The *Bacillus firmus*–*Bacillus lentus* Complex and pH 7·0 Variants of Some Alkalophilic Strains

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Variants capable of growth at pH 7·0 were obtained from 174 alkalophilic strains (optimum pH 9·7) of the genus *Bacillus* by successive transfer on media with decreasing pH values. Nearly half of the pH 7·0 variants were like strains of the *B. firmus*–*B. lentus* complex. The remaining pH 7·0 variants grew at 50 °C and were assigned to two groups closely related to *B. lentus*.

INTRODUCTION

During a taxonomic study of the genus *Bacillus*, begun in 1936, continued until 1952 and then pursued sporadically until the present, 1625 strains were assembled in the collection of the late Dr N. R. Smith, U.S. Department of Agriculture (NRS Collection). In addition, strains from many investigators were received for identification, and a small percentage of these were kept, either for further study or because they belonged to species poorly represented in the NRS Collection. From 1936 to 1962, this collection acquired merely twelve strains of *B. firmus*, five of *B. lentus* and eight designated *B. firmus*–*B. lentus* intermediates. In 1962, these 25 strains were augmented by six of *B. firmus*, three of *B. lentus* and eight intermediates that were isolated from sea water or a salt marsh (Turner & Jervis, 1968; Gordon *et al.*, 1977). At the same time, two ATCC strains of *B. firmus* and two of *B. lentus* were acquired. Although these ATCC strains were replicates of NRS strains, they had been maintained under different conditions for a long time. For this reason they were treated as separate strains.

In 1970, we received for taxonomic examination a collection of alkalophilic strains of *Bacillus* species that had been isolated mainly from soil, dung and water and grew optimally at pH 9·7 (Aunstrup *et al.*, 1972). Since some of the physiological tests for the identification of *Bacillus* species could not be performed at this high pH value (Gordon, 1981), the alkalophilic strains could not be compared with the descriptions of recognized species. By successive transfer on media with decreasing pH values, variants of the alkalophilic strains were obtained that would grow on media at pH 7·0 and could, therefore, be compared with known strains. Among the first 105 variants examined, there were 27 (26%) that resembled the 20 strains of *B. firmus* in our collection.

In the following report, pH 7·0 variants of all the 162 alkalophilic strains received in 1970 and of 12 alkalophilic strains from other culture collections are described and compared with strains of the *B. firmus*–*B. lentus* complex.

METHODS

Strains. The strain numbers and sources of the alkalophilic isolates of Aunstrup *et al.* (1972) are listed in Table 1. Twelve alkalophilic reference strains are presented in Table 2 together with their history and the species name

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Table 1. *Source and strain numbers of alkalophilic isolates*

Source	Strain numbers
Soil	BB 3, 10, 16, 16(1), 17 to 22, 24, 25, 49; C 300 to 302, 304, 311 to 314, 323, 324, 334, 335, 337 to 343, 346, 348, 354 to 358, 360, 372 to 374, 412, 413; PB 9, 16, 17, 19, 20, 25, 33, 35, 38(1), 38(2), 40, 41; SB 14a, 14b, 37; VB 3, 5 to 7, 11
Dung	BB 30, 31, 34 to 37, 40; BC 4, 6, 8, 12 to 17; C 336, 347, 349, 351 to 353, 364, 366 to 369, 371, 375, 376, 379a, 380a, 382b, 384 to 390, 392 to 404, 410, 411; SB 5; TB 2, 4, 10, 13 to 15, 18, 19
Water	BB 1, 7, 23, 41, 43, 45; C 350, 377; SB 16; TB 6, 11, 12, 16, 17
Lime pit of tannery	BC 1 to 3, 7, 9 to 11; C 365, 370, 378, 383
Compost	C 303; PB 39; SB 32
Horn meal	TB 9
Air contaminants	C 325, 326; VB 1, 2

Table 2. *History of alkalophilic reference strains*

Strain no.	Name when received, source, strain name or number
NRS 1548	<i>Bacillus alcalophilus</i> Vedder (1934); T. Gibson, University of Edinburgh (1967); NCTC 4553*; A. Vedder (1)
NRS 1554	<i>B. alcalophilus</i> ; T. Gibson (1921); NCTC 4554; A. Vedder (2)
ATCC 21522	<i>B. alkalophilus</i> ; R. L. Gherna, ATCC; K. Horikoshi (221; soil) (Horikoshi, 1971)
ATCC 21591	<i>B. alcalophilus</i> subsp. <i>halodurans</i> Boyer <i>et al.</i> (1973); R. L. Gherna, ATCC; K. Horikoshi (A-59; soil)
ATCC 27557	<i>B. alcalophilus</i> subsp. <i>halodurans</i> ; R. L. Gherna, ATCC; E. W. Boyer (NRRL B-3881*)
NCIB 9218	<i>B. circulans</i> Jordan; J. M. Shewan, NCIB; M. E. Chislett (RU 38) (Chislett & Kushner, 1961)
NRRL B-3881	<i>Bacillus</i> sp.; W. C. Haynes, U.S. Department of Agriculture; E. W. Boyer
ATCC 21592 to 21596	<i>Bacillus</i> spp.; R. L. Gherna, ATCC; K. Horikoshi (A-40, 124-1, 169, 135, 27-1; soil)

* Designated the type strain by Boyer *et al.* (1973).

under which they were received. In addition, 46 strains of the *B. firmus*-*B. lentus* complex (Gordon *et al.*, 1977) also served as reference strains.

Development of pH 7.0 variants. At intervals of 5 to 10 d, the alkalophilic cultures were grown successively on soil extract agar (Gordon *et al.*, 1973) at pH 9.0, pH 8.5, pH 8.0, pH 7.5 and pH 7.0 [media at the higher pH values were prepared by the addition of a sterile solution of 1 M-sodium sesquicarbonate to the autoclaved media (Aunstrup *et al.*, 1972)]. The resulting cultures were transferred monthly on soil extract agar (pH 7.0) for 6 months or more before they were examined for their microscopic appearance and physiological reactions. In a few instances, growth and viability were enhanced by the addition of 3% (w/v) NaCl to the soil extract agar.

Properties examined. The media and methods described by Gordon *et al.* (1973, 1977) were used in this study, with the following modifications.

Sporulation. If a culture on soil extract agar did not sporulate after 3 d to 7 d incubation, it was grown on soil extract agar with 3% (w/v) NaCl and/or on soil extract yeast agar and re-examined. Soil extract yeast agar, a modification of the medium of Gounot (1967), contained: Difco yeast extract, 1 g; dibasic potassium phosphate, 0.2 g; agar, 15 g; soil extract, 1000 ml (pH 7.0). The soil extract was prepared by autoclaving 500 g of air-dried, sifted garden soil with 500 ml of tap water in a 2 l flask for 30 min, with the same precautions as used in preparing soil extract agar (Gordon *et al.*, 1973). The cooled supernatant was carefully decanted, filtered through paper and made up to 500 ml with tap water.

Temperature of incubation. Unless stated otherwise, strains whose maximum temperature for growth was 45 °C or higher were incubated at 37 °C; other strains were incubated at 28 °C.

Anaerobic growth. Because BBL dehydrated anaerobic agar without glucose or E_h indicator (Gordon *et al.*, 1973) was no longer available, BBL thioglycollate fluid medium (without glucose or E_h indicator) with 1.5% (w/v) agar was substituted. If a culture, inoculated from one in nutrient broth, failed to grow aerobically on this medium, the test was repeated with an inoculum of a culture grown in nutrient broth containing 3% (w/v) NaCl.

Maximum temperature for growth. Slants of soil extract agar with and without 3% (w/v) NaCl were used for determining growth at the various temperatures. Growth on one or both of the agars was recorded as positive.

Acid production from carbohydrates. In addition to nutrient agar with 3% (w/v) NaCl and 0.008% (w/v) phenol red, basal ammonium phosphate agar with 3% (w/v) NaCl and 0.0008% (w/v) bromocresol purple [20 ml of a 0.04% (w/v) solution per litre] served as a base for each carbohydrate. Because some cultures formed enough alkali from organic nitrogen in nutrient agar to neutralize the acid from some of the carbohydrates, cultures on the nutrient agar substrate were observed at 1, 2, 3, 5, 7, 10, 14, 21 and 28 d. Cultures on the ammonium phosphate substrate were observed at 7, 14, 21 and 28 d. Acid production from a carbohydrate in one or both of the substrate agars during incubation was recorded as positive.

Sensitivity to bacteriophage. If a culture failed to grow on the plates of nutrient agar used for demonstrating lysis by the bacteriophage for *B. firmus* ATCC 14575, the test was repeated on soil extract agar. All plates were incubated at 28 °C and observed at 1, 2 and 3 d.

Formation of urease. The substrate broth was adjusted to pH 6.5 before sterilization. All cultures were incubated at 28 °C and examined at 7 and 14 d for an alkaline colour in the tubes containing urea relative to the control tubes.

Deamination of phenylalanine. Cultures on phenylalanine agar were tested for phenylpyruvic acid (Gordon *et al.*, 1973) after 14 and 21 d incubation.

RESULTS

Lyophilized cultures of the alkalophilic isolates received for this study (Table 1) were revived in nutrient broth, pH 9.7, and grown on soil extract agar, pH 9.0 to 9.7. With three exceptions (strains C 351, 364, 378), the cultures were Gram-positive; all but eight (BB 36; C 351, 358, 360, 372, 374, 383, 413) were motile; and all but 11 (BB 45; BC 3, 7; C 371, 400; SB 14a, 14b, 16; TB 17; VB 3, 5) sporulated.

Examination of the variants of these 162 alkalophilic isolates and of the 12 alkalophilic reference strains, all of which grew and were maintained for five to eight years on media at pH 7.0, resulted in their assignment to the *B. firmus*–*B. lentus* complex (Tables 3 and 4). Re-examination of the 46 reference strains of this complex (Gordon *et al.*, 1977) by the modified tests used in this study placed two strains (NRS 1569 and 1573) of the 18 previously designated as *B. firmus*–*B. lentus* intermediates within *B. lentus*, and the spectrum-like arrangement of the remaining 16 intermediate strains was similar to that reported previously (Gordon *et al.*, 1977).

Among the pH 7.0 variants of 174 alkalophilic strains, *B. firmus* was represented by 47 (27%), including ATCC 21592, 21593 and 21596 (Tables 3 and 4). The microscopic appearance of these variants was more uniform than that of the other groups of variants. The rods were 0.7 to 0.9 µm in width, and those of 37 of the cultures appeared singly, in pairs or in short chains. Their appearance is compared with that of strains of *B. firmus* and *B. lentus* in Fig. 1 (a–d). Cultures of the remaining 10 variants also contained long chains and filaments like those illustrated in Fig. 1 (e, f), and three of the 10 had many shadow, or poorly stained forms. Sporulation was not observed in 11 strains (BC 2, 8; C 387, 400; PB 20, 38(1); SB 14a, 14b, 16; VB 3, 5). Cultures of the remaining 36 strains formed central, subterminal or terminal ellipsoidal spores, which swelled the sporangia slightly if at all (Fig. 1).

Of the pH 7.0 variants, 17 were assigned as intermediates between *B. firmus* and *B. lentus* on the basis of their formation of acid from seven carbohydrates and by their sensitivity to bacteriophage for the type strain of *B. firmus* (Table 5). Intermediate pH 7.0 variants that had the same reactions as reference intermediate strains for the seven carbohydrates and to the bacteriophage were BC 7 (same as NRS 1562); C 325, 373, 403, 413, PB 38(2), ATCC 21591, NCIB 9218 (NRS 1148); and NRS 1554 (NRS 1565). The rods formed by the 17 intermediate variants were 0.7 to 1.0 µm in diameter and appeared singly, in pairs or in short chains. Three of the variants (BC 4, 7, 10) did not sporulate. The ellipsoidal, subterminal and terminal spores of six cultures [C 325, 403, 413; PB 38(2); NCIB 9218; ATCC 21591] definitely swelled the sporangia, while sporangia of the remaining eight cultures were slightly swollen by their central and subterminal spores.

Table 3. *Some physiological properties of strains of the B. firmus-B. lentus complex*

	NRS and ATCC reference strains*			pH 7.0 variants of alkalophilic strains					
							<i>B. lentus</i> groups		
	<i>B. firmus</i>	Intermediates	<i>B. lentus</i>	<i>B. firmus</i>	Intermediates	1	2	3	
No. of strains . . .	20	16	10	47	17	18	17	75	
Property†									
Hydrolysis of hippurate	80	31	80	0	59	28	100	100	
Growth in 7% NaCl	95	81	80	100	100	100	100	100	
Utilization of:									
Citrate	5	31	10	4	24	44	100	100	
Propionate	0	13	0	0	0	28	88	69	
Growth at 50 °C	0	0	0	0	6	0	100	100	
Acid from:									
Arabinose	0	0	100	0	12	100	100	100	
Glucose	100	100	100	100	94	100	100	100	
Mannitol	90	100	100	89	88	94	100	100	
Mannose	0	69	100	0	65	100	100	100	
Melibiose	0	50	100	0	35	100	100	100	
Raffinose	0	56	100	0	35	100	100	100	
Sorbitol	0	6	30	0	12	56	82	0	
Sucrose	100	100	100	100	94	100	100	100	
Xylose	0	31	100	0	41	100	100	100	
Sensitivity to bacteriophage	100	31	0	100	88	0	0	100	
Decomposition of:									
Casein	100	94	30	91	76	83	94	91	
Gelatin	90	94	40	79	65	94	100	100	
Tyrosine	15	13	0	0	0	0	0	0	
Production of urease	0	13	60	0	6	0	0	0	
Reduction of nitrate to nitrite	65	13	40	0	18	0	94	3	
Deamination of phenylalanine	85	19	20	100	35	6	0	1	

* Strains described by Gordon *et al.* (1977).

† Positive properties of all strains were: formation of catalase, hydrolysis of starch and growth at 35 °C. Negative properties were: production of acetoin and dihydroxyacetone, anaerobic growth, egg-yolk reaction, growth in Sabouraud dextrose broth and agar and resistance to lysozyme.

Table 4. *Assignment of pH 7.0 variants of alkalophilic strains to groups of the B. firmus-B. lentus complex*

Group	No. of strains	Strain numbers
<i>B. firmus</i>	47	BB 20, 22, 24, 40; BC 1, 2, 8; C 324, 326, 338, 340, 342, 343, 346 to 350, 352, 353, 355, 379a, 387, 395, 399 to 402, 404; PB 20, 33, 35, 38(1), 39; SB 14a, 14b, 16, 37; VB 1 to 3, 5, 6, 11; ATCC 21592, 21593, 21596
<i>B. firmus-B. lentus</i> intermediates	17	BB 16; BC 3, 4, 7, 10; C 325, 373, 403, 413; PB 9, 19, 38(2); ATCC 21591, 21594; NCIB 9218; NRS 1548, 1554
<i>B. lentus</i>		
Group 1	18	BB 45; C 301, 302, 304, 311, 323, 334 to 337, 339, 341, 368, 369, 392; PB 25; SB 5; ATCC 21595
Group 2	17	BB 3, 7, 23, 30; C 300, 303, 312 to 314, 360, 365, 372, 374; SB 32; TB 13; VB 7; ATCC 21522
Group 3	75	BB 1, 10, 16(1), 17 to 19, 21, 25, 31, 34 to 37, 41, 43, 49; BC 6, 9, 11 to 17; C 351, 354, 356 to 358, 364, 366, 367, 370, 371, 375 to 378, 380a, 382b, 383 to 386, 388 to 390, 393, 394, 396 to 398, 410 to 412; PB 16, 17, 40, 41; TB 2, 4, 6, 9 to 12, 14 to 19; ATCC 27557; NRRL B-3881

Fig. 1. Cells and sporangia of cultures grown on soil extract agar for 3 d at 28 °C: (a) pH 7.0 variant of alkalophilic strain C 338 assigned to *B. firmus*; (b) ATCC 14575, type strain of *B. firmus*; (c) pH 7.0 variant of strain C 304 assigned to *B. lentus* Group 1; (d) ATCC 10840, type strain of *B. lentus*; (e, f) pH 7.0 variants of strains C 374 and 300 assigned to *B. lentus* Group 2. The bar markers represent 2 µm.

The 110 pH 7.0 variants representing *B. lentus* were divided into three groups by growth at 50 °C, sensitivity to bacteriophage and reduction of nitrate to nitrite (Table 3). Of the three groups, the 18 strains of Group 1 were most like the reference strains of *B. lentus*.

Table 5. *Reactions of pH 7.0 variants assigned to the B. firmus-B. lentus intermediates*

Strain	Acid production from carbohydrates*							Other properties†							
	Ara	Man	Mel	Raf	Sor	Suc	Xyl	SB	C	G	U	N	P	H	50 °C
Typical <i>B. firmus</i>	—	—	—	—	—	+	—	+	+	b	—	b	+	—	—
BB 16	—	—	—	—	—	+	+	+	+	—	—	+	—	—	—
BC 3	—	—	—	—	—	+	+	+	+	—	—	+	—	—	—
BC 4	—	—	—	—	—	+	+	+	+	—	—	+	+	+	+
PB 9	—	—	—	—	—	—	—	v	+	+	+	+	—	+	—
C 325	—	+	—	—	—	+	—	+	+	+	—	—	—	+	—
C 373	—	+	—	—	—	+	—	+	+	+	—	+	—	+	—
C 403	—	+	—	—	—	+	—	+	+	—	—	—	—	+	—
C 413	—	+	—	—	—	+	—	+	—	—	—	—	—	+	—
PB 38(2)	—	+	—	—	—	+	—	+	+	—	—	—	—	+	—
ATCC 21591	—	+	—	—	—	+	—	+	+	—	—	—	—	+	—
NCIB 9218	—	+	—	—	—	+	—	+	—	+	—	—	—	+	—
PB 19	—	—	+	+	—	+	—	+	+	+	—	—	+	+	—
BC 7	—	+	+	+	—	+	—	—	+	+	—	—	—	—	—
NRS 1548	—	+	+	+	—	+	+	+	+	+	—	—	+	—	—
NRS 1554	—	+	+	+	—	+	+	—	+	+	—	—	+	—	—
BC 10	+	—	+	+	+	+	+	+	+	—	—	+	—	—	—
ATCC 21594	+	+	+	+	+	+	+	v	—	—	—	—	—	—	—
Typical <i>B. lentus</i>															
Group 1	+	+	+	+	b	+	+	—	b	b	b	b	—	b	—
Group 2	+	+	+	+	b	+	+	—	+	+	—	+	—	+	+
Group 3	+	+	+	+	—	+	+	+	+	+	—	—	—	+	+

b, Some strains of the taxon were positive and others negative; v, cultures of the same strain were sometimes positive and sometimes negative.

* Ara, arabinose; Man, mannose; Mel, melibiose; Raf, raffinose; Sor, sorbitol; Suc, sucrose; Xyl, xylose.

† SB, sensitive to bacteriophage for *B. firmus* ATCC 14575; C, decomposition of casein; G, decomposition of gelatin; U, production of urease; N, reduction of nitrate to nitrite; P, deamination of phenylalanine; H, hydrolysis of hippurate; 50 °C, growth at 50 °C.

Morphologically their rods varied from 0.6 to 0.9 µm in width, and those of half the cultures appeared singly, in pairs and in short chains (Fig. 1c); the other cultures grew in long chains and filaments. Four of these pH 7.0 variants (BB 45; C 323, 337, 369) did not produce spores, and the central and subterminal ellipsoidal spores of the remaining 14 cultures did not swell the sporangia appreciably.

Group 2 *B. lentus* strains could be distinguished from those of Group 1 by their growth at 50 °C and reduction of nitrate to nitrite (Table 3). The rods of these 17 strains were 0.7 to 1.0 µm wide, and eight of the cultures also produced long chains and filaments (Fig. 1e, f). All but one strain (C 365) formed spores that were ellipsoidal, central or subterminal and did not noticeably swell the sporangia.

The cellular morphology of Group 3 strains was similar to that of the Group 2 strains but the cultures grew at 50 °C and were sensitive to the bacteriophage. Nine of these pH 7.0 variants (BB 37, 41, 43; BC 15, 17; C 412; TB 11, 12, 19) did not produce spores. The ellipsoidal, central and subterminal spores of 15% of the cultures of the remaining strains did not swell the sporangia while spores of the other 85% of the cultures swelled the sporangia slightly, but the free spores were no wider than the rods.

The specificity of the bacteriophage used in this study for strains of the *B. firmus*-*B. lentus* complex was examined by testing representative strains of a number of species. In addition to the sensitivity or resistance of the 220 strains recorded in Table 3, 402 strains representing *B. subtilis*, *B. pumilus*, *B. licheniformis* and ten other species were resistant to the bacteriophage.

Of the 51 strains representing species *incertae sedis* (Gordon *et al.*, 1973), 47 were resistant. The four phage-sensitive strains were ATCC 12905 (designated *B. macroides*), ATCC 14574 (*B. badius*), NRS 1117 (*B. epiphytus*), and NRS 1118 (*B. filicolonicus*).

DISCUSSION

The sensitivity to acid of strains of the *B. firmus*–*B. lentus* complex indicates that some of them might have their counterparts among the pH 7.0 variants of alkalophilic strains of the genus. According to our tests and observations, strains of *B. firmus*, *B. lentus* and a number resembling previously designated intermediate strains appeared among the variants (Table 5). The strains that grew at 50 °C, assigned here to *B. lentus* Groups 2 and 3, were not matched by previously studied strains, although the overall pattern of their properties (Table 3) was like that of the NRS and ATCC strains of the *B. firmus*–*B. lentus* complex.

After thorough investigation, the utilization in the classification of the genus *Bacillus* of properties independent of the metabolism of the strains, i.e. DNA homology (Priest, 1981), lipid composition (Minnikin & Goodfellow, 1981) and pyrolysis gas–liquid chromatography (O'Donnell & Norris, 1981), is expected to be very valuable. If study of these and other strains by different methods confirms the four groupings made here and sharply reduces the number of *B. firmus*–*B. lentus* intermediates, the recognition of three or four different species may be justified. With our present knowledge of these strains, however, we prefer to place them loosely in the *B. firmus*–*B. lentus* complex.

We regret that time did not permit re-examination of the strains representing species *incertae sedis* in the NRS Collection, particularly of the four that were sensitive to the bacteriophage for *B. firmus* ATCC 14575.

After five to eight years of cultivation on agar at pH 7.0, cultures of some of the pH 7.0 variants sporulated abundantly (Fig. 1) and might be expected to survive in test tube culture and in a natural environment as long as unmanipulated strains of the genus. Cultures of other variants, however, did not sporulate, and their viability was limited to a few months.

We can only speculate on the possibility that marine forms of the *B. firmus*–*B. lentus* complex were transferred by chance to other environments and that the hardier cells survived and their descendants became adapted to their new surroundings. Despite the futility of such speculation, the taxonomist should be alert to the possibility that strains with an unusual and seemingly dominant characteristic or strains isolated from unusual sources may be closely related to strains of long-recognized species.

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REFERENCES

- AUNSTRUP, K., OUTTRUP, H., ANDRESEN, O. & DAMBMANN, C. (1972). Proteases from alkalophilic *Bacillus* species. In *Proceedings of the Fourth International Symposium on Fermentation Technology Today*, pp. 299–305. Edited by G. Terui. Osaka, Japan: Society of Fermentation Technology.
- BOYER, E. W., INGLE, M. B. & MERCER, G. D. (1973). *Bacillus alcalophilus* subsp. *halodurans* subsp. nov.: an alkaline-amylase-producing alkalophilic organism. *International Journal of Systematic Bacteriology* **23**, 238–242.
- CHISLETT, M. E. & KUSHNER, D. J. (1961). A strain of *Bacillus circulans* capable of growing under highly alkaline conditions. *Journal of General Microbiology* **24**, 187–190.
- GORDON, R. E. (1981). One hundred and seven years of the genus *Bacillus*. In *The Aerobic Endospore-forming Bacteria: Classification and Identification*, pp. 1–15. Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press.
- GORDON, R. E., HAYNES, W. C. & PANG, C. H.-N. (1973). *The Genus Bacillus. Agriculture Handbook* No. 427. Washington, D.C.: United States Department of Agriculture.
- GORDON, R. E., HYDE, J. L. & MOORE, J. A., JR (1977). *Bacillus firmus*–*Bacillus lentus*: a series of

- one species? *International Journal of Systematic Bacteriology* **27**, 256–262.
- GOUNOT, A. M. (1967). Rôle biologique des arthro-bacters dans les limons souterrains. *Annales de l'Institut Pasteur* **113**, 923–945.
- HORIKOSHI, K. (1971). Production of alkaline enzymes by alkalophilic microorganisms. Part 1. Alkaline protease produced by *Bacillus* no. 221. *Agricultural and Biological Chemistry* **35**, 1407–1414.
- MINNIKIN, D. E. & GOODFELLOW, M. (1981). Lipids in the classification of *Bacillus* and related taxa. In *The Aerobic Endospore-forming Bacteria: Classification and Identification*, pp. 59–90. Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press.
- O'DONNELL, A. G. & NORRIS, J. R. (1981). Pyrolysis gas–liquid chromatographic studies. In *The Aerobic Endospore-forming Bacteria: Classification and Identification*, pp. 141–179. Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press.
- PRIEST, F. G. (1981). DNA homology in the genus *Bacillus*. In *The Aerobic Endospore-forming Bacteria: Classification and Identification*, pp. 33–57. Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press.
- TURNER, M. & JERVIS, D. I. (1968). The distribution of pigmented *Bacillus* species in saltmarsh and other saline and non-saline soils. *Nova Hedwigia* **16**, 293–297.
- VEDDER, A. (1934). *Bacillus alcalophilus* n. sp.; benevens enkele ervaringen met sterk alcalische voedingsbodems. *Antonie van Leeuwenhoek* **1**, 143–147.