Bacillus cohnii sp. nov., a New, Obligately Alkaliphilic, Oval-Spore-Forming *Bacillus* Species with Ornithine and Aspartic Acid Instead of Diaminopimelic Acid in the Cell Wall

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A group of 20 alkaliphilic *Bacillus* strains in which all strains revealed the same unique combination of properties—obligate alkaliphily, oval spores distending the sporangium, and ornithine and aspartic acid instead of diaminopimelic acid in the cell wall—was examined. Most of the strains had been isolated by a five-step enrichment and isolation procedure. The G+C content was determined to span a range from 33.5 to 35.0 mol%. Unsaturated fatty acids amounted to 17 to 28% of the total cellular fatty acids. Through DNA-DNA hybridization experiments 11 strains could be grouped in one species. Low homology values with the type strains of validly published *Bacillus* species with similar G+C contents suggest that these strains belong to a hitherto undescribed species for which the name *Bacillus cohnii* is proposed. The type strain of the new species is strain RSH (= DSM 6307).

More than 100 years ago, in 1872, Ferdinand Cohn described the genus *Bacillus* (8). Sixty-two years later Vedder isolated and described the first alkaliphilic *Bacillus* species: *Bacillus alcalophilus* (35). Although a considerable number of alkaliphilic *Bacillus* strains have been isolated in the past years, *B. alcalophilus* has been until now, the only validly published obligately alkaliphilic *Bacillus* species.

Gordon and Hyde were the first, in 1982, to present a comprehensive work on the taxonomy of alkaliphilic *Bacillus* strains (18). To be able to apply standard phenotypical tests, they adapted the strains to neutral pH conditions and arranged them in five groups. They placed the strains loosely in the *Bacillus firmus*-*Bacillus lentus* complex (17). On the basis provided by these authors it was possible in 1985 to rearrange the strain groupings by applying genotypical tests and subsequent physiological tests under alkaline conditions (14). Groups in which phenotypical characteristics correlated with a distinct G+C content were found. During these investigations a few strains were found to differ from all others. They revealed a combination of properties which had not been described before in the genus *Bacillus*: oval spores and no diaminopimelic acid (Dap) in the cell wall.

Cell walls with a cross-linkage between the peptide side chains other than via Dap had been found within the genus *Bacillus* until today only with the round-spore-forming species. This coincided with the more recent findings of Stackebrandt et al. (32) and Ash et al. (2) that these organisms (together with a number of related nonsporeformers) build a separate cluster when the 16S rRNA nucleotide sequences are compared. Alexander and Priest (1) found with their numerical analysis distinctive round-spored taxa. In a previous comprehensive study on the numerical classification of the genus *Bacillus* (28) Priest et al. stated, "It is evident that the genus *Bacillus* now encompasses some 80 taxa of approximate species rank that can be assigned to five or more cluster groups. The latter should be used as a framework for redefining the current genus and splitting it into several genera." A possible separation of the round-spore-forming organisms from the genus *Bacillus*, which presently spans a

moles percent G+C range far too large as to be acceptable for a single genus, has therefore been discussed. Unfortunately, the convincing uniform picture had been disturbed by the finding that a round-spore-forming species, "*Bacillus aminovorans*," contains Dap in the cell wall (32) and the occurrence of round- and oval-spore-forming *Bacillus sphaericus* strains. However, *B. sphaericus* has now been separated into several DNA homology groups (1), and it is not yet clear whether the oval-spore-forming *B. sphaericus* strains can be found in a distinct group or whether they also lack Dap. The strains described in the present study add to this disturbance.

Further distinguishing properties of the Dap-less strains were as follows: the terminally positioned spore swells the sporangium, and growth occurs at around pH 9.7 and up to 47°C. Relatively high DNA homology values among these strains and low homology values with all other tested strains (14) supported the unique position of these organisms among the alkaliphiles.

In this work additional strains with these properties were isolated and together with the strains of Fritze et al. (14) subjected to phenotypical and genotypical tests.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Four strains were obtained from the National Collections of Industrial and Marine Bacteria Ltd., Torry Research Station, Aberdeen, Scotland. One strain was provided by K. Aunstrup, Novo Industri, Copenhagen, Denmark, and one strain came from H.-G. Trüper, Bonn, Germany. Nine strains were isolated from samples collected in the area of Braunschweig from several places which were up to 10 km apart. Five strains received from the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DSM), were isolated from the Wadi Natrun, Egypt. The reference strains (type strains) used were obtained from the DSM. These strains are not listed in Table 1 (*B. circulans* DSM 11^T, *B. alcalophilus* DSM 485^T, *B. lentus* DSM 9^T, *B. pantothenicus* DSM 26^T, *B. marinus* DSM 1297^T, *B. fusiformis*, DSM 2898^T, *B. insolitus* DSM 5^T, and *B. sphaericus* DSM 28^T).

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TABLE 1. List of bacterial strains used in this study

Name	Source and/or collection number ^a	Strain designation	Reference(s)	Isolation source
<i>B. circulans</i>	DSM 2528	RU 38	8, 15, 19	Contamination on an agar plate with a high pH value
	NCIB 9218			
<i>Bacillus</i> sp.	NCIB 10284	C 325	2a, 15, 19	Infection on an alkaline agar plate
<i>Bacillus</i> sp.	NCIB 10318	C 373	2a, 15, 19	Garden soil, city
<i>Bacillus</i> sp.	NCIB 10327	C 413	2a, 15, 19	Loam, grass field
<i>Bacillus</i> sp.	K. Aunstrup	PB38(2)	2a, 15, 19	
<i>Bacillus</i> sp.	H. G. Trüper	M 5	15	Mono Lake, California
<i>Bacillus</i> sp.	DSM	5 A		Water + sand, Wadi Natrun, Egypt
<i>Bacillus</i> sp.	DSM	5'A		Water + sand, Wadi Natrun, Egypt
<i>Bacillus</i> sp.	DSM	14 B		Water + sand, Wadi Natrun, Egypt
<i>Bacillus</i> sp.	DSM	15 B		Water + sand, Wadi Natrun, Egypt
<i>Bacillus</i> sp.	DSM	15'B		Water + sand, Wadi Natrun, Egypt
<i>Bacillus</i> sp.	Isolated for this work	RSA		Old horse feces
<i>Bacillus</i> sp.	Isolated for this work	RSB		Old horse feces
<i>Bacillus</i> sp.	Isolated for this work	RSF		Soil, horse meadow
<i>Bacillus</i> sp.	Isolated for this work	RSHT ^T		Soil, horse meadow
<i>Bacillus</i> sp.	Isolated for this work	RSQ		Old horse feces
<i>Bacillus</i> sp.	Isolated for this work	RST		Old horse feces
<i>Bacillus</i> sp.	Isolated for this work	RSU		Soil from field
<i>Bacillus</i> sp.	Isolated for this work	RS2		Soil from field
<i>Bacillus</i> sp.	Isolated for this work	RS6		Soil from field

^a NCIB, National Collection of Industrial Bacteria (now National Collections of Industrial and Marine Bacteria [NCIMB]), Torry Research Station, Aberdeen, Scotland; DSM, DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Enrichment and isolation. Samples were taken from alkaline or possibly alkaline environments such as soil of horse meadows and fields or from old horse feces. Five steps of enrichment and selection were applied to isolate strains with the properties mentioned above. (i) For pasteurization, 1 g of sample material was mixed with 5 ml of water, and 1 ml of the supernatant was heated at 80°C for 10 min. (ii) For incubation, after autoclaving, the pH value of a meat extract-peptone medium (nutrient broth; Difco) was adjusted to 9.7 by adding sodium sesquicarbonate solution to a final concentration of 0.1 mol/liter (3). Tubes were inoculated with 1 to 2 drops of the pasteurized suspension and incubated aerobically at 45°C for 1 to 2 days. (iii) The cultures were streaked onto agar plates with a pH value of 9.7 (same medium as above plus agar) to obtain single colonies. The plates were incubated at 45°C or at 28 to 30°C for 2 to 7 days. Only a few morphologically different types of colonies developed on these plates. (iv) Upon microscopical examination of cells of the colonies, cells with the morphological properties described above were found only in the seldom-occurring, small, cream white colonies. These were selected, and a pure culture was made. (v) The test for the presence of Dap was thin-layer chromatography according to the method of Kutzner (23).

Chemotaxonomical characterization. These tests were performed by using previously described methods: cell wall analysis (29), analysis of the fatty acids (24, 26), and analysis of the polar lipids and quinones (4, 34).

Physiological characterization. Unless otherwise stated, the strains were characterized by using the modified methods of Gordon et al. (16). After autoclaving, the pH value of the media was adjusted to 9.7 by adding sodium sesquicarbonate solution to a final concentration of 0.1 mol/liter (3). The media used for nitrate reduction and phenylalanine deamination tests had to be acidified prior to examining the activity of the strains. The following tests were performed by using previously described methods: KOH test (19), oxidase

test (22), aminopeptidase activity test (test strips; Merck, Darmstadt, Germany) (6), Tween test (30), pullulanase test (27), and 4-methylumbelliferone glucuronidase (MUG) test (13). Splitting of urea was tested by inoculating the medium (per liter of H₂O: urea, 20 g; KH₂PO₄, 9.1 g; Na₂HPO₄ · 12H₂O 9.5 g; yeast extract, 0.1 g; phenol red, 0.01 g; distributed in 1-ml amounts into test tubes) with 2 to 3 drops of a heavy suspension of a culture grown overnight and washed twice with saline. For the test for obligate alkaliphily, the meat extract-peptone agar medium was buffered at pH 7 by adding the McIlvaine citrate-phosphate buffer. No growth at this pH value indicated that the particular strain is alkaliphilic, not only alkalitolerant.

Isolation of DNA. The DNA was isolated by using hydroxylapatite (hydroxylapatite for column chromatography, Bio-Gel HTP; Bio-Rad, Richmond, Calif.) as described by Cashion et al. (5); DNA solutions obtained by this method were dialyzed shortly against 0.1× SSC (1× SSC: 0.15 M NaCl plus 0.015 M sodium citrate) and were concentrated with 2-butanol by using the method of Stafford and Bieber (33).

G+C content. DNA solutions were dialyzed against 500 volumes of 1× SSC at 4°C for 3 days with frequent change of buffer. The G+C content was determined by using a Gilford model 2600 spectrophotometer equipped with a thermoprogrammer. Thermal denaturation (T_m) values were calculated from computer-analyzed melting curves of preparations in 1× SSC by using the method of Marmur and Doty (25) with modifications from Huß (20). The DNA concentration of the samples was adjusted to an A_{260} of 0.4 in 1× SSC. In each measurement, a DNA sample of *Escherichia coli* K-12 wild type DSM 498 (G+C = 51.7%) was used as a reference and the moles percent G+C content was calculated from $2.44 \times (T_m - 69.37)$ (10). Each experiment was carried out three times.

DNA-DNA hybridization. DNA-DNA homology values were determined spectrophotometrically from the renaturation rates of the individual DNA samples and their mixtures

FIG. 1. Photomicrograph of the strain RSH^T. Bar = 10 μ m.

by using the method of De Ley et al. (12) as described by Fritze et al. (14). Optimal renaturation temperature (T_{OR}) was calculated from the equation given by Gillis et al. (15). Recording of the decrease in A_{260} for 30 min as well as calculation of the renaturation rates with linear regression was done by computer. The degree of binding (D) was calculated from the equation given by De Ley et al. (12). Each experiment was carried out two to three times.

RESULTS

Twenty *Bacillus* strains with the properties of growth at pH 9.7 and 47°C, no Dap in the cell wall, and terminally located oval spores that distend the sporangium have been examined (Fig. 1). When analyzed, all 20 strains showed a cell wall structure classified as A₄ β -murein according to the nomenclature of Schleifer and Kandler (29). In the type of cell wall found here, Dap is replaced by ornithine and aspartic acid forms an interpeptide bridge (L-Orn-D-Asp) (Fig. 2).

The G+C content of all 20 strains spans from 33.5 mol%

FIG. 2. Structure of the cell wall. G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid; L-Ala, L-alanine; Asp, aspartic acid; D-Glu, D-glutamic acid; L-Orn, L-ornithine.

TABLE 2. G+C values in numerical order

Strain(s)	Mol% G+C content
15'B	33.5
RSA	33.9
14 B	34.1
PB38(2), NCIB 10284, RSF, RSQ, RST, RSU	34.5
NCIB 10327, NCIB 10318, RSH ^T	34.6
M 5, 15 B, 5 A, 5'A	34.7
DSM 2528, RS2, RS6	34.8
RSB	35.0

(strain 15'B) to 35.0 mol% (strain RSB) (Table 2), suggesting that the strains might belong to one species. De Ley (11) recommends that the G+C range of members of one species should not exceed the interval of ± 1 mol%. At the same time it cannot be assumed that strains with similar G+C contents necessarily belong to one species.

A differentiation into taxonomically relevant groups was obtained from the results of the DNA-DNA hybridization experiments (Table 3). The 70% border recommended by Wayne et al. (36) as the lowest homology value for two strains belonging to the same species was employed. On the basis of these experiments it was possible to arrange the strains into six DNA homology groups (Table 3), of which each might represent an individual species. Between groups 1 to 5 homology values of 45 to 63% D were found. Strain 14B (homology group 6) showed only 23% homology to strain NCIB 10318 (homology group 1).

Fatty acids with a chain length of 14 to 17 carbon atoms were detected. Only small amounts of C₁₈ fatty acids were found. The C_{15:0} iso and anteiso fatty acids predominate, with the C_{15:0} anteiso fatty acids usually outweighing the C_{15:0} iso fatty acids. Of all fatty acids determined, the iso and anteiso branched fatty acids amounted to the biggest proportion. The percentage of unsaturated fatty acids ranges from 19% (strain 5A) to 35% (strain 14B).

A pattern of polar lipids containing diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol was detected with all 20 strains.

Menaquinone 7 was determined to be the main lipoquinone fraction for all of the 20 strains (95.8 to 100%). Only strain RST contained significant amounts of menaquinone 3 (27.8% menaquinone 3; 72.2% menaquinone 7).

All 20 strains showed a relatively uniform pattern of physiological and biochemical properties. The data for properties differing among the strains are listed in Table 4. Positive reactions for all strains were recorded for the oxidase and catalase tests, growth in the range from 25 to 45°C, and hydrolysis of Tween 60, hippurate, and gelatin. Negative reactions for all strains were recorded for lysis by KOH, growth at 6 and 50°C, deamination of phenylalanine, splitting of urea, and the presence of Dap in the cell wall.

DISCUSSION

One of the DNA homology groups ($D > 70\%$) described in the study on alkaliphilic *Bacillus* strains of Fritze et al. (14) comprised three strains: PB38(2), NCIB 10284, and NCIB 10327. Three further strains, NCIB 10318, DSM 2528, and M 5, showed 52 to 56% homology to this group. With each other these revealed 72% (NCIB 10318 and DSM 2528), 55% (NCIB 10318 and M 5), and 57% (M 5 and DSM 2528) homology. All of these six strains had the unusual combina-

TABLE 3. DNA-DNA homologies

Homology group	Strain	% Homology ^a with:										
		NCIB 10318	DSM 2528	RSH ^T	PB38(2)	NCIB 10284	NCIB 10327	15 B	15'B	5 A	5'A	M 5
1	DSM 2528	77										
	RSA	79	—									
	RSB	83	—									
	RSF	79	—									
	RSH ^T	85	—									
	RSQ	80	—	—								
	RST	83	—	—								
	RSU	81	—	—								
	RS2	81	—	—								
	RS6	82	—	—								
2	PB38(2)	54	—	53								
	NCIB 10284	57	—	—	86							
	NCIB 10327	60	(56)	—	(85)	(96)						
3	15 B	58	—	—	—	—	—					
	15'B	58	—	—	54	—	—	89				
4	5 A	62	—	—	—	—	—	—	63			
	5'A	59	—	—	50	—	—	—	57	89		
5	M 5	60	(57)	—	—	—	(52)	—	45	60	—	
6	14 B	23	—	—	ND	ND	ND	ND	ND	ND	ND	ND

^a —, not determined since DNA-DNA homology values compared with those of selected representatives of a homology group indicate that they are in the same species (>70%) or are not in the same species (<70%). ND, not determined. Values in parentheses are taken from the study of Fritze et al. (14). Values were calculated as the means of two to three determinations.

tion of phenotypical properties in common: growth at pH 9.7 and 47°C, no Dap in the cell wall, and terminally positioned oval spores swelling the sporangium. More strains with this combination of properties were easily isolated with the described method.

Within the gram-positive bacteria the structure of the cell wall is of considerable taxonomic value. The vast majority of the hitherto described *Bacillus* species reveal a cell wall with the direct linked *meso*-Dap type. To date, only a few *Bacillus* species not containing Dap in their cell walls are described. All these species form round spores. The cell walls of all 20 strains examined in this study show a structure classified as A₄B-murein type (L-Orn-D-Asp), which has not been described before in the genus *Bacillus*. A relatively similar type of cell wall can be found with the above-mentioned round-spore-forming *Bacillus* species, e.g., *B. insolitus*, in which diaminopimelic acid is replaced by ornithine and the peptide chains are linked through glutamic acid (L-Orn-D-Glu); *B. sphaericus*, in which lysine is the diamino acid and aspartic acid is the interpeptide bridge (L-Lys-D-Asp); and *B. pasteurii*, in which a combination of lysine and alanine take over the task of the diamino acid and aspartic acid again is the interpeptide bridge. A recent study revealed that with *B. marinus* the linkage is through L-lysine directly with a glycine bound to the carboxyl group (22a).

When regarding the composition of the cellular fatty acids of the organisms, the considerable amount of unsaturated fatty acids is striking. A similar proportion of unsaturated fatty acids had been found by Kaneda (21) for the group F organisms. Kaneda examined 19 *Bacillus* species for their fatty acid compositions and grouped them accordingly. Group F consisted of the round-spore-forming psychrophilic organisms *B. insolitus* and *B. globisporus* revealing 17 to 28% unsaturated fatty acids.

Close relationship to the mentioned species may also be seen through the relatively similar physiological behavior of the strains presented here with respect to their restricted range of carbohydrates from which acid may be produced (results not shown).

The pattern of polar lipids and quinones found with our strains seems to be typical for the genus *Bacillus* as it is presently described. Most of the *Bacillus* strains investigated until now possess phosphatidylglycerol and phosphatidylethanolamine. Collins and Jones reported that all *Bacillus* strains examined until now contain only menaquinones and as the main fraction usually menaquinone 7 (9).

All 20 strains examined in this study showed more or less similar behavior in most physiological and biochemical tests. On the basis of the DNA-DNA hybridization experiments with which six distinct homology groups could be found, it was possible to further interpret the physiological data. Although a much larger number of physiological properties needs to be determined for thorough characterization of these strains, a few of those might already be useful to distinguish between some of the homology groups presented here.

In Table 5 some differential characteristics of the six homology groups and of the type strains of *Bacillus* species in the same G+C range are shown. Homology group 2 can be separated from homology group 1 through the inability of its strains to reduce nitrate to nitrite. The strains of homology group 3 grow, in contrast to strains of group 1, in the presence of 10% NaCl. Strain M 5, the only strain of homology group 5, grows also, although weakly, in the presence of 10% NaCl. Strain 14 B, the only member of group 6, differs from strains of group 1 in its inability to split MUG and by its narrower temperature range for growth. Only homology group 4 cannot be distinguished through the

TABLE 4. Differential phenotypic and genotypic properties of the strains^a

Strain	Amino-peptidase activity	Growth at (°C):				Hydrolysis of:				Growth in presence of NaCl:			Reduction of NO ₃ to NO ₂	Splitting of MUG	Homology group
		10	47	49	Tween 20	Tween 40	Tween 80	Pullulan	Casein	Starch	5%	10%			
NCIB 10318	-	+	+	-	-	-	+	+	+	+	+	-	+	+	1
DSM 2528	-	+	+	-	+	+	+	+	-	+	+	-	+	+	1
RSA	-	+	+	-	+	+	+	+	+	+	+	-	+	+	1
RSB	-	+	-	-	+	+	+	+	+	+	+	-	+	+	1
RSF	-	+	+	-	+	+	+	+	+	+	+	-	+	+	1
RSH ^T	-	+	+	-	+	+	+	+	+	+	+	-	+	+	1
RSQ	-	+	+	-	+	+	+	+	+	+	+	-	+	+	1
RST	-	+	+	-	-	+	+	-	+	±	±	-	+	+	1
RSU	-	+	+	-	-	-	+	+	+	+	+	-	+	+	1
RS2	-	+	+	-	+	-	+	+	+	+	+	-	+	+	1
RS6	-	+	+	-	+	+	+	+	+	+	+	-	+	+	1
PB38(2)	-	+	+	±	+	+	+	+	+	+	+	-	-	+	2
NCIB 10284	-	+	+	-	+	+	+	+	+	+	+	-	-	+	2
NCIB 10327	-	+	+	-	+	+	+	+	+	+	+	-	-	+	2
15 B	-	+	+	-	+	+	+	+	+	+	+	+	+	+	3
15'B	-	+	+	-	+	+	+	+	+	+	+	+	+	+	3
5 A	-	+	+	-	+	+	+	+	+	+	+	-	+	+	4
5'A	-	+	+	-	+	+	+	+	+	+	+	-	+	+	4
M 5	-	+	+	-	+	+	+	+	+	+	+	-	+	+	5
14 B	+	-	-	-	+	+	-	+	+	+	+	±	+	+	6
Differing from the majority	14 B	14 B	14 B	RSB, PB38(2)	NCIB 10318, RST, RSU	NCIB 10318, RSU, RS2	NCIB 10318, RSU, 14 B	RST	DSM 2528	RST	RST	15 B, 15'B, M 5	PB38(2), NCIB 10284, NCIB 10327	14 B	

^a +, positive reaction; -, negative reaction; ±, variable reaction.

TABLE 5. Some differential characters of the six homology groups and of *Bacillus* species in the same G+C range^a

Group or strain	Growth at (°C):		Hydrolysis of:					Growth in presence of NaCl:		Nitrate reduction	Splitting of MUG	G+C (mol%)	Dap	Alkaliphile	Oval spores	Swollen sporangia
	10	47	Hippurate	Gelatin	Pullulan	Casein	Starch	5%	10%							
Homology groups																
1	+	+	+	+	+	+	+	+	-	+	+	33.9-35.0	-	+	+	+
2	+	+	+	+	+	+	+	+	-	-	+	34.5-34.6	-	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	33.5-34.7	-	+	+	+
4	+	+	+	+	+	+	+	+	-	+	+	34.7	-	+	+	+
5	+	+	+	+	+	+	+	+	±	+	+	34.7	-	+	+	+
6	-	-	+	+	+	+	+	+	-	+	-	34.1	-	+	+	+
Strains ^b																
<i>B. sphaericus</i> DSM 28 ^T	-	-	-	+	-	+	-	-	-	-	-	37.3	-	-	-	+
<i>B. insolitus</i> DSM 5 ^T	+	-	+	-	ND	-	-	-	-	-	ND	35.9	-	-	-	-
<i>B. marinus</i> DSM 1297 ^T	+	-	-	+	-	-	-	+	-	-	-	39.3	-	-	-	+
<i>B. pantothenicus</i> DSM 26 ^T	-	-	-	-	-	-	+	-	-	+	-	36.9	+	-	±	+
<i>B. alcalophilus</i> DSM 485 ^T	+	-	-	+	+	+	+	-	-	-	-	37.0	+	+	+	-
<i>B. lentus</i> DSM 9 ^T	-	-	+	-	+	-	+	+	-	-	+	36.3	+	-	+	-
<i>B. circulans</i> DSM 11 ^T	-	+	-	+	+	-	±	+	-	-	+	35.5	+	-	+	+

^a +, positive reaction; -, negative reaction; ±, variable reaction; ND, not determined.

^b Physiological-biochemical data for all strains except *B. insolitus* are from Fritze et al. (14). G+C values of all strains are from Fahmy et al. (12a).

presently chosen characters. The strains of groups 1 to 5 may be seen as members of closely related species, whereas the low homology value of strain 14 B (homology group 6) with group 1 and its different behavior in several phenotypic tests might indicate that this strain belongs to a more distantly related species.

The results shown suggest that the alkaliphilic oval-spore-forming strains with L-Orn-D-Asp instead of Dap in the cell wall represent a major group within the genus *Bacillus* that possibly contains several species occurring ubiquitously. Homology group 1 described in this work consists of 11 strains, which is a solid basis for a description of a species as recommended by Sneath (31).

Through DNA-DNA hybridization experiments with the type strains of other *Bacillus* species with similar G+C contents it was shown previously (14) and in this study that there is no relationship within species level of these type strains to homology group 1 of this study. The following *Bacillus* species type strains were examined, and only low homology values were obtained: *B. alcalophilus* DSM 485 ($D = 25\%$), *B. circulans* DSM 11 ($D = 10\%$), *B. lentus* DSM 9 ($D = 8\%$), *B. pantothenicus* DSM 26 ($D = 16\%$), *B. marinus* DSM 1297 ($D = 38\%$), *B. fusiformis* DSM 2898 ($D = 15\%$), *B. insolitus* DSM 5 ($D = 19\%$), and *B. sphaericus* DSM 28 ($D = 17\%$).

Strain DSM 2528 was isolated and described by Chislett and Kushner in 1961 (7) as *B. circulans* RU 38. In a later study (14) it was shown that the DNA-DNA homology value is only 17% between DSM 2528 (RU 38) and the type strain of *B. circulans*, DSM 11. According to the present results, strain DSM 2528 belongs to a different species, which is described here.

On the basis of the given data the organisms of homology group 1 are considered strains of a new species, for which the name *Bacillus cohnii* is proposed. A description of this species is given below.

Description of *B. cohnii* sp. nov. *Bacillus cohnii* (coh'nii. M.L. gen. n. from Cohn; named after the German botanist and bacteriologist Ferdinand Cohn, who described the genus *Bacillus* in 1872).

Cells from overnight cultures are gram positive (no lysis

by KOH; no aminopeptidase activity). Cell width, 0.6 to 0.7 µm; peritrichously flagellated. Spores oval and terminally located; sporangia swollen. Colonies are cream white on alkaline meat extract-peptone medium. After incubation at 45°C for 2 days the diameter of the colonies is 1 to 2 mm. Catalase and oxidase positive. Obligately alkaliphilic. Minimum temperature of growth, 10°C; maximum temperature of growth, 47°C (one strain negative). Growth at 5% NaCl; no growth at 10% NaCl. Nitrate is reduced. MUG is hydrolyzed. Hydrolysis of starch, pullulan (one strain negative), hippurate, Tween 20 (three strains negative), Tween 40 (three strains negative), Tween 60, Tween 80 (one strain negative), gelatin, and casein (one strain negative) occurs. Urea is not hydrolyzed; phenylalanine is not deaminated. The cell wall is an A₄B-murein with ornithine as the diamino acid and aspartic acid as the interpeptide bridge. The main proportion of the polar lipids consists of diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol. The main quinone is menaquinone 7 (96 to 100%) (one strain exhibits significant amounts of menaquinone 3: 28%). C_{15:0} iso and anteiso fatty acids represent the main fraction of fatty acids (46 to 54%). The proportion of unsaturated fatty acids amounts to 22 to 27%. The G+C content is 33.9 to 35.0 mol%, as determined by the thermal denaturation method (strain RSH^T, 34.6 mol% G+C).

Most of the strains were isolated from soil samples and feces collected in Denmark and in Germany. One strain was found as a contaminant on an agar plate in Great Britain. The type strain is strain RSH (= DSM 6307).

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