JOURNAL OF BACTERIOLOGY, Dec. 1971, p. 1384 1389
Vol. 108, No. 3

Spore Appendages and Taxonomy of *Clostridium sordellii*

L. J. RODE, LEODOCIA POPE, CAMILLE FILIP, AND LOUIS DS. SMITH

Department of Microbiology, The University of Texas, Austin, Texas 78712, and Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060

Received for publication 9 August 1971

Twenty-five strains of *Clostridium sordellii* were divided into two groups on the basis of spore fine structure. Sixteen strains formed spores with smooth tubular appendages, and nine strains formed spores which lacked appendages. The other properties of the 25 strains were relatively constant. Since the minor strain variability which was encountered did not correlate with spore appendage status, fragmentation of this species on the basis of spore appendage status is not advocated.

Among the common *Clostridium* species, spores with appendages are known for *C. bifermentans* (3, 5, 9, 8), *C. botulinum* type E (2), *C. cochlearium* (4), and *C. sordellii* (3). Only one morphological type of spore appendage is known for *C. botulinum* type E (3) and one for *C. cochlearium* (4). On the other hand, five distinctive appendage types have been described for strains of *C. bifermentans* (7).

The occurrence of spore appendages in *C. sordellii* was first noted by Hodgkiss, Ordal, and Cann (3) for two of five strains examined. Because of the close taxonomic relationship between *C. bifermentans* and *C. sordellii*, and in view of the diversity of spore appendage morphology observed for *C. bifermentans*, we have now examined 25 strains of *C. sordellii*. Our objective has been to gain knowledge of appendage incidence and possible appendage morphological diversity in this species and to ascertain whether correlations of possible taxonomic significance may exist between spore fine structure and other properties.

MATERIALS AND METHODS

C. sordellii strains. Designations and sources of the 25 strains used in this study are shown in Table 1.

Spore production. Growth and sporulation were on the surface of 2% agar plates of Brain Heart Infusion (Difco) medium which contained sodium thioglycollate (0.5 g/liter). Plates were incubated for 7 days at 30 C in an anaerobic environment (6).

Electron microscopy. Carbon replicas of free spores were used for the routine electron microscope survey appraisal of spore structure. Spore suspensions were washed with demineralized water prior to preparation of replicas according to methods described previously (7).

Negative stains were made with 2% uranyl acetate, *p*H 4.3.

Specimens were examined with an Hitachi HS-7S electron microscope with double condenser and 50-kv accelerating voltage. Micrographs were taken on Kodak contrast process Ortho film.

Conventional taxonomic appraisal of strains. The strains of *C. sordellii* used in this study were examined by using the procedures given in *Outline of Clinical Methods in Anaerobic Bacteriology* (1).

RESULTS

Conventional taxonomic appraisal: constant strain features. The strains of *C. sordellii* were gram-positive rods, 1 to 1.5 μm in diameter and 3 to 4.5 μm in length, with oval spores, subterminal in position. Flagella, when present, were peritrichous. All strains liquefied gelatin, digested the casein in milk, produced indole, reduced nitrate, produced gas from peptone, and fermented glucose. All strains but 5913 and 4666B produced phospholipase. All strains except 2972 fermented maltose, only 4958 fermented glycerol, and only 5916-1 fermented galactose. No strain produced catalase or lipase on egg-yolk agar, and no strain fermented adonitol, amygdalin, cellobiose, cellulose, dulcitol, erythritol, esculin, glycogen, inositol, inulin, lactose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sorbose, starch, sucrose, trehalose, or xylose.

Conventional taxonomic appraisal: variable strain features. The 25 strains exhibited variability in regard to motility, digestion of meat, production of H_2S, hydrolysis of esculin, fermentation of fructose, tolerance to 6.5% NaCl, hy-

drolysis of hippurate, hemolytic activity, inhibition by Gantrisin, and mouse toxicity. These data are summarized in Table 2.

Electron microscopy: constant spore features. Spores of all 25 strains were oval and possessed an exosporium. The spore surfaces were rough and ill-defined in appearance, and they exhibited no prominent characteristic features (Fig. 1).

Electron microscopy: variable spore features. Sixteen of the 25 strains produced spores with appendages (Table 2). Although variability of spore size between strains was noted, such variability occurred also within spore populations of a given strain and is commonly encountered.

Appendage characterization in C. sordellii. Spore appendages of the 16 strains were all smooth tubes. Considerable variability was noted, however, with respect to number of appendages per spore, appendage length, and appendage width (Table 3). The number of appendages per spore was small (one to four), appendage length varied from 0.4 to 2.3 μm, and appendage width varied from 57 to 80 nm (Table 3).

The spore in Fig. 1 is illustrative of the appendage-bearing spore of *C. sordellii*. The appendages penetrate the prominent exosporium which is most readily observed at one or both spore ends. Appendage fine structure by negative stain (Fig. 2) appears identical to that described previously for the smooth tubular appendages of some *C. bifermentans* strains (9), and it is notably dissimilar to the hexagonal fine structure of the exosporium (Fig. 3). The latter structure appears similar to the exosporium of spores of *C. bifermentans* (9), *C. botulinum* type F (3), and OS strains of *C. botulinum* type E (3).

TABLE 1. *Clostridium sordellii strain designations and sources*

Strain no.[a]	Source
2013	ATCC 9714[b]
2025	CDC 224[c]
2027	CDC 861[c]
2656	McClung 128
2972	Prevot 3013
3058	Human infection
4463	McClung 2720
4538	Alpaca infection[d]
4539B	Alpaca infection[d]
4540	Alpaca infection[d]
4666B	Sheep infection
4847	Prévot 9/43c
4850	Prévot 12/16c
4852	Prévot 06/43D
4958	Prévot 365A
5061	Prévot 4529
5913	—[e]
5914	—[e]
5915	—[e]
5916-1	—[e]
5917	—[e]
5918	—[e]
5919	—[e]
5920	—[e]
5921	—[e]

[a] Accession numbers of the Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Va., which identify the strains for the present work.

[b] ATCC, American Type Culture Collection, Rockville, Md.

[c] CDC, Center for Disease Control, Atlanta, Ga.

[d] Isolated by Manuel Moro.

[e] Isolated by one of us (L.DS.S.) from various animal or human infections.

DISCUSSION

This survey of 25 strains of *C. sordellii* provides perspective with regard to spore fine structure in relation to other strain properties. Although 64% of the strains formed spores with appendages (Table 2), the appendages were all of a single type (Table 3; Fig. 1 and 2). This is in marked contrast to *C. bifermentans* wherein five distinctive spore appendage types have been described (5, 8, 9). *C. sordellii* appears heterogeneous with respect to spore appendage formation (nine strains did not form appendage-bearing spores) but homogeneous with respect to spore appendage type.

The appendages are smooth tubes, as was first noted for two strains of *C. sordellii* by Hodgkiss, Ordal, and Cann (3). They can be detached readily from the spore and often fragment during preparative procedures for electron microscopy. This tended to complicate assessment of appendage status in certain cases, especially where appendages were sparse. Many spores in such preparations lacked appendages, but detached fragments could be detected.

The homogeneity of the 25 strains in other regards is apparent and consistent with their designation as *C. sordellii*. Although some strain variability was noted (Table 2), no strain was thereby excluded from the species. Spore appendage status did not correlate with any of the other variable properties.

In view of the foregoing, and consistent with the proposal advanced earlier for *C. bifermentans* (7), fragmentation of the species on the basis of spore appendage status is not advocated. Designation of the two spore types as "varieties" of *C. sordellii* is proposed.

TABLE 2. *Variable properties of 25 strains of Clostridium sordellii*

Strain no.	Spore appendages	Motility	Meat digestion	H_2S production	Esculin hydrolysis	Fructose fermentation	NaCl (6.5%) tolerance	Hippurate hydrolysis	Hemolysis	Inhibited by Gantrisin	Toxicity
2013	+	+	+	−	−	+	−	+	+		+
2025	+	+	+	−	−	+	−	+	−		−
2027	+	+	−	−	+	+	−	+	+		−
2972	+	+	+	−	−	+	−	+	−		−
3058	+	+	−	−	+	−	−	+	+		−
4463	+	−	+	+	−	+	−	−	+		−
4538	+	+	+	+	−	−	−	−	−	−	−
4539B	+	+	+	+	−	+	+	+	+		
4540	+	+	+	+	−	−	+	+	+		−
4666B	+	+	+	+	−	+	−	−	+		
4847	+	+	+	+	−	+	+	−	−	+	−
4852	+	−	−	+	−	+	−	+	−	+	−
4958	+	+	+	+	+	−	−	−	−		−
5061	+	+	+	+	+	+	−	−	−	−	−
5914	+	+	+	+	+	+	−	+	+	−	−
5920	+	+	+	+	−	−	+	+	+	−	−
2656	−	+	+	−	+	+	−	+	−	+	+
4850	−	−	+	+	+	+	+	+	−		−
5913	−	+	+	+	−	+	+	+	−	−	−
5915	−	+	+	+	−	−	+	+	+	−	
5916-1	−	+	+	+	+	+	+	+	+	−	−
5917	−	+	−	+	−	−	−	+	+	+	+
5918	−	−	+	+	−	−	−	+	+	+	+
5919	−	−	+	+	−	−	−	+	+	+	+
5921	−	−	+	+	−	−	−	+	+	+	−

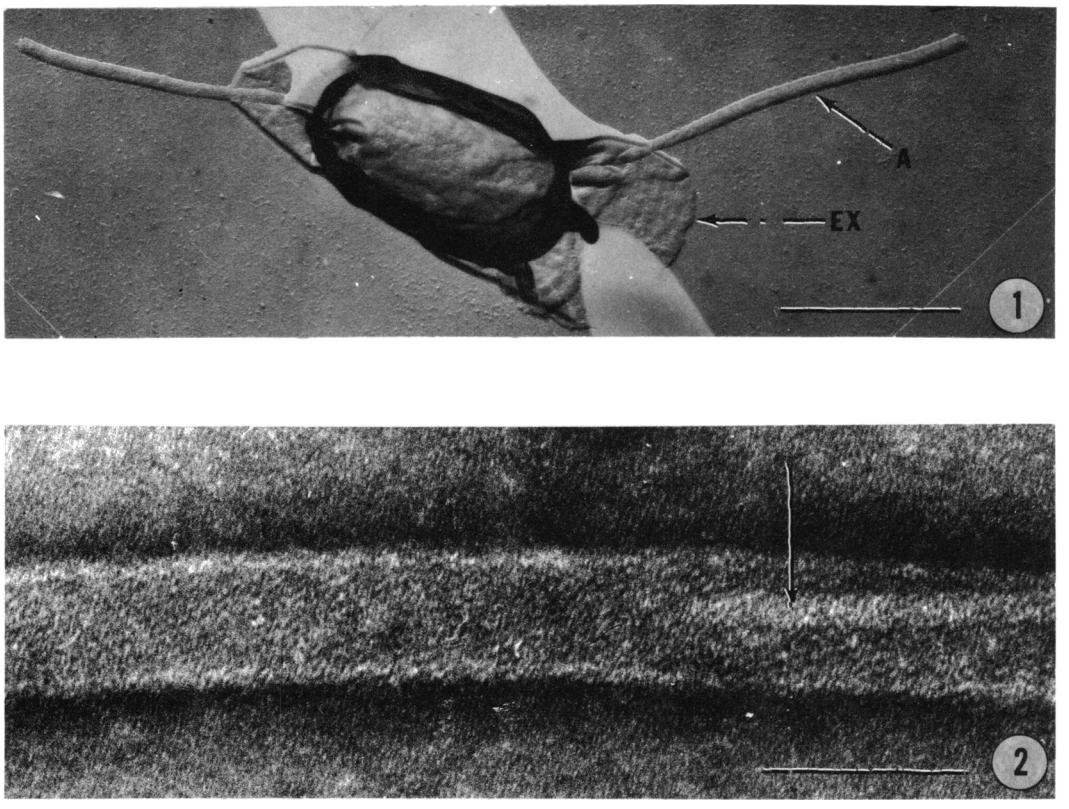

FIG. 2. *Detail of the smooth tubular appendage of Clostridium sordellii 2972. A flagellum fragment (arrow) is present. Negative stain. Bar represents 0.1 μm.*

TABLE 3. *Spore characterization in 16 strains of Clostridium sordellii*

Strain no.	Appendage type	No. of appendages per spore[a]	Appendage length (μm)[a]	Appendage width (nm)[b]	Spore exosporium[c]
2013	Smooth, tubular	1	1.5	70	+
2025	Smooth, tubular	1	0.6	64	+
2027	Smooth, tubular	1	1.6	70	+
2972	Smooth, tubular	2	2.3	80	+
3058	Smooth, tubular	4	0.6	62	+
4463	Smooth, tubular	2	1.4	66	+
4537	Smooth, tubular	1	0.4	57	+
4539B	Smooth, tubular	1	1.2	69	+
4540	Smooth, tubular	1	0.7	73	+
4666B	Smooth, tubular	1	1.4	69	+
4847	Smooth, tubular	1	0.4	62	+
4852	Smooth, tubular	1	1.4	64	+
4958	Smooth, tubular	1	2.1	80	+
5061	Smooth, tubular	1	2.0	71	+
5914	Smooth, tubular	1	2.2	62	+
5920	Smooth, tubular	1	1.5	79	+

[a] Maximum observed.

[b] This dimension is designated as appendage width rather than as appendage diameter because the tubular appendages tend to flatten during specimen preparation for electron microscopy.

[c] Exosporia were detected also for the nine strains (Table 2) which formed spores lacking appendages.

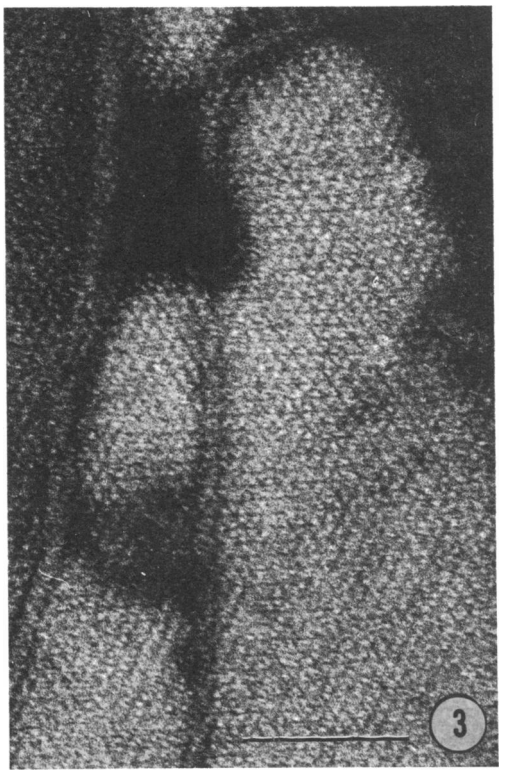

FIG. 3. *Hexagonal fine structure of the exosporium of Clostridium sordellii 2972, which contrasts with the fine structure of the appendage (Fig. 2). Negative stain. Bar represents 0.1 μm.*

ACKNOWLEDGMENTS

We thank Manuel Moro for the strains of *C. sordellii* that he isolated from infections in alpacas.

This investigation was supported by research grant GB-17677 from the National Science Foundation and Public Health Service research grant AI-02830 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Anaerobe Laboratory, Virginia Polytechnic Institute and State University. 1970. Outline of clinical methods in anerobic bacteriology, 2nd rev. Virginia Polytechnic Institute and State Univ., Blacksburg, Va.
2. Hodgkiss, W., and Z. J. Ordal. 1966. Morphology of the spore of some strains of *Clostridium botulinum* type E. J. Bacteriol. **91**:2031-2036.
3. Hodgkiss, W., Z. J. Ordal, and D. C. Cann. 1967. The morphology and ultrastructure of the spore and exosporium of some *Clostridium* species. J. Gen. Microbiol. **47**: 213-225.
4. Pope, L., and L. J. Rode. 1969. Spore fine structure in *Clostridium cochlearium*. J. Bacteriol. **100**:994-1001.
5. Pope, L., D. P. Yolton, and L. J. Rode. 1967. Appendages of *Clostridium bifermentans* spores. J. Bacteriol. **94**:1206-1215.
6. Rode, L. J., M. A. Crawford, and M. G. Williams. 1967. *Clostridium* spores with ribbon-like appendages. J. Bacteriol. **93**:1160-1173.
7. Rode, L. J., and L. DS. Smith. 1971. Taxonomic implications of spore fine structure in *Clostridium bifermentans*. J. Bacteriol. **105**:349-354.
8. Samsonoff, W. A., T. Hashimoto, and S. F. Conti. 1970. Ultrastructural changes associated with germination and outgrowth of an appendage-bearing clostridial spore. J. Bacteriol. **101**:1038-1045.
9. Yolton, D. P., L. Pope, M. G. Williams, and L. J. Rode. 1968. Further electron microscope characterization of spore appendages of *Clostridium bifermentans*. J. Bacteriol. **95**:231-238.