Proposal for transfer of *Pelagibacillus goriensis* Kim *et al.* 2007 to the genus *Terribacillus* as *Terribacillus goriensis* comb. nov.

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The phenotypic, chemotaxonomic and genotypic characteristics of two recently described genera were compared. *Terribacillus saccharophilus* and *Pelagibacillus goriensis* (both type species of their respective genera) are similar in many phenotypic characteristics and in their fatty acid profiles, both have MK-7 as the major menaquinone and they have similar G + C contents. Comparison of the 16S rRNA gene sequence of *P. goriensis* CL-GR16^T with those of *T. saccharophilus* 002-048^T and *Terribacillus halophilus* 002-051^T showed high degrees of sequence similarity, respectively 99.8 and 98.9 %. Phylogenetically, these three taxa are closely related and form a coherent cluster in the phylogenetic tree. The genomic relatedness of *P. goriensis* DSM 18252^T with *T. saccharophilus* JCM 21759^T and *T. halophilus* JCM 21760^T is 51.7 and 35.6 %, respectively, which is well below the value of 70 % recommended for delineation of species. *P. goriensis* differs from *T. saccharophilus* in motility, the Voges–Proskauer test and acid production from and utilization of some carbohydrates. Based on these analyses, we conclude that *Pelagibacillus* should not be considered a separate genus and hence *Pelagibacillus goriensis* should be transferred to the genus *Terribacillus* as *Terribacillus goriensis* comb. nov., with the type strain CL-GR16^T (=KCCM 42329^T =DSM 18252^T).

In the course of a study of bacterial and archaeal diversity of a landfill site, many bacteria were isolated and characterized. During this process, a strain designated 3LF 22T was found to show 99.8 % 16S rRNA gene sequence similarity to two recently described novel taxa, *Terribacillus saccharophilus* (An *et al.*, 2007) and *Pelagibacillus goriensis* (Kim *et al.*, 2007). Because of sequence similarity of strain 3LF 22T with these two taxa, we examined their taxonomic positions. On the basis of the data available, we propose the unification of the two genera and the transfer of the sole species of the genus *Pelagibacillus* to the genus *Terribacillus*.

The genera *Terribacillus* and *Pelagibacillus* belong to *Bacillus* group 1 (Ash *et al.*, 1991), along with many halophilic and halotolerant members of genera like *Halobacillus* (Spring *et al.*, 1996), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Gracilibacillus* (Wainø *et al.*, 1999), *Oceanobacillus* (Lu *et al.*, 2001), *Filobacillus* (Schlesner *et al.*, 2001), *Lentibacillus* (Yoon *et al.*, 2002), *Paraliobacillus* (Ishikawa *et al.*, 2002), *Tenuibacillus* (Ren & Zhou, 2005a), *Pontibacillus* (Lim *et al.*, 2005), *Salinibacillus* (Ren & Zhou, 2005b), *Thalassobacillus* (Garcia *et al.*, 2005), *Halolactibacillus* (Ishikawa *et al.*, 2005), *Ornithinibacillus* (Mayr *et al.*, 2006) and *Paucisalibacillus* (Nunes *et al.*, 2006). These bacteria either do not grow or grow very weakly without added salt in the medium, but growth is stimulated by the addition of salt.

The 16S rRNA gene sequences of *Terribacillus saccharophilus* 002-048^T, *Terribacillus halophilus* 002-051^T and *Pelagibacillus goriensis* CL-GR16^T were retrieved from the NCBI nucleotide database. In order to decipher the exact taxonomic position of the three strains, we also retrieved the 16S rRNA gene sequences of 17 type species of other halophilic/halotolerant genera of *Bacillus* group 1 along with sequences of other representative members of *Bacillus* groups 1 and 2, aligned them using the CLUSTAL_X software (Thompson *et al.*, 1997) and edited the alignment manually. Pairwise sequence similarities were calculated using the BioEdit software package (Hall, 1999). A continuous stretch of 1389 nucleotides without any ambiguous bases or gaps was used to construct a phylogenetic tree based on the algorithm of Jukes & Cantor (1969) using the neighbour-joining method (Saitou & Nei, 1987) and with the TREECON software package (Van de Peer & De Wachter, 1997). The topology of the constructed tree was evaluated using 1000 replicates. A tree based on the maximum-parsimony method was also constructed using the PHYLIP software package version 3.5c (Felsenstein, 1993). DNA–DNA hybridization was performed between *P. goriensis* DSM 18252^T and its phylogenetically close relatives *T. saccharophilus* JCM 21759^T and *T. halophilus* JCM 21760^T using the membrane filter method (Tourova & Antonov, 1987) as described by Reddy *et al.* (2003).

Analysis of 16S rRNA gene sequences indicated that *P. goriensis* KCCM 42329^T shared a high degree of sequence similarity (99.8 %) with *T. saccharophilus* IAM 15309^T, the type strain of the type species of the genus *Terribacillus*, and 98.9 % with *T. halophilus* IAM 15310^T. This high level of sequence similarity is generally not observed between strains belonging to different genera (Ludwig *et al.*, 1998). The three strains showed relatively low sequence similarity to other halophilic and halotolerant bacilli like *Ornithinibacillus bavariensis* (a maximum of 94.7 % similarity to the type strain), *Salirhabdus euzeyi* (94.3 %), *Paucisalibacillus globulus* (94.2 %), *Salinibacillus aidengensis* (93.9 %) and *Oceanobacillus picturae* (93.8 %) and less than 93.0 % to other members of *Bacillus* rRNA group 1. As shown in Fig. 1, the three strains along with strain 3LF 22T formed a distinct and tight cluster supported by a high bootstrap value of 100 %, thus emphasizing the fact that these four strains are phylogenetically closely related. A tree constructed using the maximum-parsimony method showed a similar topology (refer to Fig. 1). Moreover, members of these two genera also share many phenotypic characteristics (Table 1). For example, *T. saccharophilus* and *P. goriensis* have similar fatty acid profiles, with anteiso-C_{15:0} as the major fatty acid followed by anteiso-C_{17:0} and iso-C_{15:0}, similar major menaquinones (MK-7) and similar G+C contents (Table 1). These data support the results of phylogenetic analyses that the taxonomic position of the genus *Pelagibacillus* needs re-examination, and we propose that *P. goriensis* be transferred as a species of the genus *Terribacillus*. DNA–DNA hybridization of *P. goriensis* DSM 18252^T with *T. saccharophilus* JCM 21759^T and *T. halophilus* JCM 21760^T was 51.7 and 35.6 %, respectively.

Since the genomic relatedness is below the value of 70 % recommended for delineation of bacterial species (Wayne *et al.*, 1987), *P. goriensis* should be treated as a separate species within the genus *Terribacillus*. Results of DNA–DNA hybridization studies also revealed that strain 3LF 22T shared 79.8 % similarity at the genomic level with *P. goriensis* DSM 18252^T and 26.2 % with *T. saccharophilus* JCM 21759^T. Although *P. goriensis* and *T. saccharophilus* share a number of characteristics (Table 1), they differ in some phenotypic traits like the Voges–Proskauer test, β -galactosidase activity, optimal NaCl concentration for growth, acid production from melibiose and utilization of D-xylose, D-glucose, D-fructose, sucrose, glycerol and mannitol. These differences in phenotypic properties may be used to delineate the two taxa.

Reclassifications such as that proposed here may be required when two different research groups working on novel strains at the same time communicate their manuscripts for publication at about the same time. It appears that, in this case, the two research groups would probably not have known which of the strains was going to be described first, as the database containing the sequence information would have displayed them as unpublished at that time. A similar situation arose when the same strain, ATCC 19623 (originally described as a strain of *Zooglea ramigera*), was described almost simultaneously as a member of the novel genera *Crabtreeella* (Xie & Yokota, 2006) and *Shinella* (An *et al.*, 2006); *Crabtreeella saccharophila* is a later homotypic synonym of *Shinella zoogloeoides* (<http://www.bacterio.cict.fr/c/crabtreeella.html>). It is therefore quite possible that these situations can arise inadvertently.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences and reconstructed using the neighbour-joining method showing the relationship between *T. saccharophilus*, *P. goriensis* and closely related genera. Bootstrap values >50 % are shown at nodes as percentages of 1000 replications. The sequence from *Paenibacillus polymyxa* IAM 13419^T was used as an outgroup. Bar, 0.02 substitutions per site. Dots indicate nodes that were also recovered using the maximum-parsimony method.

Table 1. Comparative phenotypic characteristics of the type strains of *T. saccharophilus*, *T. halophilus* and *P. goriensis*

Strains: 1, *T. saccharophilus* 002-048^T (data from An *et al.*, 2007) 2, *P. goriensis* CL-GR16^T (Kim *et al.*, 2007); 3, *T. halophilus* 002-051^T (An *et al.*, 2007). The three strains share many characteristics such as swollen sporangia, no requirement for NaCl for growth, being positive for catalase activity and gelatin hydrolysis and being negative for oxidase activity, anaerobic growth, reduction of nitrate, H₂S production, arginine dihydrolase, lysine decarboxylase, tryptophan deaminase, urease and indole production. All three strains have MK-7 as their major menaquinone. Fatty acids that were present in amounts less than 0.5 % in all three strains are not shown. NA, Data not available; w, weakly positive.

Characteristic	1	2	3
Spore shape*	E, S	E	E, S
Spore position†	ST, T	C, ST	ST, T
Motility	—	+	—
Voges–Proskauer test	+	—	+
Acid production from:			
Amygdalin, melibiose	—	+	—
Glucose, sucrose	+	+	—
Rhamnose	—	w	—
Utilization of:			
L-Arabinose	—	—	+
Citrate, xylose	—	+	—
D-Fructose, D-glucose, glycerol, mannitol, sucrose	+	—	+
Galactose, raffinose, trehalose	+	+	—
Melibiose	+	NA	—
Production of β -galactosidase	—	+	—
Growth temperature range (°C)	5–45	15–43	5–45
pH range for growth	6–10	5.5–9.0	5–10
NaCl concentration for growth (% w/v)			
Range	0–16	0–14	0–19
Optimum	1–5	0–2	1–5
DNA G + C content (mol%)	44.0	43.0	45.8
Fatty acids (%)			
C _{14:0}	1.2	1.9	1.4
C _{15:0}	NA	0.7	NA
C _{16:0}	1.3	2.9	1.4
C _{14:1} ω 5c	NA	0.8	NA
iso-C _{14:0}	2.3	2.6	1.1
iso-C _{15:0}	7.6	9.1	5.6
anteiso-C _{15:0}	63.5	65.6	47.6
iso-C _{16:0}	5.5	4.2	5.3
anteiso-C _{17:0}	17.9	11.0	34.8
iso-C _{17:0}	0.7	0.4	1.1

*E, Ellipsoidal; S, spherical.

†C, Central; ST, subterminal; T, terminal.

Emended description of the genus *Terribacillus* An *et al.* 2007

Terribacillus [Ter.ri.ba.cil'lus. L. n. *terra* earth; L. masc. n. *bacillus* a small staff; N.L. masc. n. *Terribacillus* earth (soil) bacillus (rod)].

The description of the genus is the same as that given by An *et al.* (2007). In addition, the cells may be motile or non-motile and β -galactosidase-positive or -negative. The Voges–Proskauer test may be positive or

negative. The DNA G + C content is in the range 43.0–46.0 mol%.

Description of *Terribacillus goriensis* (Kim *et al.* 2007) comb. nov.

Terribacillus goriensis (go.ri.en'sis. N.L. masc. adj. *goriensis* from Gori, where the type strain was isolated).

Basonym: *Pelagibacillus goriensis* Kim *et al.* 2007.

The description is the same as that given for *Pelagibacillus goriensis* by Kim *et al.* (2007).

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