ELECTRON MICROSCOPE SURVEY OF THE SURFACE CONFIGURATION OF SPORES OF THE GENUS *BACILLUS*

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Many taxonomic studies of species of the genus *Bacillus* have been made, but not any based upon the surface structure of spores. This was due in a large measure to the lack of a suitable method of preparation. Recently, Bradley and Williams (1957) and Franklin and Bradley (1957) showed, by means of electron microscopy and using carbon replicas, that the spores of some species of the aerobic sporeforming bacteria have different surface patterns. The sculpturing of the spore surface may be pronounced and complex as in *Bacillus polymyxa*, or indistinct as in *Bacillus stearothermophilus*. These authors suggested that the different surface configurations might be sufficiently well defined to be of value in the classification of this genus.

Accordingly, an examination has been made of a total of 19 species and 4 varieties, included in the classification of Smith *et al.* (1952), and 1 species not listed in their scheme, to assess the taxonomic value of this method.

METHODS

Preparation of spores. The organisms were cultured according to the method of Williams *et al.* (1957), and all species were incubated at 37 C except *B. stearothermophilus* and *Bacillus calidolactis*, which were incubated at 65 C. Clear observation of the surface patterns is dependent upon the cleanness of the spore suspensions, so that the following procedure was adopted. When sporulation was well advanced, the spores were harvested in sterile distilled water and shaken for 3 to 4 hr with glass beads. They were then washed 10 times with distilled water to remove vegetative cell material and debris. Suspensions which remained dirty after repeated washing were further cleaned by the method of Brown *et al.* (1957) involving the use of lysozyme.

The more important morphological and physiological characteristics of the strains used in the investigation, as indicated by Smith *et al.* (1952), were also studied to ascertain any major differences in biochemical reactions which might exist between these and typical strains of the species concerned.

Preparation of carbon replicas. The replica method described in detail by Bradley and Williams (1957) was used as follows. Specimen support grids were coated with Formvar films, and drops of spore suspension applied and allowed to dry. The spores, now mounted on the Formvar films, were then coated with a layer of evaporated carbon, and the Formvar washed away from the carbon film with chloroform. The spores were dissolved by immersing the grids in a mixture of chromic and permanganic acids. After washing and drying, the replicas were shadowed with gold/palladium at an angle of 2:1(26½°).

The electron micrographs shown were printed as negatives at a final magnification of 6700 ×.

The concentration of the spore suspensions was not critical and could be judged satisfactorily upon the basis of turbidity.

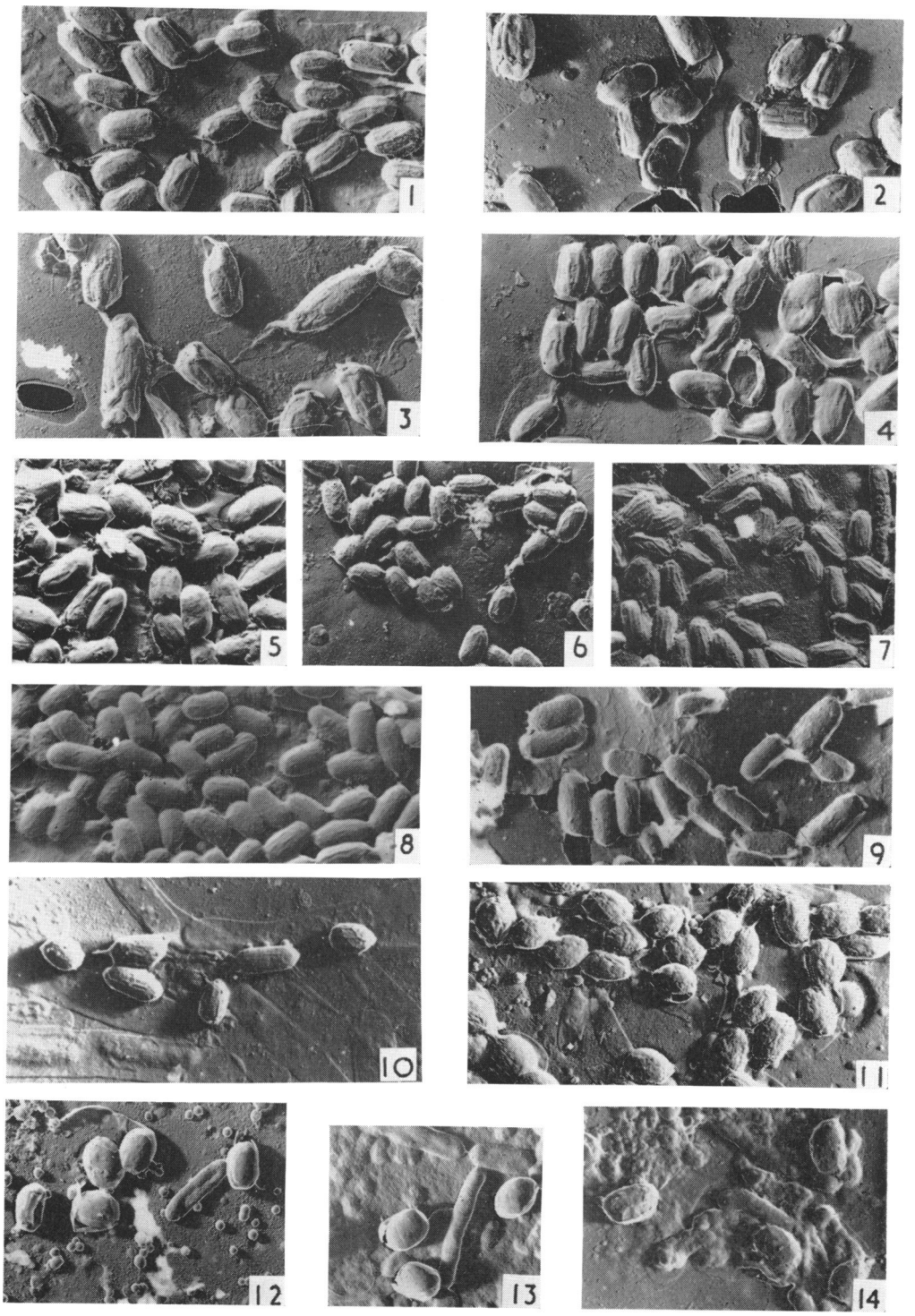
RESULTS

Details of the strains studied and the results obtained are given below. Electron micrographs of the spores are shown in figures 1 to 76. The species are grouped according to the classification of Smith *et al.* (1952).

Group 1. Sporangia not definitely swollen.

Bacillus subtilis, *B. subtilis* var. *aterrimus*, and *B. subtilis* var. *niger*

(1) Strains and sources:—*B. subtilis* CN 2745, the Wellcome Bacterial Collection (figure 1); *B. subtilis* 22, NIRD stock culture (figure 2); *B. subtilis* NCIB 8159, National Collection of Industrial Bacteria (figure 3); *B. subtilis* var. *aterrimus* CN 2192, the Wellcome Bacterial Collection (figure 4); *B. subtilis* var. *niger* CN 4008, the Wellcome Bacterial Collection (figure 5).



FIGS. 1–14

Figure 1. Bacillus subtilis strain CN 2745.
Figure 2. B. subtilis strain 22.
Figure 3. B. subtilis strain NCIB 8159.
Figure 4. B. subtilis var. *aterrimus* strain CN 2192.
Figure 5. B. subtilis var. *niger* strain CN 4008.
Figure 6. B. pumilus strain CN 787.
Figure 7. B. pumilus strain CN 807.
Figure 8. B. pumilus strain CN 607.
Figure 9. B. firmus strain CN 2936.
Figure 10. B. firmus strain CN 2196.
Figure 11. B. lentus strain CN 2789
Figure 12. B. lentus strain CN 3321.
Figure 13. B. lentus strain CN 3321.
Figure 14. B. lentus strain CN 2750.

(2) Biochemical reactions:—The reactions of all strains were typical of this species and the respective varieties.

(3) Electron microscopy:—The surface structure of the spores of *B. subtilis* has been described elsewhere (Bradley and Williams, 1957). Strains CN 2745 and 22 possess generally similar spores, although the ribbing is slightly less pronounced in the former. An additional feature is evident in strain NCIB 8159, in which some spores (*Ca.* 5 per cent) are almost double the length of others; these long spores are ribbed similarly to normal spores. The strains of *B. subtilis* var. *aterrimus* and *B. subtilis* var. *niger* examined here do not show any fundamental differences from the parent species.

(4) Description of the spores of *B. subtilis* (including varieties):—Spores cylindrical; ends well rounded; size 1 to 1.5 μ (occasionally 3 μ) by 0.8 μ; surface ribbed, the ribs usually longitudinal and rather irregular, sometimes transverse at the ends of the spores, the extent of the ribbing varying with strains.

Bacillus pumilus

(1) Strains and sources:—CN 787 (figure 6), CN 807 (figure 7), CN 607 (figure 8), all from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—The reactions of all three strains were typical of this species. Knight and Proom (1950) discussed the similarity of this species to *B. subtilis* and described the existence of a number of "intermediate" strains.

(3) Electron microscopy:—The spores of *B. pumilus* are very similar to those of *B. subtilis* except in size, *B. pumilus* spores being much smaller. Because of the constancy of their size, they can be distinguished with a reasonable degree of certainty by this feature, although they are more likely to be confused with *B. firmus*, a species also possessing small spores.

(4) Description of the spores of *B. pumilus*:—Spores cylindrical; ends well rounded; size 1 by 0.5 μ; surface ribbing usually similar to *B. subtilis*, although in some cases the surface is nearly smooth.

Bacillus firmus

(1) Strains and sources:—CN 2936 (figure 9), CN 2196 (figure 10), both from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—The reactions of both strains were typical of this species.

(3) Electron microscopy:—The spores of this species closely resemble those of *B. pumilus*, but are slightly larger and more cylindrical. Their size is not as consistent, and the shapes of the spores vary from very short cylinders and ovals to long cylinders, even within a single strain. The most important strain differences, as with *B. subtilus* and *B. pumilus* are variations in the degree of ribbing.

(4) Description of the spores of *B. firmus*:—Spores usually long, cylindrical, but sometimes oval; size 1 to 1.8 μ by 0.5 to 0.8 μ; surface smooth or ribbed similar to *B. pumilus*.

Bacillus lentus

(1) Strains and sources:—CN 2789 (figure 11), CN 3321 (figures 12 and 13), CN 2750 (figure 14), all from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—Strains CN 2750 and CN 3321 hydrolyzed starch; otherwise the reactions were typical of the species.

(3) Electron microscopy:—The spores of *B. lentus* differ from those previously described, but bear a close resemblance to those of *B. megaterium*. The spore surfaces are partially obscured by surface contamination in strain CN 2750, but it is thought that the roughness of the spores of strain CN 2789 is genuine, because it does not cover the surrounding substrate. It must be noted that the spores are covered with vegetative cell debris, but it seems improbable that this would impart the observed degree of roughness. The spore surface may thus vary from rough to perfectly smooth (strain CN 3321). It was difficult to obtain clean spore suspensions of this species.

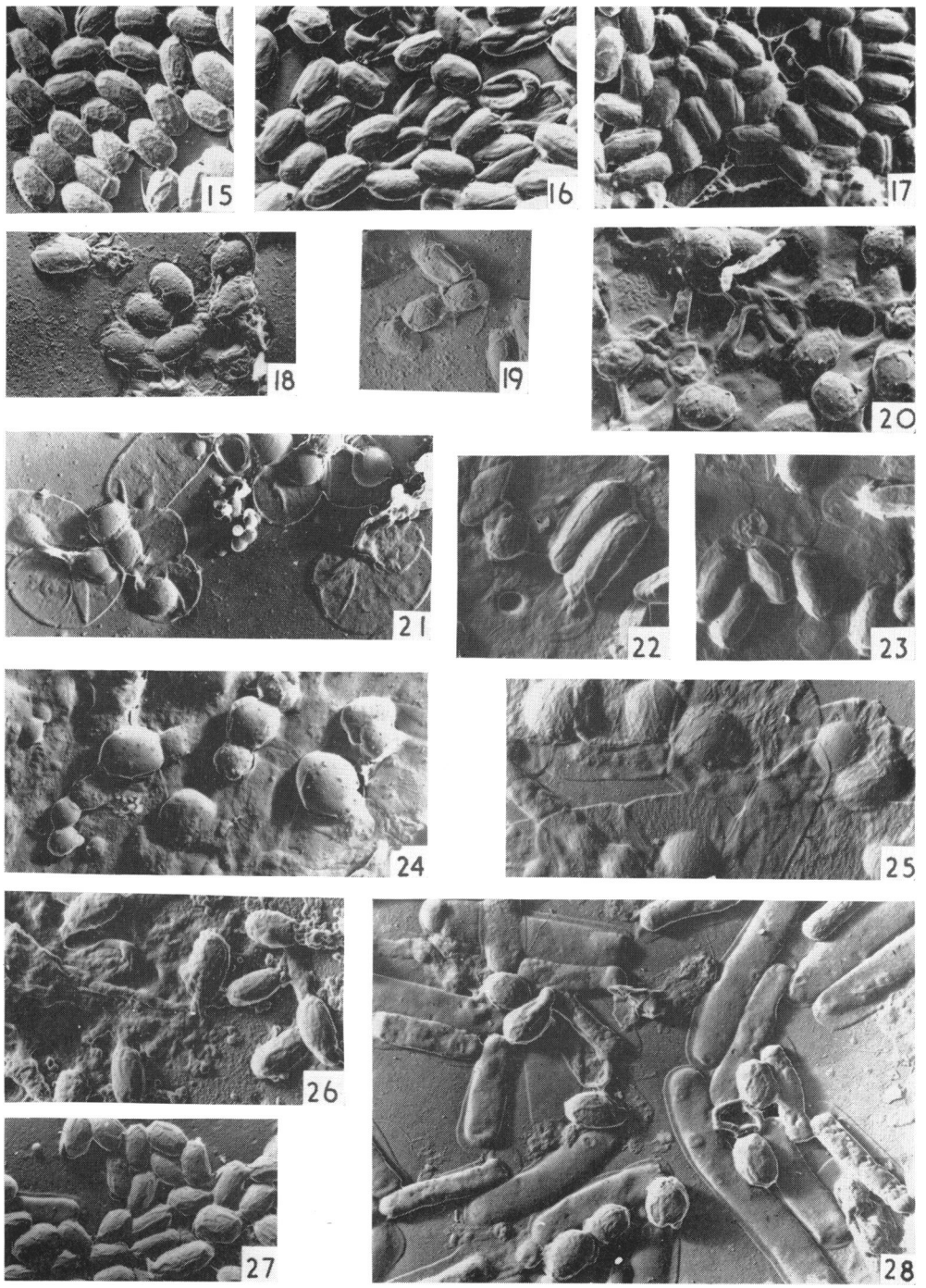
(4) Description of the spores of *B. lentus*:—Spores oval, occasionally spherical; size 1.1 by 0.9 μ; surface smooth or rough without conspicuous ribs.

Bacillus licheniformis

(1) Strains and sources:—92, NIRD stock culture (figure 15). NCIB 6816 (figure 16), NCIB 7224 (figure 17), both from the National Collection of Industrial Bacteria.

(2) Biochemical reactions:—The reactions of all strains were typical of this species.

(3) Electron microscopy:—This species has been described elsewhere (Bradley and Williams, 1957). The sculpturing of the spore surface is very similar to that of *B. subtilis*, except for a



FIGS. 15–28

Figure 15. Bacillus licheniformis strain 92.

Figure 16. B. licheniformis strain NCIB 6816.

Figure 17. B. licheniformis strain NCIB 7224.

Figure 18. B. megaterium strain 126.

Figure 19. B. megaterium strain 126.

Figure 20. B. megaterium strain CN 2193.

Figure 21. B. cereus strain 109.

Figure 22. B. cereus var. *thuringiensis* strain CN 4138.

Figure 23. B. cereus var. *thuringiensis* strain CN 4138.

Figure 24. B. cereus strain 103.

Figure 25. B. cereus var. *mycoides* strain CN 1409.

Figure 26. B. coagulans strain CN 2832.

Figure 27. B. coagulans strain CN 2835.

Figure 28. B. coagulans strain CN 2919.

longitudinal groove, which is a unique distinguishing feature. It has been suggested that this is a line of weakness in the spore coat (Franklin and Bradley, 1957). The groove is sometimes very shallow (strain 92), and in this form it appears to be associated with more conspicuous ribbing.

(4) Description of the spores of *B. licheniformis*:—Spores oval; size 1 to 1.8 μ by 0.6 to 0.9 μ; variable degree of ribbing similar in nature to *B. subtilis*, or surface nearly smooth; a single longitudinal groove, the depth of which varies according to the strain, distinguishing from all other species.

Bacillus megaterium

(1) Strains and sources:—126, NIRD stock culture (figures 18 and 19), CN 2193, the Wellcome Bacterial Collection (figure 20).

(2) Biochemical reactions:—Strain CN 2193 failed to hydrolyze starch and gave a positive Voges-Proskauer reaction. Strain 126 was typical of this species.

(3) Electron microscopy:—It was extremely difficult to prepare clean spore suspensions of this species. The spores were often covered with vegetative cell debris, and it was difficult to recognize any definite features from the electron micrographs. Attempts at cleaning the suspensions, by means of the lysozyme treatment, produced little improvement, as can be seen from figure 20. The spores resemble those of *B. lentus*, although there appears to be a greater proportion of spherical or nearly spherical spores.

(4) Description of spores of *B. megaterium*:—Spores spherical to oval; size 1 to 1.6 μ by 1 μ; surface structure difficult to observe because of contaminating vegetative cell material, but probably smooth or only very slightly ribbed.

Bacillus cereus, *B. cereus* var. *mycoides*, and *B. cereus* var. *thuringiensis*

(1) Strains and sources:—109 (figure 21), 103 (figure 24), both NIRD stock cultures. Variety *thuringiensis*, CN 4138 (figures 22 and 23), variety *mycoides*, CN 1409 (figure 25), both from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—The reactions of all strains were typical of the species and its respective varieties.

(3) Electron microscopy:—Spores of the strains of *B. cereus* and *B. cereus* var. *mycoides* examined appear to be similar. The existence of an "exosporium" surrounding the spores distinguishes them from all other species of this group. Lysozyme treatment failed to remove this without, in some way, affecting the spores (figure 24). The "exosporium" is more closely associated with the spore itself than the vegetative remains occasionally persisting in spore suspensions of other species. It is of interest that the spores of three of the strains examined were spherical and not oval, a feature at variance with most published accounts of this species. However, spores of *B. cereus* var. *thuringiensis* were oval and did not resemble the spores of the parent species; they were more like those of *B. subtilis* although the biochemical tests showed the strain to be more closely allied with *B. cereus*. The spores can be distinguished from those of *B. subtilis* by the great variation in their length, some spores being very long. The length variation is evenly graded from short to long, thus distinguishing the spores from those of *B. subtilis* strain NCIB 8159, where most of the spores are of constant size with relatively few which are very large. Vegetative cell walls were difficult to remove, but it is not certain whether they are as closely associated with the spores as the "exosporia" of the parent species.

(4) Description of spores of *B. cereus* and *B. cereus* var. *mycoides*:—Spores usually spherical; size variable 0.9 to 2.0 μ diameter; spores surrounded by an "exosporium" obscuring the surface, but probably smooth underneath.

(5) Description of spores of *B. cereus* var. *thuringiensis*:—Spores generally cylindrical, often markedly so, but sometimes oval; size 1.5 to 2.5 μ by 0.9 μ; surface smooth to very slightly ribbed; vegetative cell walls difficult to remove.

Bacillus coagulans

(1) Strains and sources:—CN 2832 (figure 26), CN 2835 (figure 27), CN 2919 (figure 28), all from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—Strain 2919 did not hydrolyze starch, otherwise the reactions of the strains were typical of the species.

Strains of this species may form either group 1 or group 2 type spores; the types formed by the strains examined here were as follows: CN 2832, spore type 2; CN 2835, spore type 2; CN 2919, spore type 1.

(3) Electron microscopy:—The two types of

spores appear to be different, the group 1 spores being slightly smaller and less distinctly ribbed than those of group 2. It was difficult to obtain spores of group 2 free from vegetative cell debris. An interesting feature of this species lies in a structure noted on the surface of residual vegetative cells. This consists of a raised region surrounding a hole or depression (figure 28), somewhat resembling a yeast bud scar in appearance (Bradley, 1956). These structures, which were not visible in a young culture of vegetative cells where spores were absent, cannot be explained at present, but further investigation is planned. The feature may be useful for identification purposes.

(4) Description of spores of *B. coagulans*:—Group 1 spores: Spores usually cylindrical, sometimes oval; size 1 to 1.5 μ by 0.6 μ; surface indistinctly ribbed.

Group 2 spores: Spores oval to cylindrical; size 1.2 to 1.5 μ by 0.8 μ; surface indistinctly ribbed.

Group 2. Sporangia definitely swollen by oval spores.

Bacillus polymyxa

(1) Strains and sources:—138 (figure 29), NIRD stock culture; CN 2494 (figure 30), CN 1417 (figures 31 to 32), both from the Wellcome Bacterial Collection; 153 (figure 33), NIRD stock culture; CN 3677 (figure 34), from the Wellcome Bacterial Collection.

Bacillus macerans

(1) Strains and sources:—CN 1013 (figure 35), CN 2204 (figure 36), CN 2614 (figure 37), all from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—The reactions of all strains were typical of their respective species. *B. polymyxa* and *B. macerans* are similar in many respects, particularly their ability to form gas from carbohydrates under normal conditions. In this respect, they are unique in the genus *Bacillus*.

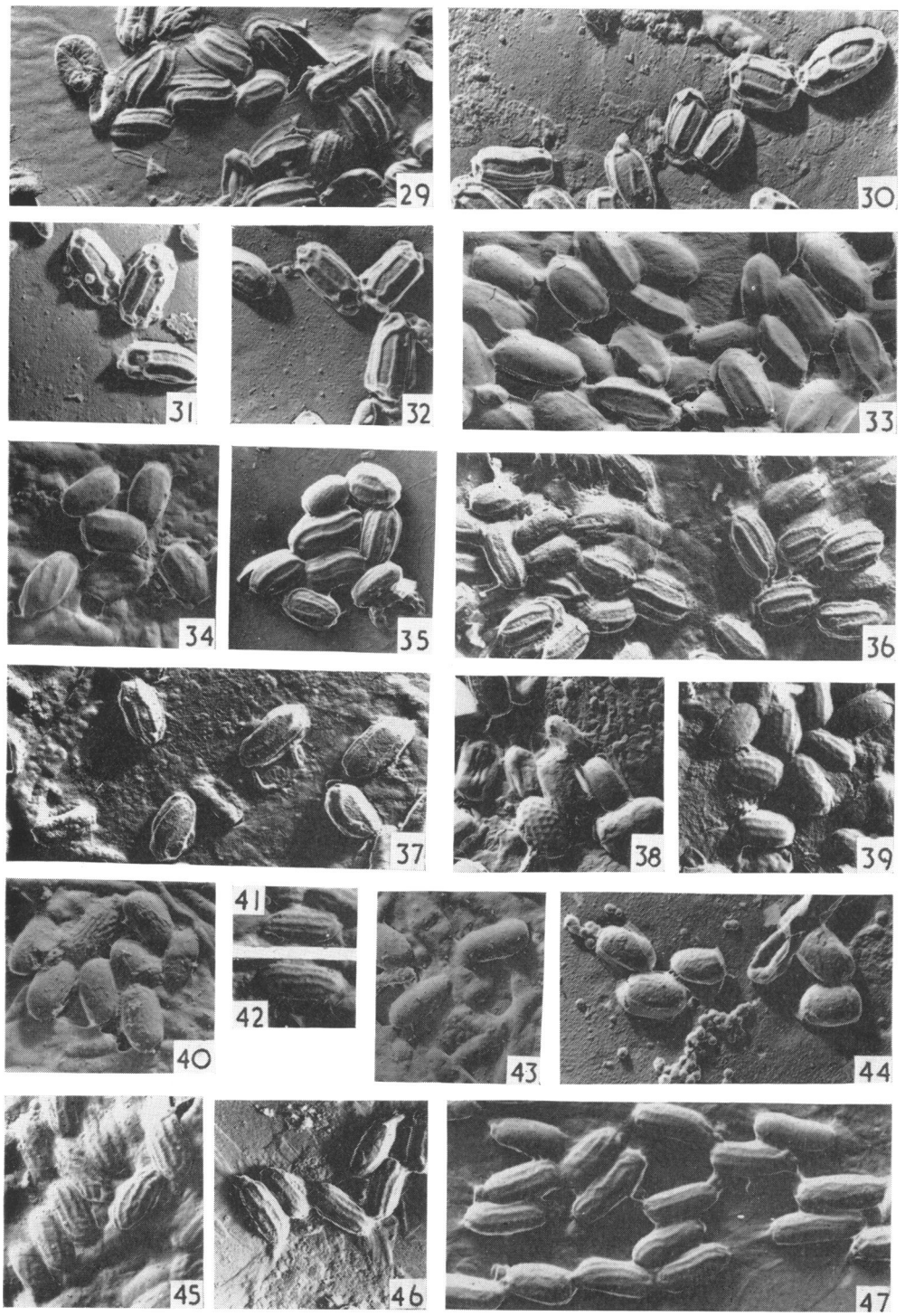
(3) Electron microscopy:—Spores of *B. polymyxa* and *B. macerans* are extremely similar in surface appearance and a number of strains of both species were studied in an unsuccessful attempt to distinguish them. A previous examination of strains of these species (Franklin and Bradley, 1957) showed that their spores were identical and a proposed structure of a "perfect" spore of this species was described. It is now known, however, as a result of the work reported here, that this type of ribbing is only valid for the most commonly occurring spore so far encountered. Two strain differences have been found within the species *B. polymyxa* amongst the strains studied here. One of these is in full agreement structurally with a strain studied by van den Hooff and Aninga (1956) in which the ribs form a reticulate structure at the ends of the spores. This study was previously discussed in a preliminary examination using carbon replicas (Franklin and Bradley, 1957) in which only the most common type of spore was found. The difference between the results of van den Hooff and Aninga (1956) and those of Franklin and Bradley (1957) was explained, either as an effect of the replica technique used by the former authors, or as a strain difference. Strains CN 2494 and CN 1417 appear to be similar to that described by van den Hooff and Aninga (1956), confirming their observations. The difference between the reticulate spores and the more common type is one of strain variation, therefore, and not one of technique. The other notable strain difference is shown by *B. polymyxa* strain 153 (figure 33), where two sets of ribs are entirely absent. Strain CN 3677 merely shows a variation in the height of the ribs as is the case with *B. macerans* strain CN 2204. With the other two strains of *B. macerans*, however, the spores are of the more common type.

It is unlikely that these two species can be separated by the surface structure of the spores although very marked differences are noted within the species *B. polymyxa*. It is possible that these differences may also occur within *B. macerans*. Dondero and Holbert (1957) showed the existence of a delicate exosporium surrounding *B. polymyxa* spores but this was not evident from the carbon replica method used here.

(4) Description of spores of *B. polymyxa* and *B. macerans*:—Spores oval to cylindrical; size 1.1 to 2.1 μ by 0.9 to 1.2 μ; surface always ribbed, generally very heavily; ribs parallel, longitudinal, usually numbering eight but sometimes only four; ribs either in the form of loops or reticulate networks at the ends of the spores; type of ribbing unique, facilitating identification.

Bacillus alvei

(1) Strains and sources:—CN 3186 (figure 45), CN 2198 (figure 46), CN 2771 (figure 47), all from the Wellcome Bacterial Collection.



FIGS. 29–47

Figure 29. Bacillus polymyxa strain 138.
Figure 30. B. polymyxa strain CN 2494.
Figure 31. B. polymyxa strain CN 1417.
Figure 32. B. polymyxa strain CN 1417.
Figure 33. B. polymyxa strain 153.
Figure 34. B. polymyxa strain CN 3677.

(2) Biochemical reactions:—All three strains failed to hydrolyze starch, but otherwise the reactions were typical of this species.

(3) Electron microscopy:—It is probable that spores of this species are unique because of their shape. The spores of all three strains examined here are easily identified by their length and type of ribbing. Gibson and Topping (1938), Knight and Proom (1950) and Smith *et al.* (1952) discussed the difficulties of differentiating and placing organisms in the *B. macerans*, *B. circulans*, and *B. alvei* group of species and have indicated the existence of "intermediates." These "intermediates" might provide a spectrum of variation in surface patterns, linking the species.

Vegetative cell walls were difficult to remove from the spore suspensions and could usually be seen in electron micrographs of this species.

(4) Description of the spores of *B. alvei*:—Spores long, cylindrical with rounded ends; size 1.8 to 2.2 μ by 0.8 μ; surface distinctly ribbed; ribs parallel and longitudinal; vegetative cell walls usually present but not masking surface structure; spores frequently found side by side in rows.

Bacillus circulans

(1) Strains and sources:—152 (figures 38 and 39) and 151 (figures 41 and 42), both NIRD stock cultures. CN 2201 (figures 40 and 43) and CN 2526 (figure 44), both from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—Strain CN 2201 did not hydrolyze gelatin and strain CN 2526 was unable to grow anaerobically in shake culture; otherwise the reactions of the strains were typical of this species.

(3) Electron microscopy:—The species *B. circulans* was described by Gibson and Topping (1938) as "exhibiting variations in several directions." This was also observed in the nature of the spore surfaces of this species. Knight and Proom (1950) indicated the close relationship existing between *B. circulans* and *B. alvei* and described a number of "intermediate" strains. One of the strains of *B. circulans* studied here (strain 152) was found to have the somewhat indistinct parallel longitudinal ribs characteristic of *B. alvei*, but it differed from this species in the shape of the spores, as can be seen from the plates. Strain 151 also exhibited ribbing similar to spores of *B. alvei* but failed to give a positive egg-yolk reaction distinguishing it from this species. It seems probable, therefore, that in the same way that a number of intermediate strains with different biochemical characteristics link *B. circulans* with *B. alvei*, so there may be a number of different spore shapes and surface patterns linking the smooth spores of some strains of *B. circulans* with the long, ribbed spores of *B. alvei*.

(4) Description of the spores of *B. circulans*:—Spores oval; size 1.4 μ by 0.9 to 1.2 μ; surface smooth or sometimes patterned with rather indistinct longitudinal parallel ribs; spores occasionally reticulated (figure 38).

Bacillus brevis

(1) Strains and sources:—SM 820 (figure 48), 179 (figure 49), 145 (figure 50), all NIRD stock cultures; NCTC 5098 (figures 51, 52, 53), National Collection of Type Cultures.

(2) Biochemical reactions:—The reactions of all strains were typical of this species.

(3) Electron microscopy:—The surface structure of the spores of *B. brevis* is constant and unique. No strain differences were detected and the species is easily identified in the electron microscope.

(4) Description of the spores of *B. brevis*:—Spores oval or nearly cylindrical; size 0.9 to 1.5 μ by 0.7 μ; surface smooth except for one or two distinct parallel longitudinal ribs, often terminating midway along the spores; very faint networks occasionally present.

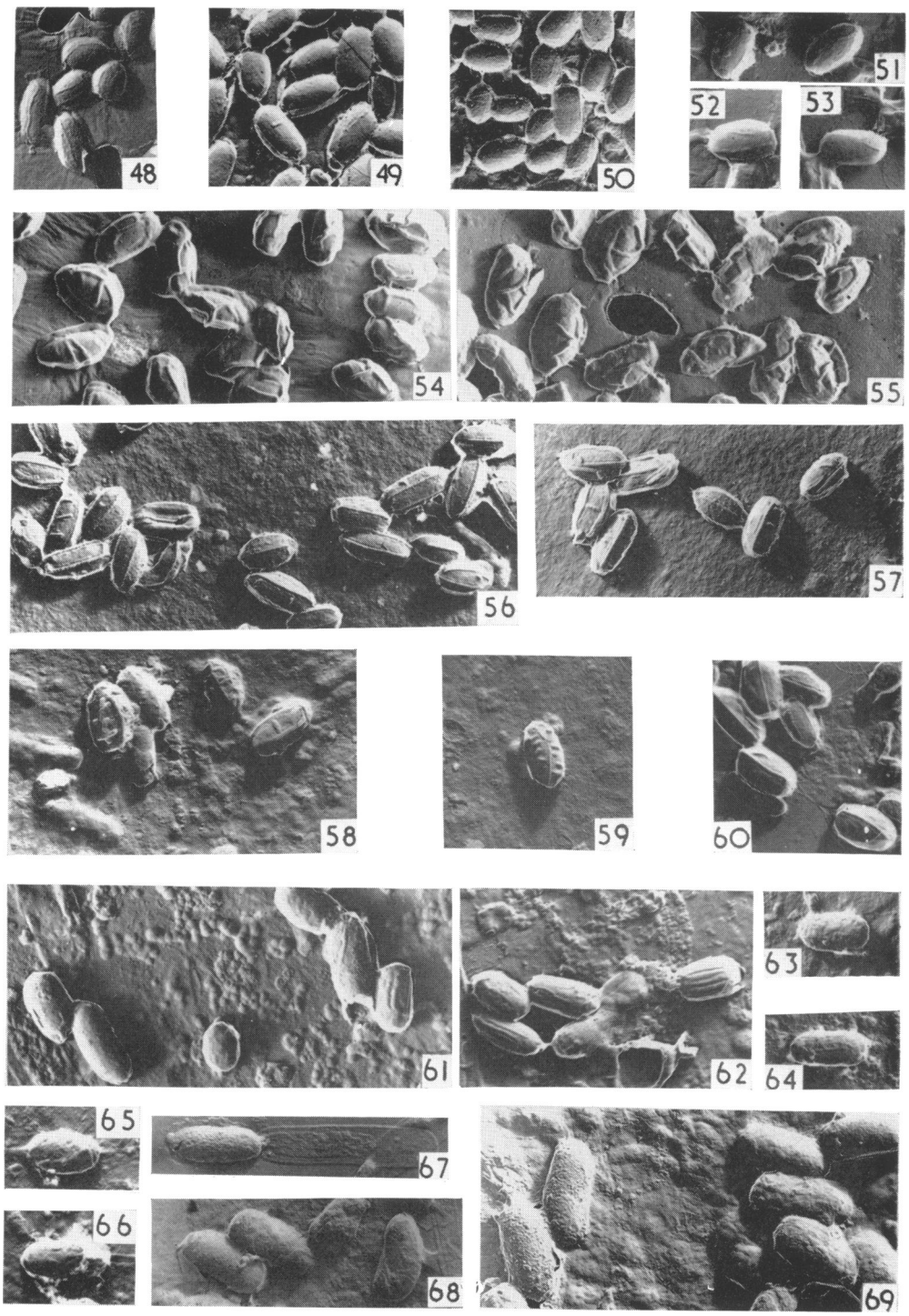
Bacillus laterosporus

(1) Strains and sources:—CN 2197 (figure 54), from the Wellcome Bacterial Collection; NCIB 8213 (figure 55), from the National Collection of Industrial Bacteria.

(2) Biochemical reactions:—The reactions of all strains were typical of this species.

(3) Electron microscopy:—The characteristic

Figure 35. *B. macerans* strain CN 1013.
Figure 36. *B. macerans* strain CN 2204.
Figure 37. *B. macerans* strain CN 2614.
Figure 38. *B. circulans* strain 152.
Figure 39. *B. circulans* strain 152.
Figure 40. *B. circulans* strain CN 2201.
Figure 41. *B. circulans* strain 151.
Figure 42. *B. circulans* strain 151.
Figure 43. *B. circulans* strain CN 2201.
Figure 44. *B. circulans* strain CN 2526.
Figure 45. *B. alvei* strain CN 3186.
Figure 46. *B. alvei* strain CN 2198.
Figure 47. *B. alvei* strain CN 2771.



FIGS. 48–69

Figure 48. Bacillus brevis strain SM 820.
Figure 49. B. brevis strain 179.
Figure 50. B. brevis strain 145.
Figure 51. B. brevis strain NCTC 5098.
Figure 52. B. brevis strain NCTC 5098.
Figure 53. B. brevis strain NCTC 5098.
Figure 54. B. laterosporus strain CN 2197.
Figure 55. B. laterosporus strain NCIB 8213.

spores of *B. laterosporus* are easily identified in the light microscope by a distinct "C" shaped body around the spore which was shown by Hannay (1957) to be an extension of the spore-coat. They are also readily distinguished in the electron microscope by this feature, together with a unique surface configuration.

(4) Description of the spores of *B. laterosporus*: —Spores oval; spore coat extending to form a "C"-shaped protuberance on one side; size 1.5 to 1.9 μ by 0.9 to 1.3 μ; surface ornamented with well-defined, sparse branching ridges occasionally forming very loose networks.

Bacillus pulvifaciens

(1) Strains and sources:—CN 3621 (figures 56 and 57), CN 3622 (figures 58 and 59), CN 3623 (figure 60), all from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—The reactions of all strains were typical of this species.

(3) Electron microscopy:—This species bears a superficial resemblance to *B. polymyxa* strain 153 (figure 33), but a close inspection of the electron micrographs shows that the longitudinal ribs are not of the same form; there is a minute groove on either side of the base of each rib, a unique feature which facilitates identification. In addition, short ribs are sometimes found at right angles to the longitudinal ribs.

(4) Description of the spores of *B. pulvifaciens*: —Spores oval; size 1.3 to 1.9 μ by 0.7 to 1.0 μ; four longitudinal ribs with fine grooves on either side of the base; a few transverse ribs sometimes present; the unique configuration is easily identified.

Bacillus stearothermophilus

(1) Strains and sources:—159 (figures 67 and 68), 204 (figure 69), both NIRD stock cultures.

(2) Biochemical reactions:—Both strains grew well at 65 C and gave the typical reactions of this species.

(3) Electron microscopy:—It was difficult to prepare clean spore suspensions, the spores being usually covered with a layer of contaminating material which imparted a rough appearance. There appears to be no surface structure present.

(4) Description of the spores of *B. stearothermophilus*:—Spores cylindrical; size 2.4 μ by 1.0 to 1.3 μ; spores difficult to obtain free from surface contamination, but otherwise smooth; vegetative cell walls sometimes persisting in the spore suspensions.

Bacillus calidolactis

(1) Strains and sources:—188 (figure 61), 187 (figure 62), 190 (figures 63, 64, 65, 66), all NIRD stock cultures.

(2) Biochemical reactions:—Smith *et al.* (1952) did not include this species in their classification of the genus *Bacillus*. All three strains examined grew at 65 C, distinguishing them from *B. coagulans*, and failed to hydrolyze gelatin, distinguishing them from *B. stearothermophilus*.

(3) Electron microscopy:—Because of the ability to grow at 65 C, the only confusion likely to arise in the identification of this species is with strains of *B. stearothermophilus*. Spores of both species are rather similar, but some ribbing is usually present in *B. calidolactis*. There are a few smooth spores present, but these differ from those of *B. stearothermophilus* which are rough in appearance because of the surface contamination present.

(4) Description of the spores of *B. calidolactis*: —Spores oval to cylindrical; size 1.2 to 2.2 μ by 0.9 μ; surface usually patterned with irregularly spaced, parallel, longitudinal ribs, often indistinct; occasional smooth spores are present.

Group 3. Sporangia swollen by round spores.

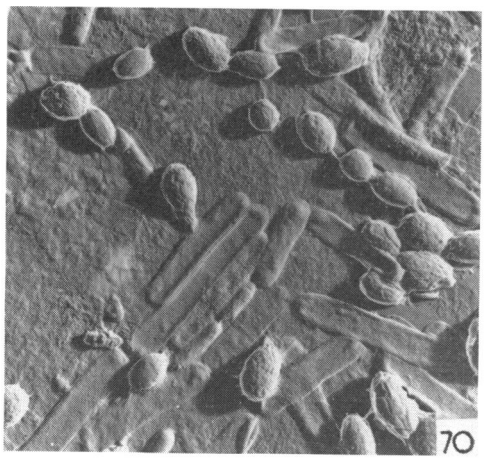
Bacillus pantothenticus

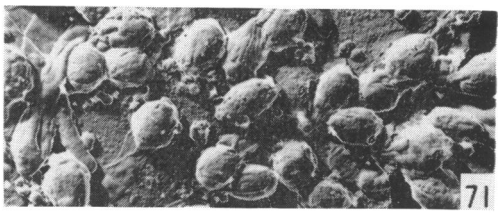
(1) Strains and sources:—CN 3043 (figure 70), CN 3019 (figure 71), both from the Wellcome Bacterial Collection.

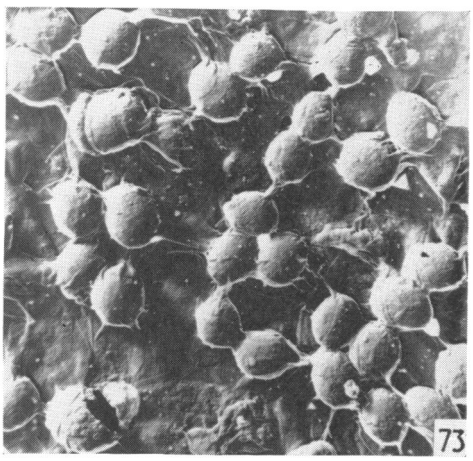
(2) Biochemical reactions:—Strain CN 3019 failed to hydrolyze starch, otherwise the reactions of both strains were typical of this species.

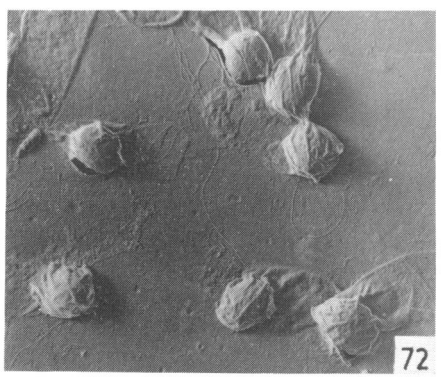
(3) Electron microscopy:—This species would

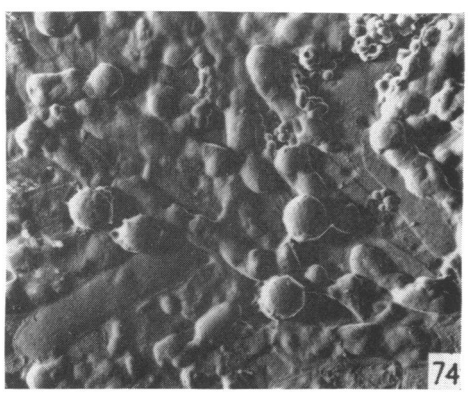
Figure 56. B. pulvifaciens strain CN 3621.
Figure 57. B. pulvifaciens strain CN 3621.
Figure 58. B. pulvifaciens strain CN 3622.
Figure 59. B. pulvifaciens strain CN 3622.
Figure 60. B. pulvifaciens strain CN 3623.
Figure 61. B. calidolactis strain 188.
Figure 62. B. calidolactis strain 187.
Figure 63. B. calidolactis strain 190.
Figure 64. B. calidolactis strain 190.
Figure 65. B. calidolactis strain 190.
Figure 66. B. calidolactis strain 190.
Figure 67. B. stearothermophilus strain 159.
Figure 68. B. stearothermophilus strain 159.
Figure 69. B. stearothermophilus strain 204.

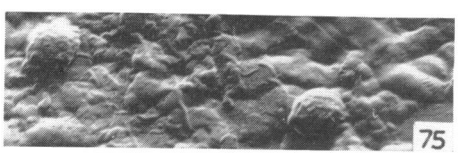












FIGS. 70–76

Figure 70. Bacillus pantothenticus strain CN 3043.

Figure 71. B. pantothenticus strain CN 3019.

Figure 72. B. sphaericus strain 183.

Figure 73. B. sphaericus strain 184.

Figure 74. B. sphaericus strain CN 2105.

Figure 75. B. pasteurii strain 179.

Figure 76. B. pasteurii strain 179.

seem to be intermediate between groups 2 and 3 because of its ability to produce both round and oval spores. The spores have a rough appearance, but exhibit no marked surface configuration.

(4) Description of the spores of *B. pantothenticus*:—Spores spherical to oval; size 0.6 to 1.2 μ by 0.7 μ; surface slightly rough; spores difficult to obtain free from vegetative cells.

Bacillus sphaericus

(1) Strains and sources:—183 (figure 72), 184 (figure 73), both NIRD stock cultures; CN 2105 (figure 74) from the Wellcome Bacterial Collection.

(2) Biochemical reactions:—All strains were inactive biochemically, showing no marked deviations from descriptions of this species.

(3) Electron microscopy:—Truly spherical spores are produced by this species, and the spore surfaces are smooth. The species is therefore easily identified, although it is similar to *B. pasteurii*.

(4) Description of the spores of *B. sphaericus*:—Spores spherical; size 1 to 1.3 μ diameter; surface smooth; spores difficult to obtain free of vegetative cells.

Bacillus pasteurii

(1) Strains and sources:—179 (figures 75, 76), NIRD stock culture.

(2) Biochemical reactions:—The reactions of this strain were typical of the species. Eleven strains of *B. pasteurii* obtained from various sources were cultured on a medium (pH 8.5) containing 2 per cent urea. Good growth occurred with nearly all cultures, but only one strain (179) produced spores.

(3) Electron microscopy:—This species produces spherical spores, but they are rough, not smooth as in the case of *B. sphaericus*.

(4) Description:—Spores spherical; diameter 1.0 μ; uniform size; surface rough in appearance with occasional irregular ribbing.

DISCUSSION

The various surface structures of spores of the genus *Bacillus* shown in the electron micrographs are not only of considerable morphological interest, but might also prove to be a useful method for the identification of species. It is interesting to consider the efficiency of this method for each group of species. In group 1, spores of *B. licheniformis* and *B. cereus* can be identified immediately. Those not belonging to either of these species, including varieties, can be readily placed into one of the following pairs of species: (a) *B. subtilis*, *B. coagulans*; (b) *B. pumilus*, *B. firmus*; (c) *B. lentus*, *B. megaterium*. This alone will simplify identification by other methods. A more careful examination of the surface structures should suggest to which one of the pair the organism belongs.

In group 2, the following species are easily identified: *B. brevis*, *B. laterosporus*, *B. pulvifaciens*, *B. stearothermophilus*, and *B. calidolactis*. Group 2 spores not belonging to these species can be placed in one of the following pairs: (a) *B. polymyxa*, *B. macerans*; (b) *B. circulans*, *B. alvei*. It is not possible to separate pair (a), but a study of shapes and types of surface configuration may differentiate between the species of pair (b), or indicate an intermediary position.

In group 3, each species is unique and can be identified easily.

In general, therefore, the surface structure is of great value in the identification of organisms of this genus. The method provides for identifying the component species in a mixture, without the necessity of separating them physically.

A full investigation of certain pairs of closely related species might help in clarifying the existing classification, by relating surface structures of the spores to the biochemical and nutritional characteristics.

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SUMMARY

A survey of the surface configuration of spores of 19 species and 4 varieties of the genus *Bacillus* has been carried out by electron microscopy using a carbon replica technique.

The majority of the species are readily distinguished by the patterns on the spore surfaces. The remainder can be narrowed down to pairs with similar sculpturing.

Spore surfaces may be smooth, as in *B. sphaericus*, or complex, as in *B. polymyxa* and *B. macerans*.

Concise descriptions of spores of the different species examined are given and these indicate the value of the method as a taxonomic aid.

REFERENCES

BRADLEY, D. E. 1956 A carbon replica technique for microbiological specimens applied to the study of the division of *Saccharomyces cerevisiae* with the electron microscope. J. Roy. Microscop. Soc., **75**, 254–261.

BRADLEY, D. E. AND WILLIAMS, D. J. 1957 An electron microscope study of the spores of some species of the genus *Bacillus* using carbon replicas. J. Gen. Microbiol., **17**, 75–79.

BROWN, W. L., ORDAL, Z. J., AND HALVORSON, H. O. 1957 Production and cleaning of spores of putrefactive anaerobe 3679. Appl. Microbiol., **5**, 156–159.

DONDERO, N. C. AND HOLBERT, P. E. 1957 The endospore of *Bacillus polymyxa*. J. Bacteriol., **74**, 43.

FRANKLIN, J. G. AND BRADLEY, D. E. 1957 A further study of the spores of species of the genus *Bacillus* in the electron microscope using carbon replicas, and some preliminary observations on *Clostridium welchii*. J. Appl. Bacteriol., **20**, 467–472.

GIBSON, T. AND TOPPING, L. E. 1938 Further studies of the aerobic spore forming bacilli. Proc. Soc. Agr. Bacteriologists, **1938**, paper no. 7.

HANNAY, C. L. 1957 The parasporal body of *Bacillus laterosporus* Laubach. J. Biophys. Biochem. Cytol., **3**, 1001–1010.

KNIGHT, B. C. J. G. AND PROOM, H. 1950 A comparative survey of the nutrition and physiology of mesophilic species in the genus *Bacillus*. J. Gen. Microbiol., **4**, 508–538.

SMITH, N., GORDON, R., AND CLARK, F. 1952 Aerobic spore-forming bacteria. U.S.D.A., Agr. Monograph no. 16, U. S. Govt. Printing Office, Washington, D. C.

VAN DEN HOOFF, A., AND ANINGA, S. 1956 An electron microscope study on the shape of the spores of *Bacillus polymyxa*. Antonie van Leeuwenhoek. J. Microbiol. Serol., **22**, 327–330.

WILLIAMS, D. J., FRANKLIN, J. G., CHAPMAN, H. R., AND CLEGG, L. F. L. 1957 Methods of assessing the sporicidal efficiency of an ultra-high-temperature milk sterilizing plant. I. Experiments with suspensions of spores in water. J. Appl. Bacteriol., **20**, 43–49.