STUDIES ON ANAEROBIC BACTERIA

IV. TAXONOMY OF CULTURES OF A THERMOPHILIC SPECIES CAUSING "SWELLS" OF CANNED FOODS

L. S. McCLUNG*

Department of Agricultural Bacteriology, University of Wisconsin, Madison

Received for publication July 16, 1934

HISTORY OF STRAINS

In a previous paper (McClung, 1935), methods of culture were discussed for the isolation and routine cultivation of a group of thermophilic anaerobic spore-forming bacteria. It seemed necessary to study the technique of culture of this particular group in some detail as in many instances it was found that either media or methods which have been used by other investigators for the various groups of mesophilic anaerobes were not satisfactory for the routine study of this group. Following the development of these methods a detailed study has been made to aid in the classification of the cultures. As was earlier mentioned in the review of the literature, little has been published on the morphological and physiological characteristics of this group.

Sources of strains

In an attempt to obtain as nearly as possible a representative collection of cultures, strains were secured from the National Canners' Research Laboratories in Washington[1] and original isolations were made from soil.[2] Seven cultures were obtained from the Washington laboratory; twenty represent original isolations. Included in the first seven are strains which had been isolated from "blown" canned foods or from sugar. The soil dilutions

* Now with Research Divison, American Can Company, Maywood, Illinois.

[1] Through the courtesy of Dr. E. J. Cameron.

[2] The soil used was obtained from Dr. J. R. Esty of the Western Branch of the National Canners' Association. The samples were from the contaminated field reported by Townsend (1932).

(1:100 and 1:1000) were steamed for fifteen minutes (thus eliminating almost entirely the usual contaminants) before subcultures were made into liver infusion with 1:100,000 gentian violet (as recommended by Cameron (1930) to reduce the "flat sour" contamination) and into pea infusion which were incubated at 58°C. for enrichment.

Purification of cultures

Following several serial transplants in the enrichment media, plate cultures on pea-infusion agar were made. Serial (six or more) single colony isolations by the streak technique served as the purification procedure. Microscopic examination followed each step of the purification; in no case did forms other than the typical one (to be described later) appear after the plating began. The cultures received from the Washington laboratory were subjected to the same re-purification procedure. Single cell isolation[1] was used as the final step in the purification of six of the cultures. All cultures were then tested for both aerobic and anaerobic contaminants at 37°C. and 55°C. and for their ability to produce the "hard swell" spoilage at thermophilic temperatures in cans of peas or corn which had been previously incubated at 60°C. to insure their freedom from this group of organisms.

DISTINCTIVE TAXONOMIC CHARACTERISTICS

Morphological characteristics

Morphology. All the strains are long, slender, granulated bacilli bearing a round, strictly terminal spore which swells the rod (figs. 4 and 5). The organisms occur singly or occasionally in pairs, though never in chains. Although there is some variation in the size of the individual cells within the same culture in a

[1] *Method.* Spore suspensions were obtained from surface growth on vegetable infusion agar and single spores isolated by means of a modified Chamber's micromanipulator. The tip of the micropipette containing the isolated spore was clipped with sterile forceps and dropped into tubes of liver infusion or glucose tryptone broth (agar seal). Although only a small number of spores was isolated, a fair percentage of these germinated. Isolation of vegetative cells was not attempted as this has been shown by others to yield a lower percentage of positive cultures with other groups of anaerobic bacilli.

particular medium, the general picture is characteristic; a change of the culture medium, however, alters the cell type. In the glucose-containing media (glucose tryptone broth, etc.) the vegetative cells become shorter and thicker than those in vegetable-infusion or liver-infusion media; also no sporulation is seen. Cell measurements of a four-day-old culture on pea-infusion agar (air-dried nigrosin mount) showed the following variations: length, 3.5 to 7.5μ and width, 0.4 to 0.7μ. Spores from the same culture varied from 0.6 to 0.8μ in diameter. These are similar to the figures reported by Paine (1931).

The mode of sporulation is the most distinctive feature of the microscopic picture. On pea-infusion agar young spores may be seen as early as forty-eight hours; ninety-six to one hundred and twenty hours incubation is usually necessary for these to mature. Free spores are not often observed; the sporangium remains attached to the spore even in cultures in which spores which take a spore stain have been observed for several days.

Staining reactions. The vegetative cells of these cultures are often granulated; this is especially well revealed by methylene blue or dilute gentian violet. Granulation is most pronounced in old cultures in the process of spore formation or in cultures grown in media which are not well suited for growth. Immature spores stain more intensely with the simple stains than do the sporangia in which they are borne. Mature spores are stained only with difficulty; the methods of Dorner and of Moeller and the technique recently proposed by Schaeffer and Fulton (1933) have all been used with success. Irrespective of the method used, the heating process in the staining of the spore should be lengthened from five to ten times that proposed for the mesophilic aerobic spore-forming group.

None of the cultures of this group are stained by Gram's method. This appears to be an exception to the rule that spore-forming organisms are Gram-positive (although other exceptions may be found in the literature, *e.g.*, aerobic spore-forming bacilli causing false presumptive tests in water analyses) and is also contrary to the report by Paine (1931). Although in this paper the vegetative cells are reported to be easily decolorized the technique of

the stain was not included and thus the exact procedure can not be checked. In addition to the Gram stains made in the usual routine of study, the following experiment was carried out to test this point. Five strains were grown in liver infusion and smears prepared on acid-cleaned slides at the end of three, six, nine, twelve, fifteen, eighteen and twenty-four hours following inoculation. Quadruplicate slides were prepared and the smears of two sets received, before drying, a dilute suspension of *Lactobacillus Delbrückii*. One set of the slides prepared in this manner and one set without the added organism were stained by the Kopeloff-Beerman modification of the Gram stain (acetone-alcohol used as the decolorizing agent); the remaining two sets were stained by the Hucker modification (95 per cent alcohol as the decolorizing agent). In no case were the cells of the thermophilic species Gram-positive, though the lactobacillus did take the stain. In a few instances, not correlated with the age of the culture but probably with slight variation in decolorization, the cells of the thermophilic organism assumed a slight purple tinge; in no case were they definitely Gram-positive.

Upon examination by the Hiss method no capsule could be demonstrated. The granulose reaction with Meissner's iodine solution was negative in twenty-four-hour cultures grown in corn-liver medium.

Motility. A small percentage of the cells of a four- to six-hour-glucose tryptone broth were found to be slowly motile by microscopic examination. The inocula for this series were from active (eighteen-hour) cultures and the tubes were preheated to 60°C. before inoculation. Peritrichiate flagella are seen in stains prepared according to the methods of Gray (1926) and Craigie (1929). Older cultures (eighteen to twenty-four hours) which have been repeatedly examined by the coverslip method or, in certain instances, by the capillary tube technique of Sturges (1928) do not exhibit motility.

Colony characteristics. Deep colonies are rather difficult to obtain, for the best media for colony formation are those containing glucose. The concentration of sugar necessary for growth allows extensive gas production, resulting in bubbles and cracks

in the agar in the vicinity of the colonies. Deep colonies, in a glucose (0.5 per cent) tryptone (1 per cent) medium in which the agar concentration was reduced to 1.5 per cent, are small and lenticular.

The usual surface colony type on pea-infusion agar is of medium size (2 to 4 mm.), granular, with rough, indistinct, "feather" edges, greyish-white and often showing a slightly raised center, if the colonies are well isolated (fig. 1). On prolonged incubation (three to five weeks) at thermophilic temperatures, secondary colonies may sometimes be observed (fig. 3). Two cultures, as originally isolated, did not show the colony type described above but are, in general, smaller and without granulations, the edge being smooth (fig. 2). Recent studies have shown that the phenomenon of induced variation of bacterial cultures is not confined to the aerobic species but may be true also of the common anaerobic species. The colony types observed in this study may indicate such a phenomenon although it is not possible to state whether or not the two colony types represent true "rough" and "smooth" phases as this problem has not been extensively studied. The descriptions given are those shown by the cultures as originally isolated; they have remained stable during a period of eighteen months. The two types are not separated by the cultural reactions applied.

Physiological characteristics

Oxygen relations. All cultures are obligate anaerobes growing only in the more reduced media with strict exclusion of free oxygen. Even with media of low oxidation-reduction potential such as liver infusion with dried liver tissue, or others of similar nature, growth will not occur in unsealed tubes unless a large inoculum (1 to 2 ml.) of an active culture is used, and the medium must be previously exhausted of dissolved air by steaming. Their anaerobicity is also shown in agar shake cultures (0.1 per cent glucose, 1 per cent tryptone agar) in which there is an inhibition of growth for about $\frac{1}{2}$ inch from the surface. The failure to obtain plate cultures with vegetative inocula, as previously discussed, is also evidence for considering the group strict anaerobes.

The limiting oxidation-reduction potential for growth has not been determined. The catalase reaction is negative.

Temperature relations. With the exception of the experiment next to be described cultures for all other tests have been incubated at 58° to 60°C. This is well within the optimum range for growth as is shown by the following series in which a study was made of the temperature relations. The temperatures chosen were 20°, 30°, 37°, 42°, 55°, 62° and 70°C. Both spore and vegetative inocula were used for all cultures. In the case of the vegetative cells the inoculum was a 1:100 dilution of an active liver-infusion culture; this had been shown in preliminary experiments to be sufficiently dilute so that the lag phase would be pronounced with unfavorable conditions. A similar dilute inoculum for the spores was prepared from a stock suspension which had been heated sufficiently to kill the vegetative cells. The medium was liver infusion, tubed and sterilized over particles of dried liver. Duplicate tubes from both spore and vegetative inocula were made for every culture at each temperature and, following inoculation, all tubes were sealed with a plug of agar.

A summary of all the results of this experiment may be seen in table 1; the abundance of growth, as indicated by the volume of gas produced and the turbidity of the infusion, is indicated numerically. The results were so nearly uniform that it is unnecessary to report the specific details for each culture. In general, the cultures at thermophilic temperatures (55°C. and 62°C.) from the spore inocula were identical with those from vegetative inocula. At lower temperatures, however, growth initiation from the spore inocula was a little slower than from the vegetative inocula. These results show that the optimum temperature is thermophilic; although, if prolonged periods of incubation are used, germination of spores and growth is possible at lower temperatures. The practical importance of this slow growth as a factor in spoilage losses in canned foods is obvious.

Heat resistance. The exact heat resistance of these cultures, has not yet been determined. That the resistance is high has been repeatedly shown in experimental packs of non-acid products by the control laboratories and by the fact that the spoilage

produced by these organisms in commercial canning is due to the survival of the process temperatures by the spores. Confirmatory evidence is found in the fact that the soil dilutions used for enrichment material in this study were subjected to steaming and also that all spore suspensions used as inocula in the experiments reported in this study were steamed from fifteen to twenty-five minutes.

TABLE 1

General results on temperature relations of cultures receiving vegetative inocula

TIME	TEMPERATURE OF INCUBATION							
	20°C.	30°C.	37°C.	42°C.	47°C.	55°C.	62°C.	70°C.
days								
1	0	0	0	0	1	4	4	0
2	0	0	0	0	2	—	—	0
5	0	0	0	2	3	—	—	0
10	0	0	1	3	4	—	—	—
20	0	0	2	3	—	—	—	—
30	0	±1	2	—	—	—	—	—

"1, 2, 3, 4," symbols indicating progressive stages of growth; "0," no visible growth; "—," discarded.

Diagnostic cultural characteristics

Growth in complex media. The members of this group are not separated by the differential media usually considered in the classification of anaerobic bacteria. The general reactions produced in the various complex media or in certain differential media by all the cultures are given in table 2. Brain mash and beef-heart infusion, which have perhaps been most widely used for general cultivation of other groups of anaerobic bacteria are not suited to this group. Growth is obtained only from large or active inocula and the reactions produced are not easily recognized or particularly distinctive. A better medium for this group is liver-infusion broth sterilized over particles of dried liver as described in the previous paper. Gas and turbidity of the infusion are easily recognizable indicators of growth; the infusion liquid also becomes lighter in color.

Proteolytic properties. In brain mash, beef-heart infusion, etc.,

the cultures do not exhibit proteolytic powers; this confirms the conclusion of Paine (1931) which was based upon formol titrations. Further, they are not gelatinolytic as shown by the Frazier technique, using both tannic acid and mercuric chloride as precipitants, applied to five day old cultures in tryptone agar containing 0.1 per cent glucose to promote growth.

TABLE 2

Reactions following growth in complex media

MEDIUM	REACTION
Brain mash	Slight turbidity with transient gassing. No blackening, even on prolonged incubation. No proteolysis
Beef heart infusion	Slight turbidity. No digestion of the meat tissue or putrefactive odor
Liver infusion	Marked turbidity; violent gassing in early stages (12 to 20 hours) of growth. Liquid becomes lighter in color. Distinctive odor but no digestion of the tissue. Marked auto-agglutination; particularly if cultures are removed from thermophilic temperatures
Alkaline egg medium	Gassing (with addition of 0.5 per cent glucose) but no digestion of coagulated albumin. No visible growth in absence of sugar
Blood agar plates	Slight growth only. It is not possible to determine the type of hemolysis due to the temperature of incubation. (Horse blood; tryptone base)
Coagulated serum slants	Slight or no growth. No liquefaction
Corn-liver mash (5 per cent)	Gassing in early stages of growth. Slight clearing after 24 hours
Litmus milk (with reduced Fe)	Gas production in the early stages during which time the litmus is reduced. A hard curd is formed which may be channelled by subsequent gassing. The litmus may be re-oxidized on further incubation and tricalcium citrate crystals appear after 8 to 10 days (Peterson, *et al.*, 1927)

Saccharolytic properties. Growth does not occur in the absence of a fermentable carbohydrate, whereas the saccharolytic properties of the cultures are extensive and well defined. The qualitative fermentations of the various sugars and related compounds reveal an unusual uniformity of results with respect to the compounds fermented by the several strains, regardless of their separate origin.

The criteria to be used in anaerobic fermentation have been a matter of dispute (Hall, 1921) and probably any one method will not be acceptable to all. In this study two criteria have been considered. First, in the base medium (1 per cent tryptone broth) alone no growth or only very slight turbidity will occur (the inoculum used in this experiment was 1 ml. of a 1:100 dilution of active liver infusion culture). Thus growth in the presence of a carbon compound becomes an indicator of the utilization of the added compound. An increase in the hydrogen ion concentration becomes an additional criterion, since, as has been previously shown, the strains are non-proteolytic; and therefore, the acid can not result from protein cleavage. For the same reason, there has been no complication due to the production of ammonia which might unite with the acids produced. Therefore, in the present case, a change of pH may be taken as a useful index of fermentation. As will be discussed later, because of the uniformity of results and the exact correlation of positive results according to the two criteria used, it was not considered necessary to test for the absence of the compound utilized, in the case of those substances for which a practical method of quantitative determination exists.

The various carbon compounds were sterilized in concentrated aqueous solutions, and added to give a concentration of 1 per cent in the sterile base. Duplicate tubes, inoculated from a 1:100 dilution of an active liver-infusion culture, were incubated at 58°C. The unsealed tubes were incubated for five days in an anaerobic jar. The results are shown in table 3. The values in the final pH column represent not the range of duplicate tubes of any one culture, but the range of the entire set of cultures. Thus, for instance, the final values for all cultures with cellobiose fell within the range of pH 4.1 to 4.4; whereas with glucose, fructose, etc., no difference in the final pH reached by all the cultures could be detected by colorimetric methods. In no case was there a disagreement between growth and change in pH.

As shown in table 3, the strains tested ferment all the mono- and disaccharides (with the exception of the methyl-pentose, rhamnose) and also all the glucosides tested. The action on the trisaccharide, raffinose, is slight, if any; the alcohols tested and

calcium lactate are not utilized. Of the polysaccharides inulin and pectin are not fermented in contrast to dextrin, glycogen and

TABLE 3

Qualitative fermentation of sugars and related compounds

	GROWTH	FINAL pH	CONTROL
Monosaccharides:			
Arabinose	+	4.6–4.7	6.2
Fructose	+	4.3	6.3
Galactose	+	4.3	6.3
Glucose	+	4.3	6.3
Mannose	+	4.3	6.3
Rhamnose	−	6.2	6.2
Xylose	+	4.2–4.3	6.3
Disaccharides:			
Cellobiose	+	4.1–4.4	6.3
Lactose	+	4.5–4.7	6.3
Maltose	+	4.3	6.4
Sucrose	+	4.4–4.8	6.3
Trehalose	+	4.3–4.8	6.3
Trisaccharide:			
Raffinose	±	6.3	6.4
Polysaccharides:			
Dextrin	+	4.3–5.2	6.3
Glycogen	+	4.3	6.4
Inulin	−	6.3	6.3
Pectin	−	6.1	6.1
Starch (corn)	+	4.6–4.9	6.4
Glucosides:			
Amygdalin	+	4.3–4.6	6.6
Esculin	+	4.9–5.2	6.5
Alpha methyl glucoside	+	4.5–4.8	6.4
Salicin	+	4.3–4.9	6.3
Alcohols:			
Erythritol	−	6.4	6.4
Inositol	−	6.3	6.3
Mannitol	−	6.4	6.4
Glycerol	−	6.3	6.3
Quercitol	−	6.3	6.3
Salt of organic acid:			
Calcium lactate	−	6.4	6.4

corn starch. Only very slight growth is obtained on potato slants, a high starch-containing medium, and no distinctive picture is pre-

sented. Slight action on arrowroot starch is observed by the giant colony technique on agar. The discrepancy of results in the case of starch hydrolysis possibly correlates with the origin of the starch—whether from grain or tuber. The action on cellulose was determined by the addition of strips of filter paper to tryptone base medium, and to the same plus 0.1 per cent glucose to insure initial growth. No disintegration of the cellulose occurred in eighteen days.

Other cultural tests. There is no reduction of nitrates (0.1 per cent) in 0.1 per cent glucose, 1 per cent tryptone broth; nitrites in a concentration of 2 p.p.m. in the same base medium are reduced. Hydrogen sulfide is not produced from tryptone base alone (with 0.1 per cent glucose) but is liberated from sodium thiosulfate (0.25 per cent) and from sodium sulfite (0.1 per cent) in the same base medium, as shown by the lead acetate paper test. The indol test (Gorè method) is negative on both twenty-four- and forty-eight-hour cultures in glucose tryptone broth.

Serological characteristics

Pathogenesis. The cultures are non-pathogenic. Active liver-infusion cultures were fed to white rats with no ill results and intravenous injection of living cultures were given to rabbits with no apparent systemic reaction.

Agglutinin production. As has been previously reported (McClung, 1934) anti-sera of high titer have been prepared for several of the cultures for studies on the agglutination reaction. The specific details of this work will be presented in a later paper of this series.

DISCUSSION

The results of the various tests reported here have usually been discussed as if for one species even though the reactions have been determined with twenty-seven cultures. This has been done since it is believed that the cultures studied represent strains of a single species. Only a few general characteristics are needed for identification and classification. These are preferential growth at thermophilic temperatures, obligate anaerobic

nature, the distinctive microscopic picture of the spore and sporangium, and the extensive saccharolytic (acid and gas produced) and non-proteolytic properties. It is believed that the species has not been previously described and in consideration of the above characteristics, the name *Clostridium thermosaccharolyticum* N. Sp. is proposed.

A review of the literature of thermophilic bacteria has revealed only a few descriptions of obligate anaerobic species, and certain points in the descriptions of some of these may be questioned. Damon and Feirer (1929), reporting the characteristics of four new species, list, in a survey of the literature, only the previous reports of Oprescu (1898), Benignetti (1905), and Bardou (1906) but consider (as does Werkman, 1929) that the organisms isolated by all of these workers have been observed to grow in the presence of free oxygen. Werkman (1929) was not able to confirm the anaerobic nature of one of the cultures, *Clostridium thermoputrificum*, described by Damon and Feirer. Of the cultures of the organisms of Damon and Feirer now existent, which I have been able to obtain, none gives the reactions of the original description. Werkman and Weaver (1927) reported the characteristics of *Clostridium nigrificans*, a thermophilic species, which is non-saccharolytic and only weakly proteolytic. Other reports of thermophilic anaerobic bacilli are those of Veillon (1922), Wollman (1931) and those concerning the anaerobic form fermenting cellulose at thermophilic temperatures, the most recent report of which is that of Snieszko (1933).

If the early reports may be disqualified on the basis that the cultures studied were not obligate anaerobes, and we turn our attention to the later literature we find that separation of the species now described from others is not difficult. The microscopic picture differentiates it from the organisms of Veillon and also from *Clostridium nigrificans*. In each case the cultural reactions also are dissimilar. The same may be said of the cultural reactions of the organism isolated by Wollman and of the four species reported by Damon and Feirer, the terminal spores of which are oval in shape, in contradistinction to the round spore produced by *Clostridium thermosaccharolyticum*. Morphologi-

cally, the species now described resembles the thermophilic cellulose-fermenting type of Snieszko (1933), but separation from this species may be accomplished on the basis of the fermentation of cellulose by Snieszko's organism and its failure to grow in the usual media, in contrast to the reverse reactions obtained with the strains in the present study.

From the more common species of the genus *Clostridium* which possess spherical terminal spores separation may be made on the basis of various cultural reactions, lack of toxin production and preference for growth at thermophilic temperature.

SUMMARY

Morphological and cultural characteristics are given of twenty-seven strains of thermophilic bacilli which are believed to represent the forms responsible for the type of spoilage of non-acid canned foods known as "hard swell." They have been found to be strictly anaerobic and to have an optimum temperature of growth at 55° to 60°C. Morphologically, they are long, slender bacilli possessing a round, terminal spore. The organism is non-proteolytic, extensively saccharolytic, and non-pathogenic. The cultures are believed to represent strains of one species; a review of the literature has failed to reveal a previous description. The name *Clostridium thermosaccharolyticum* N. Sp. is therefore suggested.

These problems concerning the technique of cultivation and the taxonomy of a certain group of thermophilic anaerobic bacteria were begun in the bacteriological laboratories at the University of Texas. To Dr. O. B. Williams and Dr. I. M. Lewis of that University, I wish to express my gratitude for the aid and advice which they gave during the early portion of these investigations. To Dr. E. B. Fred and Dr. Elizabeth McCoy of the Department of Agricultural Bacteriology at the University of Wisconsin I wish to express my appreciation for the suggestions and criticisms which they have given during the latter phases of this work and also for the provision of facilities for its completion. Members of the research staff of the laboratories of the National Canners' Asso-

ciation; namely, Dr. E. J. Cameron and Dr. J. R. Esty, have been most helpful, especially in the supplying of certain cultures and materials and also in the suggestions received during the course of personal correspondence and conference.

REFERENCES

BARDOU 1906 Thèse, Lille. Cited by Damon, S. R., and Feirer, W. A., 1925.
BENIGNETTI, D. 1905 Cited by Werkman, W. H., 1929.
CAMERON, E. J. 1930 Jour. Bact., **19,** 52.
CRAIGIE, J. 1929 Jour. Roy. Micros. Soc., **49** (Ser. III), 9–13.
DAMON, S. R., AND FEIRER, W. A. 1925 Jour. Bact., **10,** 37–46.
GRAY, P. H. H. 1926 Jour. Bact., **12,** 273–274.
HALL, I. C. 1921 Jour. Infect. Dis., **29,** 321–343.
McCLUNG, L. S. 1934 Jour. Bact., **27,** 64.
McCLUNG, L. S. 193–. Jour. Bact. **29,** 173.
OPRESCU, V. 1898 Arch. Hyg., **33,** 164–186.
PAINE, F. S. 1931 Centbl. Bakt. (etc.), 2 Abt., **85,** 122–129.
PETERSON, W. H., WILSON, P. W., McCOY, E., AND FRED, E. B. 1927 Jour. Amer. Chem. Soc., **49,** 2884–2888.
SCHAEFFER, A. B., AND FULTON, M. 1933 Science, **77,** 194.
SNIESZKO, S. 1933 Centbl. Bakt. (etc.), 2 Abt., **88,** 403–409.
STURGES, W. S. 1928 Jour. Bact., **15,** 19.
TOWNSEND, C. T. 1932 Jour. Infect. Dis., **51,** 129–136.
VEILLON, R. 1922 Ann. Inst. Pasteur, **36,** 422–438.
WERKMAN, C. H. 1929 Iowa Agr. Expt. Sta., Res. Bul. 117, 163–180.
WERKMAN, C. H., AND WEAVER, H. J. 1927 Iowa State Col. Jour. Sci., **2,** 57–67.
WOLLMAN, E. 1928 Arch. Inst. Pasteur Tunis, **17,** 253–256. Seen in abstract only. (Biol. Abst., **5,** 1143 (No. 11582), 1931).

PLATE 1

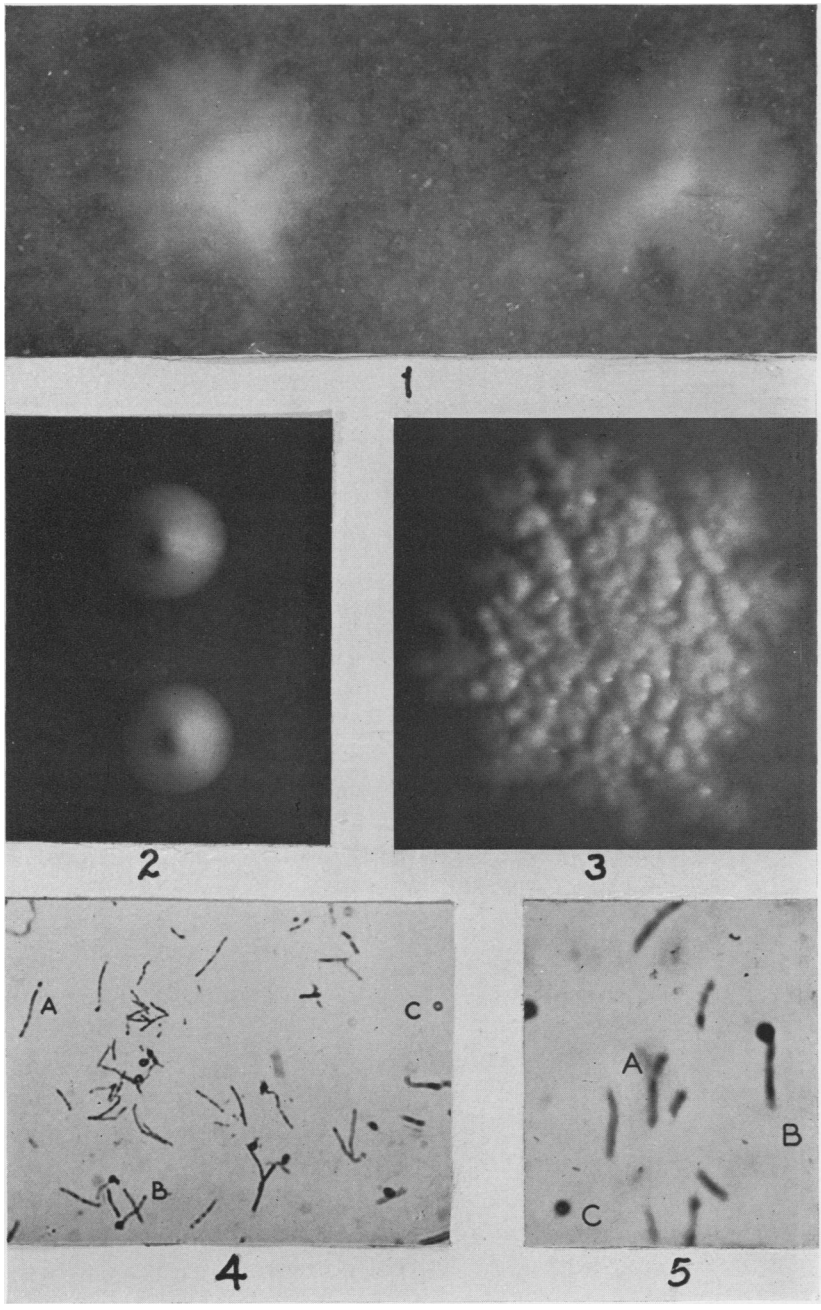
FIG. 1. Typical surface colonies showing slightly raised center and irregular edges.

FIG. 2. Surface colonies of "smooth" (?) type.

FIG. 3. Surface colony showing secondary colonies.

FIGS. 4 AND 5. Photomicrographs showing typical microscopic picture of vegetative cells and spores. In each figure: "A"—vegetative cell; "B"—vegetative cell with immature spore; "C"—mature spore. (The photomicrographs for these figures were taken by Dr. Gorton Ritchie, Department of Pathology, University of Wisconsin Medical School.)

Photographs for figures 1, 2 and 3 were taken of surface colonies on vegetable extract agar. The colonies for figures 1 and 2 had been incubated for ninety-six hours; for figure 3, for twenty-five days. The temperature of incubation was 60°C. and the magnification 8 in each case. The material for the smear for figures 4 and 5 was taken from a surface colony of the same age and type as that shown in figure 1. The slide was stained with dilute gentian violet. The magnification for figure 4 was 1000; for figure 5, 1500 (approximately).



(L. S. McClung: Studies on Anaerobic Bacteria)