strain 4-1^T was originally isolated by Maugeri *et al.* (2001) from a water sample collected at a depth of 15 m during an oceanographic cruise around the Eolian islands (Italy) at the site Vulcano – Punta Congigliara. These authors published a polyphasic taxonomic study of 18 thermophilic bacilli including strain 4-1^T, whose partial 16S rRNA gene sequence was found to be distantly related to that of the type strain of *B. methanolicus*.

Phenotypic properties of the strain published in 2001 compared with the data published within the species description in 2003 are summarized in Table 1. Further results obtained from API 50CHB analysis of the strain deposited as DSM 15084^T and *Bacillus licheniformis* DSM 13^T are given for comparison. Although the phenotypic characteristics of strains 4-1^T (as reported in 2003) and

DSM 15084^T were not identical with respect to some of the substrates tested, the profiles differ significantly from that published in 2001 for strain 4-1^T. In addition, the two species *B. aeolius* and *B. licheniformis* are similar in many of the properties detected: aerobic growth, positive result in an oxidase test and acid production from glycerol, D-xylose, ribose, mannose, glucose, fructose, inositol, mannitol, salicin, cellobiose, maltose, sucrose and turanose.

In order to verify the identity of the strain, the DSMZ and CIP independently determined the 16S rRNA gene sequence of the strains deposited as the type strain of *B. aeolius* and, in both cases, sequence analyses revealed *B. licheniformis* as the nearest phylogenetic neighbour. The sequence deposited for *B. aeolius* 4-1^T (GenBank accession no. AJ504797) does not match the sequences obtained at the DSMZ and CIP, suggesting that the wrong strain has been deposited in the culture collections. Within a stretch of more than 600 bp, the sequence submitted for strain *B. aeolius* 4-1^T differs at 18 positions when compared with the sequence obtained at the DSMZ for strain DSM 15084^T (Supplementary Fig. S1, available in IJSEM Online). Recently, seven rRNA genes were found to be present in the whole genome of *B. licheniformis* DSM 13^T. One 16S rRNA gene sequence consists of 1549 bp, and all seven sequences differed at only six positions. It is therefore unlikely that the differences detected for *B. aeolius* 4-1^T could be caused by *rrn* operon heterogeneities.

Strain DSM 15084^T was analysed using the Qualicon Riboprinter system (DuPont Qualicon) for further characterization. Cluster analysis of the riboprint pattern obtained for strain DSM 15084^T allocated the strain to the *B. licheniformis* cluster, even though the fingerprints obtained for the different strains of the species *B.*

A 16S rRNA gene sequence alignment, a dendrogram based on Riboprinting results and results of MALDI-TOF analysis are available as supplementary material with the online version of this paper.

Table 1. Acid production from different substrates as determined by the use of API 50CH

Data for strain DSM 15084^T and *B. licheniformis* DSM 13^T were obtained in this study at 55 and 37 °C, respectively, after 5 and 24 h. +, Change of indicator dye to yellow; (+), positive at 5 h time point only; w, weak reaction. Glycerol gave a positive result in all assays. The following substrates gave a negative result in all assays: erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inulin, xylitol, gentiobiose, D-lyxose, D- and L-fucose, D- and L-arabitol and gluconate.

Substrate	Strain 4-1 ^T		DSM 15084 ^T	<i>B. licheniformis</i> DSM 13 ^T
	Maugeri <i>et al.</i> (2001)	Gugliandolo <i>et al.</i> (2003a)		
L-Arabinose	–	+	–	+
Ribose	–	+	+	+
D-Xylose	–	+	w	+
Galactose	–	–	+	–
Glucose	–	+	(+)	+
Fructose	+	+	(+)	+
D-Mannose	–	+	–	+
Rhamnose	–	–	+	–
Inositol	–	+	w	+
Mannitol	–	+	w	+
Sorbitol	–	–	+	+
Methyl β-D-mannopyranoside	–	–	+	–
Methyl β-D-glucopyranoside	–	–	–	+
N-Acetylglucosamine	–	+	–	(+)
Amygdalin	–	–	w	+
Arbutin	–	–	w	+
Aesculin ferric citrate	+	–	+	+
Salicin	–	+	w	+
Cellobiose	–	+	+	+
Maltose	–	+	+	+
Lactose	+	–	+	–
Melibiose	–	+	+	–
Sucrose	–	+	(+)	+
Trehalose	+	+	–	+
Melezitose	–	+	–	–
Raffinose	–	+	+	–
Starch	+	+	w	w
Glycogen	+	–	w	w
Turanose	–	+	+	+
D-Tagatose	+	–	–	+
2-Ketogluconate	–	+	–	–
5-Ketogluconate	+	+	–	–

licheniformis vary in the intensity and number of bands (Supplementary Fig. S2).

MALDI-TOF analysis (Schumann, 2007) was applied in order to confirm that the strains deposited at the DSMZ (DSM 15084^T) and the CIP (CIP 107628^T) are identical. Spectra recorded of both strains in five replicated measurements are shown in Supplementary Fig. S3. The analysis confirmed that the two strains showed identical patterns and they can be considered identical.

The two collections have attempted to obtain a subculture of the original strain from the depositor, but the strain is no longer available in the depositor's laboratory. According

to Rule 18c of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), it is proposed that the Judicial Commission of the International Committee on Systematics of Prokaryotes places the name *Bacillus aeolius* Gugliandolo *et al.* 2003 on the list of rejected specific and subspecific epithets in names of species and subspecies of bacteria if a suitable replacement for the type strain or a neotype cannot be found within 2 years of publication of this Request.

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