

*Lysinibacillus macroides* sp. nov., nom. rev.

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'*Bacillus macroides*' ATCC 12905<sup>T</sup> (=DSM 54<sup>T</sup>=LMG 18474<sup>T</sup>), isolated in 1947 from cow dung, was not included in the Approved Lists of Bacterial Names and so it lost standing in bacteriological nomenclature. Reinvestigation of the strain, including DNA–DNA relatedness experiments, revealed that '*Bacillus macroides*' is genomically distinct from its closest relatives *Lysinibacillus xylanilyticus*, *Lysinibacillus boronitolerans* and *Lysinibacillus fusiformis* (as determined by 16S rRNA gene sequence analysis, with pairwise similarity values of 99.2, 98.8 and 98.5 %, respectively, with the type strains of these species). Further analysis showed that '*Bacillus macroides*' shares the A4x L-Lys–D-Asp peptidoglycan type with other members of the genus *Lysinibacillus* and can thus be attributed to this genus. These results, combined with additional phenotypic data, justify the description of strain LMG 18474<sup>T</sup> (=DSM 54<sup>T</sup>=ATCC 12905<sup>T</sup>) as *Lysinibacillus macroides* sp. nov., nom. rev.

Pringsheim & Robinow (1947) gave the provisional name '*Lineola longa*' to a very large, Gram-negative, filamentous bacterium of unusual morphology. Later, Pringsheim (1950) described the genus *Lineola* with '*L. longa*' as the type species, and mentioned that a subculture of '*L. longa*' had been deposited in the American Type Culture Collection (ATCC). Bennett & Canale-Parola (1965) reinvestigated the type strain (ATCC 12905<sup>T</sup>) and they found that its morphology corresponded closely to the earlier description. However, the strain formed endospores under suitable conditions and stained Gram-positive. Bennett & Canale-Parola (1965) mentioned that they sent a culture to Pringsheim, who recognized it as '*L. longa*'. In addition, Pringsheim confirmed the formation of endospores in stock cultures of '*L. longa*' kept in his laboratory. After confirmation of strain originality, Bennett & Canale-Parola (1965) concluded that '*L. longa*' should be renamed '*Bacillus macroides*'. The species was not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) because there were too few strains available and so no adequate description existed at the time the lists were compiled. As a consequence, it lost standing in bacteriological nomenclature. The species was included in the

*Species Incertae Sedis* section of *Bacillus* in *Bergey's Manual* (Logan & De Vos, 2009), with the comment that the characters of '*B. macroides*' conform to those of *Bacillus sphaericus* (recently reclassified as *Lysinibacillus sphaericus*; Ahmed *et al.*, 2007) with the exception of endospore morphology, the endospore of '*B. macroides*' being described as frankly oval and scarcely distending the sporangium.

At the beginning of this study, two strains named '*B. macroides*' were available: ATCC 12905<sup>T</sup> (=DSM 54<sup>T</sup>=LMG 18474<sup>T</sup>), on which the original description was based, and NCIMB 8796 (=NCDO 1661=LMG 18508), of which a 16S rRNA gene sequence was available (EMBL accession number X70312). A discrepancy was observed between these two strains. On the one hand, Xu & Côté (2003) demonstrated that '*B. macroides*' strain ATCC 12905<sup>T</sup> shared highest 3'-end 16S rRNA gene and 5' end 16S–23S ITS sequence similarity with *Bacillus fusiformis* (= *Lysinibacillus fusiformis*; Ahmed *et al.*, 2007) and *B. sphaericus* (= *L. sphaericus*; Ahmed *et al.*, 2007); on the other hand, '*B. macroides*' strain NCIMB 8796 showed an almost identical 16S rRNA gene sequence to *Bacillus simplex* LMG 11160<sup>T</sup>. In a polyphasic study by Heyrman *et al.* (2005), including DNA–DNA relatedness experiments, it was shown that '*B. macroides*' strain NCIMB 8796 indeed belongs to *B. simplex* and should be renamed accordingly. Since the characteristics of '*B.*

Five supplementary figures and a supplementary table are available with the online version of this paper.

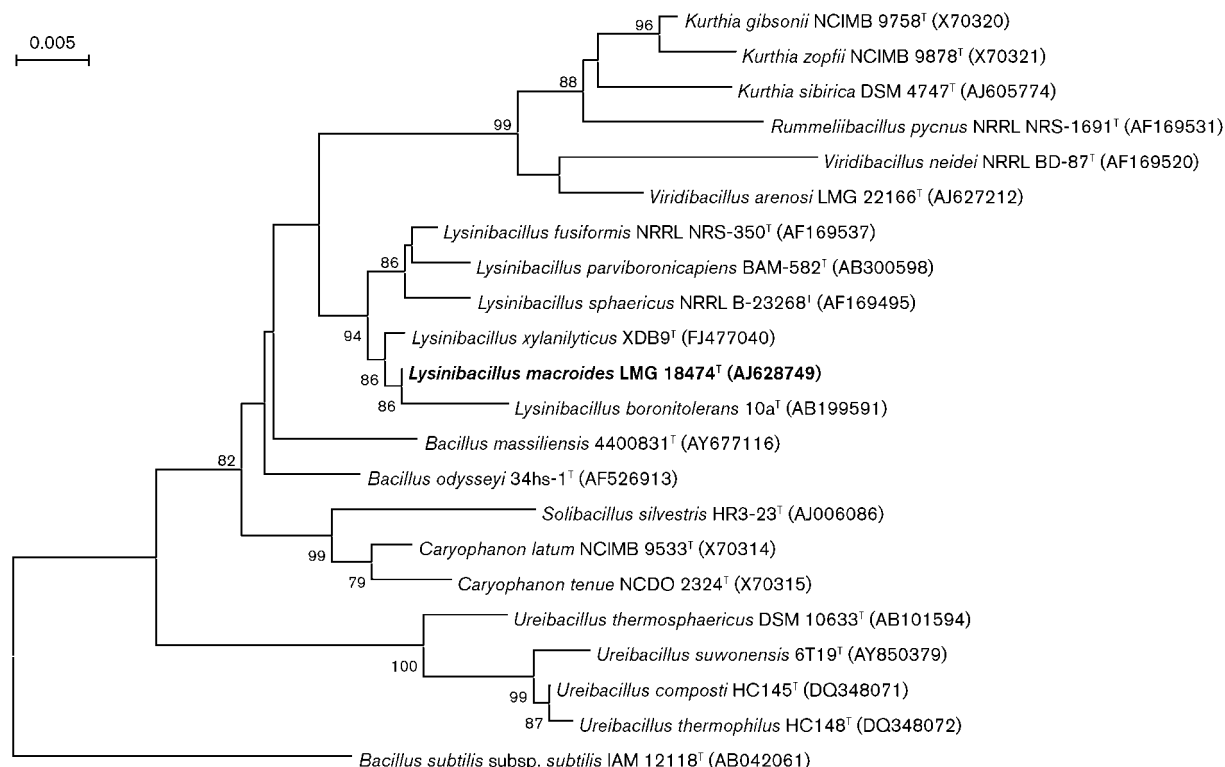
*macroides* strain ATCC 12905<sup>T</sup> conform to the original description of the species, it can be concluded that this strain is the true '*B. macroides*'. Ahmed *et al.* (2007) transferred the closest relatives of '*B. macroides*' to *Lysinibacillus* as *L. sphaericus* and *L. fusiformis*, and also described the novel species *Lysinibacillus boronitolerans*. Miwa *et al.* (2009) described another novel species of this genus, *Lysinibacillus parviboronicapiens*, isolated from soil, and Lee *et al.* (2010) proposed a xylan-degrading strain isolated from forest humus as *Lysinibacillus xylanilyticus*. Recently, Jung *et al.* (2010a) described the transfer of two *Bacillus* species, *Bacillus odyseeyi* and *Bacillus massiliensis*, to the genus *Lysinibacillus*, and described a further novel species, *Lysinibacillus sinduriensis*, isolated from tidal flat sediment samples taken from the Yellow Sea, Korea. However, this publication was retracted prior to print, due to difficulties with chemotaxonomic characterization of the type strain of *L. sinduriensis* (Jung *et al.*, 2010b).

The genus *Lysinibacillus* is characterized by a distinctive peptidoglycan composition (type A4 $\alpha$  L-Lys-D-Asp). In this study, we investigated whether LMG 18474<sup>T</sup> (=ATCC 12905<sup>T</sup>=DSM 54<sup>T</sup>) deserves separate species status, following the minimal standards for description of new taxa of aerobic endospore-forming bacteria, as recommended by Logan *et al.* (2009). Furthermore, given the recent taxonomic rearrangements, we also investigated whether LMG 18474<sup>T</sup> has the phenotypic traits that characterize members of the genus *Lysinibacillus*. For clarity, LMG 18474<sup>T</sup> is hereinafter referred to as *Lysinibacillus macroides*.

To confirm authenticity of strain LMG 18474<sup>T</sup>, the subculture used in this study, DNA and MALDI fingerprints of LMG 18474<sup>T</sup>, ATCC 12905<sup>T</sup> and DSM 54<sup>T</sup> were generated and, as can be seen in Fig. S1 (available in IJSEM Online), these profiles proved to be identical. Based on the literature discussed above, the 16S rRNA gene sequence of *L. macroides* strain LMG 18474<sup>T</sup> (AJ628749) was compared with the 16S rRNA gene sequences of type strains of all described *Bacillus* and *Lysinibacillus* species, as well as with members of the genera *Kurthia* and *Caryophanon*. Pairwise similarity values were calculated using the ARB software (Ludwig *et al.*, 2004) and were found to be highest with members of the genus *Lysinibacillus*, i.e. 99.2 % with *L. xylanilyticus* XDB9<sup>T</sup>, 98.8 % with *L. boronitolerans* 10a<sup>T</sup>, 98.5 % with *L. fusiformis* NRRL NRS-350<sup>T</sup>, 97.9 % with *L. sphaericus* NRRL B-23268<sup>T</sup> and 97.5 % with *L. parviboronicapiens* BAM-582<sup>T</sup>. Phylogenetic trees based on these 16S rRNA gene sequences were constructed by aligning all sequences based on the integrated aligner of the ARB software (Ludwig *et al.*, 2004). Refinement of the alignment was obtained by applying the bacterial position variability filter integrated in ARB, resulting in a final alignment of 1402 positions. The alignment was then exported to MEGA5 software (Tamura *et al.*, 2011) to construct neighbour-joining, maximum-likelihood and maximum-parsimony trees. The jModelTest 0.1.1 program (Posada, 2008) was applied to the dataset to determine the best fit evolutionary

model, and the resulting parameters, namely the GTR evolutionary model with 0.581 as the proportion of invariable sites value and a gamma shape value of 0.418, were applied for tree construction where appropriate. Bootstrap analysis based on 1000 replicates was performed on all three types of trees to assess the reliability of the clusters. The resulting neighbour-joining tree is represented in Fig. 1; maximum-parsimony consensus and maximum-likelihood trees are shown in Figs S2 and S3, respectively (available in IJSEM Online). In all three trees, *L. macroides*, *L. boronitolerans* and *L. xylanilyticus* grouped together and were then joined by a group including *L. sphaericus*, *L. fusiformis* and *L. parviboronicapiens*, both subgroups forming a monophyletic cluster of *Lysinibacillus* species. Based on these findings, a DNA–DNA relatedness study and determinations of the DNA G + C content were performed on *L. macroides* LMG 18474<sup>T</sup>, *L. boronitolerans* DSM 17140<sup>T</sup>, *L. xylanilyticus* CCUG 57438<sup>T</sup>, *L. sphaericus* LMG 7134<sup>T</sup>, *L. parviboronicapiens* KCTC 13154<sup>T</sup> and *L. fusiformis* LMG 9816<sup>T</sup>. For determination of the DNA G + C content and DNA–DNA hybridization, about 1 g biomass was harvested from tryptone soy agar (TSA) plates and DNA was purified as described by Logan *et al.* (2000). DNA–DNA hybridization was performed using a modification of the microplate method described by Ezaki *et al.* (1989), as described by Willems *et al.* (2001). A hybridization temperature of 32 °C was used (calculated with correction for the presence of 50 % formamide). The DNA G + C content was determined by HPLC (Mesbah *et al.*, 1989), using further specifications given by Logan *et al.* (2000). Mean DNA-relatedness values of *L. macroides* towards the type strains of *L. xylanilyticus*, *L. boronitolerans*, *L. fusiformis*, *L. sphaericus* and *L. parviboronicapiens* were 35, 31, 31, 24 and 21 %, respectively. This indicates that LMG 18474<sup>T</sup> at least represents a separate genospecies. Type strains of the closely related species *L. sphaericus* and *L. fusiformis* showed a DNA–DNA relatedness value of 25.2 %, confirming their separate species status. The DNA G + C value of *L. macroides* was 38.2 mol% (mean value of three determinations), which is highly similar to that of *L. sphaericus* and *L. fusiformis* (both 37.2 mol%). Bennett & Canale-Parola (1965) reported a DNA G + C value of 42.0 mol% for *L. macroides*, which is rather higher than the value obtained in this study.

Purified peptidoglycan preparations were obtained after disruption of cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). The amino acids and peptides in the cell wall hydrolysates [(i) 6 M HCl, 120 °C, 16 h; (ii) 4 M HCl, 100 °C, 16 h; (iii) 4 M HCl, 100 °C, 45 min] were analysed by two-dimensional ascending TLC on cellulose plates by using previously described solvent systems (Schleifer, 1985). The molar ratios of the amino acids were determined by GC and GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996). Cell material of *L. macroides* LMG 18474<sup>T</sup> and *L. boronitolerans* DSM 17140<sup>T</sup> was obtained after growth



**Fig. 1.** Neighbour-joining tree based on multiple alignment of partial 16S rRNA gene sequences (1402 positions were taken into account). Bootstrap values above 70 % (based on 1000 replications) are represented at the branch points. Accession numbers of the sequences are shown in parentheses. The 16S rRNA gene sequence of *Bacillus subtilis* subsp. *subtilis* strain IAM 12118<sup>T</sup> (AB042061) was included as an outgroup to root the tree. Bar, 0.005 substitutions per nucleotide position.

on TSA (Oxoid) for 24 h at 28 °C. Polar lipids were subsequently extracted and separated by using two-dimensional TLC according to Tindall (1990a, b). The total lipid profiles were visualized by spraying with molybdatophosphoric acid and further characterized by spraying with ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates) and  $\alpha$ -naphthol (specific for sugars). GC analysis of fatty acid methyl esters was performed starting from cells grown on plates containing 30 g trypticase soy broth (BBL, Becton Dickinson) supplemented with 15 g Bacto Agar (Difco) per litre of distilled water, and the plates were incubated for 24 h at 28 °C. Fatty acid methyl ester extraction and analysis were performed as described by Vancanneyt *et al.* (1996). For determination of cell and sporangial morphologies, isolates were grown on TSA at pH 7 and 30 °C for 24–48 h and further observed as described by Logan *et al.* (2000). Strains were characterized phenotypically as described by Logan & De Vos (2009); anaerobic growth was tested using a GasPak jar with a methylene blue indicator strip, and an aerobic control culture. *L. macroides* strain LMG 18474<sup>T</sup> was tested for carbon source assimilation using the Biotype 100 Gallery (bioMérieux), according to Logan *et al.* (2002). Additionally, *L. macroides* LMG 18474<sup>T</sup>, *L. boronitolerans* DSM 17140<sup>T</sup>, *L. sphaericus* LMG 7134<sup>T</sup>, *L. fusiformis* LMG

9816<sup>T</sup> and *L. xylanilyticus* CCUG 57438<sup>T</sup> were analysed using the Biolog Gen III characterization system (Biolog) following protocol B according to the manufacturer's instructions.

Peptidoglycan analysis revealed that strain LMG 18474<sup>T</sup> has the A4 $\alpha$  L-Lys-D-Asp peptidoglycan type, confirming the result of an earlier analysis (Schleifer & Kandler, 1972), and in agreement with the description of *Lysinibacillus* (Ahmed *et al.*, 2007). *Kurthia* species are also characterized by this type of cell wall, with lysine and aspartic acid as the diagnostic amino acids (Albert *et al.*, 2007); however, members of this genus can easily be differentiated from members of the genus *Lysinibacillus* by their inability to form endospores. Members of the genus *Ureibacillus* were characterized by an A4 $\alpha$  L-Lys-D-Asn type of peptidoglycan (Fortina *et al.*, 2001), but the recently described species *Ureibacillus composti* and *Ureibacillus thermophilus* show the A4 $\alpha$  L-Lys-D-Asp peptidoglycan type (Weon *et al.*, 2007), as observed in *Lysinibacillus* species. Albert *et al.* (2007) transferred three *Bacillus* species, *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei*, to the novel genus *Viridibacillus* based on discriminative chemotaxonomic markers. Analysis of cell wall type revealed that *Viridibacillus arvi* DSM 16317<sup>T</sup> and *Viridibacillus arenosi* DSM 16319<sup>T</sup> had the same A4 $\alpha$  L-Lys-D-Asp peptidoglycan



type, whereas *Viridibacillus neidei* NRRL BD-87<sup>T</sup> and *Bacillus pycnus* NRRL BRS-1691<sup>T</sup> (now *Rummeliibacillus pycnus*; Vaishampayan *et al.*, 2009) were characterized by the L-Lys-D-Glu peptidoglycan type (Albert *et al.*, 2007). The latter type has also been reported for *Solibacillus silvestris* (Rheims *et al.*, 1999). Despite the differences in peptidoglycan type between *V. arenosi*, *V. arvi* and *V. neidei*, all three species have been assembled within a single genus, *Viridibacillus*. This is in concordance with Abd El-Rahman *et al.* (2002), who did not wish to propose a new genus to accommodate *Bacillus psychrodurans*, *Bacillus psychrotolerans* and *Bacillus insolitus* based only upon the distinctive A4β L-Orn-D-Glu type alone. They reasoned that such single-parameter-based taxa are prone to be unstable and that such a proposal would be premature. However, very recently Krishnamurthi *et al.* (2010) did transfer these three species to the novel genus *Psychrobacillus* based on phenotypic, including chemotaxonomic, characteristics, and ribotype patterns. The view of Abd El-Rahman *et al.* (2002) is in contrast to Ahmed *et al.* (2007), who delineated *Lysinibacillus* mainly on the basis of its distinctive peptidoglycan type. A comprehensive polyphasic study of the *Bacillus* rRNA group 2 organisms (Ash *et al.*, 1991) might reveal 'good' borderlines between existing genera and potential new ones, and future rearrangements within this group can be expected. However, it must be said that it is difficult to find phenotypic differences between the members of this group as they tend to be unreactive in many routine characterization tests (e.g. API 50CHB and API Biotype 100 kits). *L. macroides*, for example, is unreactive in API 50CHB and 20E tests and did not show growth on most of the carbon sources in the Biotype 100 carbon source assimilation gallery (bioMérieux). Using the Biolog Gen III system, strains *L. macroides* LMG 18474<sup>T</sup>, *L. boronitolerans* DSM 17140<sup>T</sup>, *L. sphaericus* LMG 7134<sup>T</sup>, *L. fusiformis* LMG 9816<sup>T</sup> and *L. xylanilyticus* CCUG 57438<sup>T</sup> reacted positively for utilization of inosine, L-arginine, L-glutamic acid, L-histidine, L-serine, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, Tween 40, α-hydroxybutyric acid, β-hydroxy-DL-butyric acid, α-ketobutyric acid, diacetic acid and acetic acid. Strains were sensitive to fusidic acid, troleandomycin, minocycline, lincomycin, sodium tetradecyl sulfate, vancomycin, tetrazolium violet and tetrazolium blue. *L. macroides* LMG 18474<sup>T</sup> could be differentiated from its closest neighbours based on the Biolog Gen III system by its sensitivity to lithium chloride, aztreonam and sodium butyrate; additional differential characters based on Biolog Gen III are given in Table 1. Bennett & Canale-Parola (1965) reported '*B. macroides*' as negative for the Voges-Proskauer test and weakly positive for gelatin hydrolysis, but *L. macroides* LMG 18474<sup>T</sup> reacted positively for Voges-Proskauer and did not hydrolyse gelatin. Logan & De Vos (2009) repeated the comment of Claus & Berkeley (1986) that the endospore of '*B. macroides*' is frankly oval and scarcely distends the sporangium, a character that distinguishes it from *B. sphaericus* (= *L. sphaericus*). However, in the present work, the endospore and sporangial morphologies of *L. macroides*

LMG 18474<sup>T</sup> were not observed to be distinctly different from those of *L. sphaericus* (Fig. 2 and Fig. S4 available in IJSEM Online). The major fatty acid for *L. macroides* LMG 18474<sup>T</sup> was iso-C<sub>15:0</sub> (45.3 ± 4.6 %). Moderate amounts of iso-C<sub>16:0</sub> (12.8 ± 0.6 %), C<sub>16:1ω7c</sub> alcohol (12.3 ± 2.0), anteiso-C<sub>15:0</sub> (7.9 ± 3.0 %), C<sub>16:1ω11c</sub> (7.2 ± 2.0 %) and iso-C<sub>14:0</sub> (5.1 ± 1.1 %) were detected, whereas minor to trace amounts of iso-C<sub>17:0</sub> (3.3 ± 0.5 %), anteiso-C<sub>17:0</sub> (1.8 ± 0.6 %), C<sub>16:0</sub> (1.7 ± 0.8 %), iso-C<sub>17:1ω10c</sub> (1.3 ± 0.8) and C<sub>14:0</sub> (<1.00 %) were observed. These values (based on four replicate profiles of strain LMG 18474<sup>T</sup>) confirm the placement of strain LMG 18474<sup>T</sup> in the genus *Lysinibacillus*, which is characterized by iso-C<sub>15:0</sub> as the major fatty acid (Ahmed *et al.*, 2007). Furthermore, fatty acid profiles of the type strains of neighbouring species have been generated and are presented in Table S1 (available in IJSEM Online). Different relative amounts of anteiso-C<sub>15:0</sub> allow differentiation of *L. macroides* LMG 18474<sup>T</sup> from *L. boronitolerans* DSM 17140<sup>T</sup> and *L. fusiformis* LMG 9816<sup>T</sup>; *L. macroides* LMG 18474<sup>T</sup> can be differentiated from *L. xylanilyticus* CCUG 57438<sup>T</sup> based on a lower amount of iso-C<sub>15:0</sub>. A higher relative abundance of C<sub>16:1ω7c</sub> alcohol and the absence of anteiso-C<sub>17:0</sub> and C<sub>16:0</sub> differentiated *L. sphaericus* LMG 7134<sup>T</sup> from *L. macroides* LMG 18474<sup>T</sup>. Major polar lipids for *L. macroides* LMG 18474<sup>T</sup> and *L. boronitolerans* DSM 17140<sup>T</sup> were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Phosphoaminolipids (PAL1 to 4) and unknown lipids (L1 to 5) have also been detected in minor amounts; both profiles were highly similar but phosphoaminolipid PAL1, detected in LMG 18474<sup>T</sup>, was not present in DSM 17140<sup>T</sup> (Fig. S5a and b, available in IJSEM Online). This profile matches the polar lipid profile described for the genus *Lysinibacillus* with diphosphatidylglycerol and phosphatidylglycerol as the major components (Ahmed *et al.*, 2007). However, glycolipids, which are typical for members of the genera *Bacillus*, *Paenibacillus* and *Cohnella* (Kämpfer *et al.*, 2006), were not detected in this study, although Ahmed *et al.* (2007) reported on a ninhydrin-positive phosphoglycolipid. The profile of *L. macroides* LMG 18474<sup>T</sup> is much more complex than the profile of *Kurthia* species as reported by Goodfellow *et al.* (1980), enabling their distinction. Phenotypic and chemotaxonomic data allow the differentiation of *L. macroides* from other *Lysinibacillus* species as shown in Table 1.

Based on the data discussed above, '*B. macroides*' strain LMG 18474<sup>T</sup> is proposed as *Lysinibacillus macroides* sp. nov., nom. rev.

### ***Lysinibacillus macroides* sp. nov., nom. rev.**

*Lysinibacillus macroides* [ma.cro.i' des. Gr. adj. *makros* large or long; Gr. suff. *-eides* (from Gr. n. *eidos* form or shape) resembling, similar; N.L. masc. adj. *macroides* long in form, describing the elongated appearance of the rods].

Description is based on a single strain. Strictly aerobic, Gram-positive and Gram-negative motile rods. Cell size is

**Table 1.** Characteristics that differentiate *L. macroides* from the type strains of other *Lysinibacillus* species

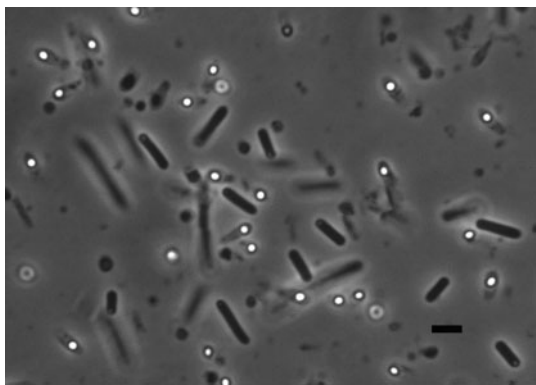
All data are from this study. Strains: 1, *Lysinibacillus macroides* LMG 18474<sup>T</sup>; 2, *Lysinibacillus boronitolerans* DSM 17140<sup>T</sup>; 3, *Lysinibacillus sphaericus* LMG 7134<sup>T</sup>; 4, *Lysinibacillus fusiformis* LMG 9816<sup>T</sup>; 5, *Lysinibacillus xylanilyticus* CCUG 57438<sup>T</sup>. All type strains gave positive results for motility, growth without NaCl, catalase activity and Voges–Proskauer test, and negative results for H<sub>2</sub>S production,  $\beta$ -galactosidase (ONPG) and indole production. +, Positive reaction; –, negative reaction; w, weak reaction; –/w, negative or weakly positive; v, variable; ND, no data available.

Characteristic	1	2	3	4	5
Cell length ( $\mu$ m)	3.0–100*	3.0–5.0	1.5–5.0	1.5–5.0	3.0–5.0
Gram reaction	v	+	+	v	+
Growth at:					
10 °C	w	–	+	–	+
45 °C	+	+	–	–	–
Growth in/at:					
5 % (w/v) NaCl	–	+	+	+	+
7 % (w/v) NaCl	–	–	–	+	–
pH 6.0	–	+	+	+	+
Oxidase	–	+	+	+	+
Nitrate reduction	–	–	+	–	+
L-Arginine dihydrolase	–	w	w	–	–
Hydrolysis of:					
Aesculin	–	–/w	–	–	ND
Casein	–	–	+	+	+
Gelatin	–	–	–	+	+
Urea	–	+	–	+	–
Acid production from:					
N-Acetyl-D-glucosamine	–	+	–	+	–
N-Acetyl- $\beta$ -D-mannoside	–	+	+	+	+
D-Glucose	–	–	–	w	–
Utilization of:					
Citrate	w	–	+	+	w
Raffinose	+	+	–	+	–
Glycerol	–	–	+	–	+
D-Fructose 6-phosphate	–	+	–	+	–
D-Gluconic acid	+	+	–	+	–
Propionic acid	+	–	+	+	+
Formic acid	–	–	+	–	+
DNA G + C content (mol%)	38.2	36.5	37.2	37.2	37.2

\**L. macroides* produces long filaments in broth culture.

0.9–1.1  $\times$  3.0–5.0  $\mu$ m on plate cultures, but long filaments of 10–100  $\mu$ m or more may be formed in broth cultures. Endospores form within 24–48 h of incubation at 30 °C on TSA containing 5 mg MnSO<sub>4</sub> l<sup>–1</sup>. Endospores are ellipsoidal but nearly spherical, lie terminally or subterminally and may swell the sporangia slightly; sporangia readily become ghost cells and endospores are released early. After 24 h incubation at 30 °C on TSA, colonies are moist and loose in texture, circular in shape, cream in colour, with irregular edges and glossy surfaces, and have diameters of 0.5–1.0 mm; colonies reach 3.0–5.0 mm in diameter after 48 h. Growth occurs at pH 7.0–9.0, with optimum growth occurring at pH 8.0; no growth occurs at pH 6.0. Growth occurs between 20 °C and 45 °C; growth is weak at 10 °C and optimum growth occurs at 30 °C. Grows

in the presence of 0 % (w/v) NaCl and in up to 4 % (w/v) NaCl, but not in 5 % (w/v). Aesculin, casein and starch are not hydrolysed. Catalase production is positive and oxidase production is negative. The Voges–Proskauer (acetoin production) test is positive, but citrate utilization, gelatin hydrolysis, nitrate reduction, and L-arginine dihydrolase, hydrogen sulfide, indole, lysine decarboxylase, ornithine decarboxylase, ONPG, tryptophan deaminase and urease production are negative. Does not produce acid or gas from carbohydrates. The amino acids L-histidine and L-proline, and the organic acids citrate, DL-lactate and  $\beta$ -hydroxybutyrate, are weakly assimilated. Raffinose, D-gluconic acid and propionic acid are also assimilated. Cell wall peptidoglycan is of the A4 $\alpha$  L-Lys–D-Asp type. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine.



**Fig. 2.** Photomicrograph of sporangia and vegetative cells of the type strain of *L. macroides* LMG 18474<sup>T</sup>, viewed by phase-contrast microscopy; spherical and slightly ellipsoidal spores lie terminally and subterminally in sporangia that are slightly swollen. Bar, 2 µm.

The predominant fatty acid is iso-C<sub>15:0</sub>; moderate amounts of iso-C<sub>16:0</sub> and C<sub>16:1</sub>ω7c alcohol are present.

The type strain is LMG 18474<sup>T</sup> (=DSM 54<sup>T</sup>=ATCC 12905<sup>T</sup>), which was isolated from cow dung. The DNA G+C content of the type strain is 38.2 mol%.

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