***Bacillus silvestris* sp. nov., a new member of the genus *Bacillus* that contains lysine in its cell wall**

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A Gram-positive, aerobic, rod-shaped, peritrichously flagellated, round-endospore-forming bacterium was isolated from a forest soil near Braunschweig, Lower Saxony, Germany, and designated strain HR3-23^T (T = type strain). Morphologically, strain HR3-23^T shows the characteristics of a member of the genus *Bacillus*. The spore position is terminal in a swollen sporangium. Comparative analysis of the 16S rDNA sequence shows strain HR3-23^T to be most closely related to *Caryophanon tenue* (95.8% 16S rRNA similarity) and to *Bacillus sphaericus* (95.4% 16S rRNA similarity). Phylogenetically, the isolate clusters among species of *Bacillus* RNA group 2. The DNA G+C content of isolate HR3-23^T is 39.3 mol%, the peptidoglycan type is A4 α (L-Lys-D-Glu), the major respiratory lipoquinone is menaquinone MK-7 and the predominant fatty acid is of the iso-C_{15:0} type. Based on the morphological, chemotaxonomic, physiological and phylogenetic properties, a new species, *Bacillus silvestris*, is proposed; strain HR3-23^T is the type strain (= DSM 12223^T).

Keywords: *Bacillus silvestris*, *Bacillus* RNA group 2, 16S rDNA analysis, phylogeny, chemotaxonomy

INTRODUCTION

The genus *Bacillus*, as defined traditionally, comprises Gram-positive, rod-shaped, endospore-forming bacteria with an aerobic or facultatively aerobic metabolism. The recognition that this genus is 'a diverse assemblage that is a grouping of convenience' (Sneath, 1986) was furthermore demonstrated a few years later by comprehensive analyses of 16S rDNA nucleotide sequences of members of this genus (Ash *et al.*, 1991; Farrow *et al.*, 1992, 1994; Rainey & Stackebrandt, 1993; Rainey *et al.*, 1994). These findings provided the basis for the dissection of the genus *Bacillus* to describe several new genera containing former *Bacillus* species, such as *Alicyclobacillus* (Wisotzkey *et al.*, 1992), *Aneurinibacillus* (Shida *et al.*, 1996), *Brevibacillus*

(Shida *et al.*, 1996), *Paenibacillus* (Ash *et al.*, 1993) and *Virgibacillus* (Heyndrickx *et al.*, 1998). Analysis of 16S rDNA has developed into a fast and reliable method for phylogenetic affiliation of a novel isolate to one of these genera.

The rRNA group 2 of the genus *Bacillus* (Ash *et al.*, 1991), comprises round-spore-forming members of the genus *Bacillus*, containing L-lysine or D-ornithine at position three of the peptide subunit (Stackebrandt *et al.*, 1987). Analysis of 16S rRNA of Gram-positive bacteria from other genera indicated that this *Bacillus* group also contains non-*Bacillus*-type organisms, such as members of *Caryophanon* and *Planococcus* (Stackebrandt *et al.*, 1987; Farrow *et al.*, 1994), *Filibacter* (Clausen *et al.*, 1985) and *Sporosarcina* (Pechman *et al.*, 1976; Farrow *et al.*, 1992).

In this work, we describe a new round-spore-forming species of the genus *Bacillus* that was isolated from forest soil. Phylogenetically and chemotaxonomically, it is related to members of *Bacillus* RNA group 2. The isolate can be distinguished from other species of this

Abbreviations: SEM, scanning electron microscopy; TEM, transmission electron microscopy; VP test, Voges–Proskauer test.

The EMBL accession number for the 16S rDNA sequence of *Bacillus silvestris* HR3-23^T is AJ006086.

group by the primary structure of the 16S rDNA and its phenotypic properties.

METHODS

Isolation of and cultural conditions for strain HR3-23^T. A soil sample was taken from a beech forest soil near Braunschweig, Germany. The sample (10 g) was placed into a Petri dish which was then stored in a jar with moistened paper cloths. Following incubation at 40 °C for 15 min, the soil was subsequently suspended in 100 ml germination solution. The germination solution consisted of 10⁻¹ concentrated glucose/yeast extract/malt extract medium (0.4 g glucose, 0.4 g yeast extract, 1.0 g malt extract, 0.2 g CaCO₃ in 1 l distilled water) with the addition of 1.0 g L-asparagine. After autoclaving, the solution was allowed to cool down to room temperature. A filter-sterilized cycloheximide solution (0.1 g dissolved in 10 ml water) was added.

Two subsequent tenfold dilutions of the soil suspension in germination solution were set up. The 10⁻³ dilution step was stirred at room temperature for 15 min. Aliquots of 100 µl were then streak-plated on different isolation media and incubated at different temperatures.

Strain HR3-23^T was isolated from a plate with Difco tryptic soy broth agar (Difco; amended with 3.0 g yeast extract l⁻¹ and 14 g agar l⁻¹ prior to autoclaving), incubated aerobically for 1 d at 25 °C. Purity of the culture was confirmed by serial plating onto the same medium. Good growth of the isolate was also observed on the R-medium of Yamada & Komagata (1972).

Determination of physiological characteristics. The following physiological tests were carried out according to Gordon *et al.* (1973): catalase and oxidase reactions, anaerobic growth, Voges-Proskauer (VP) test, temperature range for growth (5–55 °C in steps of 5 °C), presence of lecithinase, resistance to lysozyme, growth in the presence of NaCl (0, 2, 5, 7 and 10%), growth at pH 5.7, formation of acid from D-glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, formation of gas from glucose, hydrolysis of starch, decomposition of Tween 80, use of citrate and propionate, reduction of nitrate, production of indole, deamination of phenylalanine, decomposition of casein and tyrosine, liquefaction of gelatin and presence of arginine dihydrolase.

Hydrolysis of aesculin was tested according to Lanyi (1989) in a medium consisting of 10.0 g Bacto-peptone, 1.0 g sodium citrate, 1.0 g aesculin and 0.05 g iron citrate in 1 l water at pH 6.8–7.0.

A loopful of cell material of strain HR3-23^T was used to set up an overnight culture on a plate of Difco tryptic soy broth agar, incubated at 25 °C. From this culture, a suspension corresponding to McFarland standard 1 (OD₅₅₀ of 0.25; bioMérieux) was prepared in 1 ml sterile water. One drop of this suspension was added to each of the test tubes.

Growth in the VP test, NaCl tolerance and the presence of lecithinase were negative under the conditions described by Gordon *et al.* (1973), even after prolonged incubation. Therefore, these tests were repeated, adding half a volume of a 0.1% (w/v) sterile yeast extract solution to the culture suspension prior to inoculation. As growth failed or was too poor for a sound evaluation, these tests were repeated using the following media. For the VP test, a medium consisting of 5.0 g glucose, 5.0 g peptone from tryptic-digested casein, and 5.0 g KH₂PO₄ in 1 l water, pH 7.2 was used. For the test of

growth in the presence of different NaCl concentrations, culture tubes of Difco tryptic soy broth (prepared with the addition of yeast extract as described above) were set up containing various amounts of NaCl.

The lecithinase test was repeated with Difco tryptic soy broth agar medium to which 5 ml egg yolk (egg yolk emulsion; Oxoid) was added after the medium had cooled down following autoclaving.

Physiological reactions were also tested using the substrate panel of the API 50CH system (bioMérieux).

Microscopic investigations

Primary morphological characterization was done by light microscopy, including phase-contrast observations. The size and ultrastructure of cells and spores were determined by electron microscopy.

Light microscopy. For the investigation of cell morphology, 2% (w/v) agar (dissolved in water)-covered slides were prepared. A drop of culture grown in TSB medium (trypticase soy broth; Becton Dickinson) overnight was placed on the agar. Flagella were stained according to the method of Heimbrook *et al.* (1989).

Transmission electron microscopy (TEM). Cells were centrifuged, washed with PBS (0.145 M NaCl, 0.15 M sodium phosphate; Sambrook *et al.*, 1989) and fixed on ice for 1 h with a fixation solution containing 3% (v/v) glutaraldehyde and 5% (v/v) formaldehyde. After three washings with PBS, samples were embedded in 1.5% (w/v) agar in water; the solidified agar was cut into small cubes and dehydrated on ice with a graded series of acetone (10, 30, 50 and 70% for 30 min each). At the 70% acetone dehydration step, the samples were treated with 2% (w/v) uranyl acetate in 70% acetone (overnight). Samples were then further dehydrated with 90 and 100% acetone (30 min on ice) and once in 100% acetone at room temperature for 1 h.

For infiltration with epoxy resin (using Spurr's resin formula; Spurr, 1969), samples were initially treated according to the following protocol: one part 100% acetone with one part resin for 6 h; one part acetone and two parts resin overnight; pure resin for another 2 d with several changes. Samples were then transferred to gelatin capsules which were filled with pure resin. Polymerization was at 70 °C for 8 h. After trimming of the samples, ultrathin sections cut with glass knives were collected onto Formvar-coated copper grids (300 mesh) and air-dried. Counter-staining was done with a Leica Ultrastainer (uranyl acetate at 40 °C for 30 min, lead citrate at 20 °C for 3 min). Samples were examined in a Zeiss EM910 TEM at an acceleration voltage of 80 kV and at calibrated magnifications.

Scanning electron microscopy (SEM). A culture was grown on the isolation agar medium for 24 h. Agar blocks were cut from the plate, fixed in 2.5% (v/v) glutaraldehyde and dehydrated on ice in a series of increasing acetone concentrations (10, 30, 50, 70, 90 and 100% for 10 min each). Preparations were dried at the critical point of liquid CO₂, sputter-coated with gold (10 nm thickness) and examined in a Zeiss model DSM 982 SEM.

Analysis of chemotaxonomic characteristics. Cell wall and lipid analysis were carried out as described previously (Groth *et al.*, 1996) using the methods of Schleifer & Kandler (1972), MacKenzie (1987), Stead *et al.* (1992), Collins *et al.* (1977), Minnikin *et al.* (1979, 1975) and Collins & Jones (1980).

Fig. 1. Microscopic images of cells of strain HR3-23^T grown on TSB medium; arrows mark the spore positions. (a) Light microscopic image; bar, 2 µm. (b) SEM image of flagellated vegetative cells; bar, 1 µm. (c) SEM image of spores; the vegetative cell walls have already been fully lysed; bar, 0.5 µm. (d) Detail of an SEM image of a spore; remains of the cell wall of the vegetative cell are visible; bar, 0.25 µm. (e) TEM image of cut cells containing spores; the typical bi-layered structure of the endospore cell wall can be seen; bar, 1 µm.

Isolation of DNA for determination of the G + C value (mol %) and for PCR-amplification of the 16S rRNA gene. Genomic DNA was extracted by an enzymic lysis procedure as described by Rainey *et al.* (1996). G + C contents were determined by HPLC (Mesbah *et al.*, 1989). An aliquot of the isolated DNA was also used for PCR-mediated amplification of the 16S rDNA (Rainey *et al.*, 1996). The PCR products were purified by using a QIAquick PCR purification kit (Qiagen) and directly sequenced using previously described protocols (Rainey *et al.*, 1996). The sequence reaction mixtures were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems).

Phylogenetic analysis. The 16S rDNA sequence of isolate HR3-23^T was initially aligned to the sequences of a general selection of different bacteria of the DSMZ identification database and, after a first phylogenetic analysis, with members of and relatives to the RNA group 2 of the genus *Bacillus* [as defined by Ash *et al.* (1991) and Farrow *et al.* (1994)] using the ae2 editor (Maidak *et al.*, 1997). Evol-

utionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms according to the method of De Soete (1983) and also dendrograms obtained by the use of the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) were constructed. Bootstrap analysis (Felsenstein, 1985) was used to evaluate the tree topology of the neighbour-joining data by performing 500 resamplings.

RESULTS

Morphology

Strain HR3-23^T developed whitish shiny colonies on agar media. It formed peritrichously flagellated rods with a length of 0.9–2.0 µm and a width of 0.5–0.7 µm (Fig. 1a, b). Round terminal spores having a diameter of 0.5–0.7 µm were observed in swollen sporangia after growth in TSB. The ultrastructural investigation of the

spore surface showed a cauliflower-like fine structure (Fig. 1c). Remaining parts of the lysed vegetative cell wall were still found to be attached to a few of the investigated spores (Fig. 1d). In the images obtained from the ultrathin sections of cells, the typical bilayered bacterial endospore cell wall structure was observed (Fig. 1e).

Physiological characteristics

Growth of isolate HR3-23^T occurred at 10–40 °C with an optimum of 20–30 °C. The following results were obtained after cultivation at 28 °C. The organism did not grow at pH 5.7. Growth was observed on modified Difco tryptic soy broth agar (see Methods) in the presence of 0, 2 and 5 % (w/v) NaCl; no growth was observed at these NaCl concentrations in medium according to Gordon *et al.* (1973). The isolate was positive for catalase and negative for oxidase. Anaerobic growth did not occur. No acid was produced from D-glucose, L-arabinose, D-xylose, D-mannitol and D-fructose. From the 49 substrates provided in the API 50CH strip, acid was produced only from sucrose after 3 d incubation.

Production of gas from glucose was not observed and the pH of carbohydrate-containing media was not changed. From the tests of the API 50CH auxiliary panel, only utilization of glycerol (after 48 h) and ribose (after 5 d) was indicated by an increased turbidity.

The VP test and egg yolk lecithinase test were negative, indole was not formed. No hydrolysis of casein, gelatin, starch, Tween 80 or aesculin was observed. Strain HR3-23^T did not utilize citrate or propionate and did not degrade tyrosine. Deamination of phenylalanine was negative and nitrate was not reduced to nitrite. No growth occurred in the presence of lysozyme. Arginine dihydrolase activity was not detected.

Phylogenetic analysis and DNA base composition

A 1449 nt stretch of the 16S rRNA gene of isolate HR3-23^T was sequenced, representing 94.0 % of the *Escherichia coli* gene sequence (Brosius *et al.*, 1978). Similarity values calculated for the sequence of strain HR3-23^T and other bacterial sequences indicated a membership to the genus *Bacillus*. The similarity values were above 91 % for members of the *Bacillus* RNA group 2 [as defined by Ash *et al.* (1991) and Farrow *et al.* (1992)]. The highest values were found with members of the genus *Caryophanon*, i.e. *Caryophanon tenue* NCDO 2324^T (95.8 %) and *Caryophanon latum* NCIMB 9533^T (95.6 %) and with *Bacillus sphaericus* NCDO 1767 (95.4 %). The similarity of strain HR3-23^T to the 16S rDNA sequence of the type strain of the species *B. sphaericus*, ATCC 14577^T, was determined to be 94.2 %. The sequences of NCDO 1767 and ATCC 14577^T showed a similarity value of 98.5 % to each other.

Fig. 2. Phylogenetic tree of strain HR3-23^T and related organisms from *Bacillus* RNA group 2. The dendrogram was obtained by the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993). Numbers at branching points indicate percentages of occurrence of the branching order in 500 bootstrapped trees (only values of 60 and above are shown). The sequence of *Bacillus subtilis* served as a root. The scale bar represents 5 nucleotide substitutions per 100 nucleotides. Accession numbers of 16S rDNA sequences included in the dendrogram are indicated in Table 1.

Irrespective of treeing algorithms, isolate HR3-23^T branched adjacent to the pair *B. sphaericus*, *Bacillus fusiformis* and members of the genus *Caryophanon*. This branching order is also supported by high bootstrap values (Fig. 2; see also Table 1). The only variation was found in the branching order of members of the genera *Planococcus* and *Kurthia*. When the algorithm of De Soete (1983) was used, their position changed to form a lineage adjacent to *B. sphaericus* and related species (data not shown).

Chemotaxonomic properties

The fatty acid profile of isolate HR3-23^T was characterized by the predominance of iso-C_{15:0} (44.2 %), followed by iso-C_{16:1} (18.7 %) fatty acids. Smaller amounts were found for iso-C_{14:0} (2.9 %), anteiso-C_{15:0} (5.6 %), iso-C_{16:0} (6.2 %), C_{16:1} (3.1 %), iso-C_{17:0} (5.1 %), anteiso-C_{17:0} (3.0 %), iso-C_{17:1} (7.8 %), anteiso-C_{17:1} (2.8 %) and C_{17:0} (0.9 %). The peptidoglycan of isolate HR3-23^T contained lysine, glutamic acid and alanine, representing peptidoglycan type A4α (L-Lys-D-Glu) as described by Schleifer & Kandler (1972). Isoprenoid quinones were MK-7, MK-6 and MK-8 at a peak area ratio of 84:4:1. Polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, minor amounts of phosphatidylserine and one unknown phospholipid.

Table 1. Chemotaxonomic properties of strain HR3-23^T and related organisms from Bacillus RNA group 2

Strains: 1, *Kurthia zopfii* NCIMB 9878^T; 2, *Kurthia gibsonii* NCIMB 9758^T; 3, *Filibacter limicola* NCIMB 11925^T; 4, *Bacillus globisporus* NCIMB 11434^T; 5, *Bacillus psychrophilus* ATCC 23304^T; 6, *Bacillus pasteurii* NCIMB 8841^T; 7, *Sporosarcina ureae* NCIMB 9251^T; 8, *Bacillus insolitus* DSM 5^T; 9, *Planococcus kocurii* NCIMB 629^T; 10, *Planococcus citreus* NCIMB 1493^T; 11, *Bacillus fusiformis* DSM 2898^T; 12, *Bacillus sphaericus* NCDO 1767; 13, *Bacillus silvestris* HR3-23^T (= DSM 12223^T); 14, *Caryophanon tenue* NCDO 2324^T; 15, *Caryophanon latum* NCIMB 9533^T.

Strain	Accession no.	Cell wall type*	Menaquinone system†	G + C (mol%)‡
1	X70321	L-Lys-D-Asp ¹	MK-7 ¹	36.0–38.0 ¹
2	X70320	L-Lys-D-Asp ¹	MK-7 ¹	36.0–38.0 ¹
3	X70371	L-Lys-D-Glu ²	MK-7 ²	44.0 ²
4	X60644	L-Lys-D-Glu ²	MK-7 ³	39.7–39.8 ³
5	X60634	L-Lys-D-Glu ³	MK-7 ⁴	39.7–40.5 ³
6	X60631	L-Lys-Ala-D-Asp ⁴	MK-7 ⁵	38.5–38.7 ³
7	X62175	L-Lys-Gly-D-Glu ²	MK-7 ⁶	40.0–41.5 ⁴
8	X60642	Orn-D-Glu ²	MK-7 ⁵	35.9–36.1 ³
9	X62173	L-Lys-D-Glu ⁵	MK-7, MK-8 ⁷	39–41 ⁵
10	X62172	L-Lys-D-Glu ⁶	MK-7, MK-8 ⁸	48–52 ⁶
11	L14013	NA	NA	35–36 ⁷
12	X60639	L-Lys-D-Asp ²	MK-7 ⁹	37.1–37.3 ³
13	AJ006086	L-Lys-D-Glu	MK-7	39.3
14	X70315	L-Lys-D-Glu ²	MK-6 ¹⁰	41.2–41.6 ⁸
15	X70314	L-Lys-D-Glu ²	MK-6 ¹⁰	44.0–45.6 ⁸

NA, Data not available.

* Data from: 1, Shaw & Keddle (1983); 2, Stackebrandt *et al.* (1987); 3, Claus & Fritze (1989); 4, Ranftl & Kandler (1970); 5, Hao *et al.* (1985); 6, Kocur (1986).

† Data from: 1, Shaw & Keddle (1983); 2, Maiden & Jones (1984); 3, Fahmy *et al.* (1985); 4, D. Fritze, personal communication; 5, Hess *et al.* (1979); 6, Claus & Fahmy (1986); 7, Hao *et al.* (1985); 8, Kocur (1986); 9, Collins & Jones (1979); 10, Holt *et al.* (1994).

‡ Data from: 1, Shaw & Keddle (1983); 2, Maiden & Jones (1984); 3, Fahmy *et al.* (1985); 4, Claus & Fahmy (1986); 5, Hao *et al.* (1985); 6, Kocur (1986); 7, Priest *et al.* (1988); 8, Trentini (1986).

The G + C content of the DNA of isolate HR3-23^T was 39.3 mol %.

DISCUSSION

Morphological and phylogenetic analyses have clearly demonstrated that strain HR3-23^T is a member of the genus *Bacillus*. Based upon the results of physiological properties, strain HR3-23^T appears to be most similar to *Bacillus fastidiosus*, but this phenotypic relationship is due to the significant number of negative test results obtained for the isolate and *B. fastidiosus* (Claus & Berkeley, 1986). In addition, while *B. fastidiosus* requires a medium containing allantoin or uric acid (Claus & Berkeley, 1986), contains peptidoglycan with meso-diaminopimelic acid as the diagnostic amino acid and is phylogenetically a member of the *Bacillus* RNA group 1, strain HR3-23^T contains peptidoglycan with lysine as the diagnostic amino acid and clusters with the *Bacillus* RNA group 2 and its non-*Bacillus*-type relatives.

The phylogenetic relationship among the round-spore-forming bacilli and their non-spore-forming relatives has been extensively investigated and discussed by

Farrow *et al.* (1994). Their findings regarding the branching order within the RNA group 2 bacilli were confirmed by the present study. The sequence of *Filibacter limicola* was not included in the study of Farrow *et al.* (1994), but membership of this species in *Bacillus* RNA group 2 had previously been demonstrated by oligonucleotide cataloguing (Stackebrandt *et al.*, 1987). In contrast to the few branching points which are supported by a high bootstrap value, most of the branching points of the more deeply branching lineages are not statistically significant, hence the order at which these lineages evolved remains uncertain. One of the few clusters supported by high bootstrap values embraces the genus *Caryophanon* and some *Bacillus* species, including the novel isolate HR3-23^T. From a phylogenetic point of view, the latter strain and *Caryophanon* species share a common ancestry which strengthens the evolutionary origin of the morphologically different *Caryophanon* species from *Bacillus*-type ancestors.

Although the taxonomic status of the RNA group 2 bacilli has been discussed (Farrow *et al.*, 1994), no formal proposal has as yet been forwarded which transfers the L-lysine- and ornithine-containing bacilli

into one or, because of the phylogenetic intermixing with non-*Bacillus*-type genera, several new genera. Hence, any strain worthy of species rank within RNA group 2, exhibiting taxonomic properties that would identify this taxon as a member of the genus *Bacillus*, should still be described as a species of this genus. Based upon phylogenetic, chemotaxonomic and morphological properties, strain HR3-23^T represents a member of this genus; based upon phylogenetic distance and physiological properties, strain HR3-23^T represents a new species. No DNA-DNA hybridization studies were carried out, as the binary 16S rDNA similarity values obtained for strain HR3-23^T and its closest phylogenetic neighbours were low enough to exclude high DNA-DNA reassociation values that would indicate membership to one of these species (Stackebrandt & Goebel, 1994).

Description of *Bacillus silvestris* sp. nov.

Bacillus silvestris (sil.ves'tris. N.L. masc. n. *silva* forest, referring to the place of isolation).

Gram-positive, rod-shaped, peritrichously flagellated, endospore-forming, strictly aerobic organism. Round spores lie terminally in a swollen sporangium. The rods measure 0.9–2.0 µm by 0.5–0.7 µm; spore diameters are 0.5–0.7 µm. The peptidoglycan contains lysine, glutamic acid and alanine, representing peptidoglycan type A4 α . Polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, minor amounts of phosphatidylserine and one unknown phospholipid. The dominant isoprenoid quinone is of the MK-7 type. The fatty acid profile is characterized by the predominance of iso-C_{15:0} (44.2%), followed by iso-C_{16:1} (18.7%) fatty acids. Further compounds are: iso-C_{17:1} (7.8%), iso-C_{16:0} (6.2%), anteiso-C_{15:0} (5.6%), iso-C_{17:0} (5.1%), C_{16:1} (3.1%), anteiso-C_{17:0} (3.0%), iso-C_{14:0} (2.9%), anteiso-C_{17:1} (2.8%) and C_{17:0} (0.9%). Temperature range for growth is 10–40 °C with an optimum between 20 and 30 °C. Catalase-positive, oxidase-negative, VP test-negative, no pH change in the growth medium. Reactions towards the substrates provided in the API 50CH panel indicate that glycerol and ribose are utilized as sole carbon sources, but acid is not produced. No acid from and no utilization of D-glucose, erythritol, D- and L-arabinose, D- and L-xylose, adonitol, methyl β -D-xyloside, galactose, mannose, D-mannitol, D-fructose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, β -gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. Casein, gelatin, starch, Tween 80 and aesculin are not hydrolysed. Citrate or propionate are not utilized and tyrosine is not degraded. Deamination of phenylalanine and the egg yolk

lecithinase test are negative. Nitrite is not produced from nitrate, indole is not formed. The strain does not grow at pH 5.7 or in the presence of lysozyme. It grows in the presence of 0, 2 and 5% (w/v) NaCl in modified Difco tryptic soy broth agar. Arginine dihydrolase is not present. The G + C content of the DNA is 39.3 mol%, as determined by HPLC. Phylogenetically, a member of the *Bacillus* RNA group 2. Isolated from the top soil layer of a beech forest soil near Braunschweig, Lower Saxony, Germany. The type strain is HR3-23^T (= DSM 12223^T).

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