Bacteriological Studies on Sulfid Spoilage of Canned Vegetables

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BACTERIOLOGY SECTION

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Sulfid spoilage of canned vegetables is characterized by the marked blackening of the contents and the production of hydrogen sulfid by the organism responsible. Externally the can manifests no characteristics of spoilage; it does not swell or spring. The condition constitutes a new type of thermophilic spoilage due to insufficient processing. It has caused losses in both the sweet corn and pea canning industries of the middle western states. The general characteristics of the spoilage have led to the designation of "sulfid spoilage" for this type.

Sulfid spoilage in canned sweet corn has been shown to be caused by a thermophilic sporulating heat resistant anaerobe. Study of the cultural and physiological characters of the organism indicates that it is an undescribed species, for which the name *Clostridium nigrificans* has been proposed.

Processing at 118°C (245°F.) for 70 minutes has not proved effective in destroying the spores of *Clostridium nigrificans* in No. 2 cans of sweet corn.

In the light of experimental evidence now available, it seems desirable in the control of *Clostridium nigrificans* to eliminate the foci of infection in the cannery and prevent the presence of spores in the product. Prevention of contamination in the plant by avoiding the use of raw materials harboring the organism may offer a way of control if it can be shown that the organism is carried into the cannery on certain raw materials.

The effect of *Clostridium nigrificans* on different canned vegetables has been pointed out. It would seem that there is little possibility of the organism causing spoilage in vegetables other than peas and sweet corn. In a large number of cases the acidity of the canned product is sufficient to prevent growth of the organism.

*Clostridium nigrificans* occurs in the soil, in manure and on sugar and probably finds its way into the cannery in these materials, where it may set up a focus of infection.
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The canning industry of the middle western states experiences costly outbreaks of an unusual spoilage of canned sweet corn and peas which is characterized by the development of a marked blackening of the product and the evolution of a nauseating odor of hydrogen sulfid gas. Externally the can manifests no evidence of spoilage; it does not swell or spring. The condition has proved to be representative of a new type of bacterial spoilage due to underprocessing and will be referred to as "sulfid spoilage." It is known to occur spontaneously only in canned sweet corn and peas.

The spoilage is distinct from that known as "metallie" or "chemical" blackening, a condition affecting principally the heads and seams of the cans and is readily differentiated from the latter by the appearance and uniformity of the blackening throughout the contents. In sweet corn canneries, where the condition was first observed to occur, the blackening is referred to as "sulfur stinker spoilage."

EXTENT AND ECONOMIC SIGNIFICANCE OF SULFID SPOILAGE

Outbreaks of sulfid spoilage occur sporadically in sweet corn and pea canneries and give no warning of impending attack. There have been no practicable means by which the canner could become aware of the presence of the contamination before opening cans for inspection, and as a result the losses have been greatly increased. Altho spoilage generally develops within a few days if the cans are incubated at 55°C. (131°F.) occasionally development is delayed. Inasmuch as detection of the spoilage has proved difficult, outbreaks generally involve a substantial part of the season’s pack. In several instances the entire season’s pack has been so heavily contaminated that salvage was impracticable. The losses have run from that of a two day’s pack to that of the entire season.

Cameron (5) in a study of an outbreak of sulfid spoilage in a Wisconsin pea cannery showed that hot water tanks may harbor the organism responsible for the trouble and serve as sources
of infection, constantly feeding the organism into the vats. In the case cited by Cameron, when the tank harboring the organism was replaced by a metal one, spoilage was eliminated.

In 1919 an Iowa sweet corn cannery lost practically its entire pack for the season; again in 1925 another Iowa corn cannery experienced a very heavy infection in a two day’s pack. The present studies were carried out on cans from a pack in which the incidence of spoilage was estimated at 25 percent, while the records of examination of 209 cans of corn from this pack indicate an incidence of 53 percent; however, these cans were taken from a portion of the pack known to be heavily infected. This outbreak occurred in an Iowa cannery during the 1926 season and involved the entire season’s pack. Altho our records show that sulfid spoilage occurs in both sweet corn and peas, we know of no outbreak having affected any pea cannery in Iowa. On the other hand, sulfid spoilage in sweet corn appears to have been restricted largely to Iowa.

Sulfid spoilage of both peas and sweet corn is limited to the states of the middle west. In view of the habitat of the organism responsible for the blackening and the conditions surrounding an outbreak, it is difficult to understand just why sulfid spoilage should be restricted to the middle west. Inasmuch as it is characteristic and not to be confused with other types of spoilage, it seems that the condition would have been recognized and described, had it occurred elsewhere. No such descriptions of sulfid spoilage have been found in the literature. Personal communications likewise have not revealed the existence of such a spoilage outside of the middle western states.

**CHARACTERISTICS OF SULFID SPOILAGE**

The external appearance of the can is normal; there is nothing to enable one to determine the condition of the contents. The containers do not spring or swell, and little or none of the vacuum is lost. Hence, detection depends upon an examination of the contents.

When opened the sulfid spoiled can emits an odor of hydrogen sulfid. Sometimes the odor is very strong, at other times it is less marked, while rarely it is detected with difficulty. It seems to be only that of hydrogen sulfid; the presence of a putrefactive odor of protein decomposition is not detectable. That this should be true is further borne out when one studies the feeble proteolytic action of the organism responsible for the spoilage.

The appearance of sulfid spoiled sweet corn is that of a bluish-grey liquid containing numerous blackened germs and darkened kernels floating about. This appearance is uniform throughout the entire contents of the can. The metal of the can at the head
or seam may occasionally show blackening. The contents of spoiled cans after several months gradually lighten until the liquid assumes a normal color and the germs become grey.

The blackening takes place principally on the germ on the side of the growing tip. The starch of the kernel becomes grey but never assumes the deep coloration of the germ. That the blackening of the germ is due to iron sulfid formation is demonstrated by the potassium ferro-cyanide and potassium thiocyanate tests. Both show the presence of iron salts in the region of discoloration on the germ. If the contents are made acid, the iron sulfid is destroyed and the normal color is restored.

It is of interest to note that the sweet corn examined was prepared "Maine" or "cream" style as it is known. This method of preparation, in which the scrapings from the cob are added to make a product of creamy consistency, results in the presence of numerous broken kernels, a condition which has been found conducive to the development of the blackening. Maryland style corn, in which only the whole kernels are used, is much more resistant to the development of blackening.

The characteristics of sulfid spoilage in peas resemble those in sweet corn in that there is an evolution of hydrogen sulfid gas and a darkening of the liquor. Crushed peas show more pronounced darkening than those with unbroken seed coats.

**PURPOSE OF INVESTIGATION**

The present bulletin constitutes the results of studies on the bacteriology of sulfid spoilage of canned vegetables. It has been the purpose to determine the cause of the condition and to identify the microorganism responsible. The physiology of the organism has been carefully studied in order to make certain suggestions relative to its control. To this end the isolation, description and classification of the sulfid spoilage organism are given. Important in the control of bacterial spoilage is a knowledge of the habitat of the organism and the sources of contamination; these have been studied along with pure culture studies on the effect of the organism on a wide variety of canned vegetables.

**LITERATURE**

Werkman and Weaver (17) in a preliminary paper considered sulfid spoilage to be due to a thermophilic heat resistant anaerobe for which they suggested the name *Clostridium nigriificans*. Cameron (5) described the characteristics of an outbreak in a pea cannery and showed that the hot water tank was the focus of infection. When a wooden tank was replaced by a metal one, spoilage was eliminated from the cannery. James (12) in a study of the distribution of spoilage organisms found *Cl. nigriificans* abundant in soil and stable manure.
Material and Methods

The experimental part of this investigation is based on a study of twelve cases of 24 cans each of sweet corn taken from a pack of an Iowa sweet corn cannery which had suffered from contamination of the entire season's pack.

The variety of sweet corn used by this company was Country Gentleman, purchased from farmers in the vicinity of the cannery and packed "Maine style" of medium consistency.

The method used in preparing the canned corn is as follows: The corn is husked upon a wood slat conveyor, the ears then pass on this conveyor, are transferred to a steel conveyor and in turn are again transferred to a steel bucket elevator in which they are elevated to the third floor of the cannery. This bucket elevator discharges into a galvanized iron chute, thru which the ears fall upon a rubber belt. This rubber belt conveys the ears past the sorters who remove the defective and mature ears and make a separation of the ears for the purpose of grading the corn into fancy and standard grades. The standard corn is then diverted into a wooden crib. The crib is kept well protected by hard, white paint. The cut corn is discharged into a galvanized screw conveyor, which conveys the corn to the mixing tank. This mixing tank is made of copper and is heavily tinned. From the upper mixing tank the corn falls to a lower tank, which is similar in construction. From this tank it is passed into a regular Sprague-Sells filler.

All the equipment used at this plant, with the exception of the slat conveyor in the husking shed, was installed new in 1926. All of it may be termed modern sanitary cannery equipment.

The cans are filled at a temperature which insures an initial temperature of 71°C. (160°F.) followed by processing at 118°C. (245°F.) for 70 minutes.

The cooling system employed is known as the "slat conveyor cooling" method. The cans to be cooled are dumped from crates in which the cans were processed and pass thru the canal on the conveyor running at the bottom of the tank.

The storage temperature will vary from 90° to 130°F. (32° to 54°C.) in the individual cases during the first week to ten days after packing. The corn then gradually cools to the prevailing atmospheric temperature during the fall months. In the winter months the storage rooms are heated in order to prevent freezing of the corn.

A number of cans were incubated at 37°C. and at 55°C. for long periods, but no changes were observed.

Before being opened each can was carefully examined for defects, then washed and the top flamed while the can was held in an inverted position. The can opener was likewise sterilized.
Upon opening, the spoiled cans emitted a strong odor of hydrogen sulfid. The contents varied in color from bluish-grey to nearly black, depending upon the extent of spoilage. Loose blackened corn germs floated on the surface of the grey liquid. The inside tin of the cans was blackened in the more severe cases. Determinations made on 15 spoiled and five unspoiled cans showed a pH of 6.3 to 6.4 for both sets.

Details of the methods employed will be given under experimental results.

**EXPERIMENTAL**

The characteristics and manner of development of sulfid spoilage in canned sweet corn suggested that microorganisms were responsible for the trouble; various factors seemed to eliminate definitely the possibility of the spoilage being of "chemical" origin. Spoilage developed only after a definite incubation period at 55°C; it did not develop in cans kept cool. The voluminous generation of hydrogen sulfid, sometimes over night, sometimes after two or three weeks, bore the marks of bacterial action. In fact, the incidence and nature of the spoilage were suggestive of infection by an anaerobic or facultative thermophile.

Some of the first work in the laboratory indicated that the condition could be transmitted serially in sweet corn sterilized in glass tubes and heavily inoculated (5 cc.) with corn from a spoiled can. These tubes were incubated at 55°C. for one month with petrolatum layered over the corn. Incubation at 30°C. did not result in the development of spoilage and only a slow development occurred at 37°C.

Considerable difficulty was experienced in culturing the sulfid organism. Inoculated tubes of sweet corn did not consistently develop cultures of the organism; perhaps one of several would show blackening after overnight incubation at 55°C. Other tubes would fail to show any growth until a period of incubation lasting perhaps a week, two weeks, or longer; then overnight, growth developed. Some tubes never showed blackening and had to be discarded.

Microscopic examination of spoiled corn from the original cans revealed very few organisms. Occasionally a gram positive rod and what appeared to be spores were observed scattered widely throughout the slide preparations. The gram positive rods and the oval spores were the only forms found in spoiled cans. Microscopic examination of young cultures from the original cans revealed gram positive rods from 3 to 6 microns long and from 0.2 to 0.5 micron in diameter with rounded ends and subterminal spores which hardly swelled the cell walls.

Motility was difficult to determine, old cultures apparently
were non-motile, young cultures examined at one-hour intervals showed a peculiar, active motility of a relatively small percentage of the organisms. The motility observed showed an active movement, altho the organism made little real progress.

The vegetative cells showed marked ability to stain granularly. From one to six gram positive granules were often observed in cells of 24-hour cultures incubated at 55°C. Young spores retained the gram stain; older spores failed to take it.

Several cultures of the organism were isolated from the original cans of spoiled corn by anaerobic methods. These were repeatedly purified culturally.

Considerable difficulty has been experienced in preparing a medium which would consistently furnish a luxuriant growth of the organism. Numerous media suitable to the growth of anaerobes were tried, but none was found to produce a growth markedly superior to the others. Beef heart infusion agar adjusted to pH 7.2 was finally chosen as one of the stock media. To the basic media was generally added 0.1 percent ferric chloride to produce a more marked blackening. The organism has remained fastidious in its choice of a medium and one is never quite certain that a transfer will develop. It has shown an aversion toward growing on the surface of media, and it was only after the inoculated medium had been covered by a heavy layer of paraffin and incubated in an atmosphere of hydrogen that isolated surface colonies were obtained. It has seemed to us that oxygen relationships play a very important part in the success of the culture. Beef heart infusion agar plates inoculated heavily and incubated at 55°C. in an atmosphere of hydrogen have failed to produce growth; the pyrogallic acid and sodium hydroxide method was likewise unsuccessful. On the other hand, shake cultures in beef heart infusion agar have given the best colony growth as judged by the extent of the blackening of the medium around the colony, altho microscopic examination of these colonies revealed relatively few organisms present. Growth in capillary tubes filled with beef heart infusion agar was successful in isolating colonies of the organism. In all cultural studies boiling of the medium for a period of time before inoculation gave better results than did the use of unheated media.

Early in our attempts to isolate single colonies of the organism on plate culture media, it was noticed that the sulfid organism could be readily grown in a mixture of contaminating forms; in a similar manner growth of the organism could be obtained in shake cultures to within a millimeter of the top of the medium if aerobes were growing on the surface. Introduction of a small amount of CO₂ permitted growth on plate culture media under hydrogen.

A considerable number of isolations were made by different
methods from cans of spoiled corn. The physiological characteristics of five cultures were determined.

All cultures grew on a variety of media under suitable conditions. However, these conditions were not easily determined and could not be consistently duplicated. A medium of finely ground peas (200 grams), dibasic potassium phosphate (1 gram), ferric chloride (trace) and water (1 liter), adjusted to pH 7.2, proved practicable and was used from the earlier part of the investigation. Beef heart and veal infusion agar were used as stock culture media.

Cultures were conveniently transferred by placing a stock culture tube of the organism in boiling water until the agar was melted. Tubes of melted agar media were inoculated while hot with 0.1 to 1.0 cc. of the material from the stock culture, allowed to solidify and incubated at 55°C. This simple procedure served to facilitate transfer of the deeply imbedded colonies.

At present the organisms are grown in veal infusion agar.
The addition of cystine (.01 percent) appears to improve the growth. The medium is always inoculated while hot (100°C.) with a melted agar spore culture of the organism. Incubation is at 55°C. A trace of iron chloride is added to facilitate blackening of colonies.

**PHYSIOLOGICAL CHARACTERS OF THE ORGANISM**

*Action on carbohydrates, glucosides and alcohols.* The organism did not attack any of 28 sugars, alcohols or glucosides with the production of acid or acid and gas. It did not appear to be influenced by the presence of these substances in the medium. It is strictly a non-saccharolytic organism. The following substances were used in beef heart infusion agar and the results checked in the pea medium: glucose, levulose, mannose, galactose, rhamnose, sucrose, lactose, maltose, raffinose, trehalose, melizitose, arabinose, xylose, dextrin, inulin, inulín, starch, glycogen, adonitol, inositol, ducitol, erythritol, perseitol, arabitol, sorbitol, glyceroil, salicin, amygdalin and esculin.

*Action on proteins.* The organism is only feebly proteolytic. Gelatin is not liquified nor is serum digested. Cystine is attacked with the production of hydrogen sulfid.

Gelatinolysis was determined by two methods. The following modification of Frazier’s (9) method was used for this organism: beef heart infusion agar was prepared containing the infusion from 50 grams of beef heart per liter, 0.4 percent gelatin and a trace of ferric chloride. The medium was inoculated at 45°C. and drawn into capillary tubes to harden. It was then incubated at 55°C. until the colonies had developed. Long periods of incubation were required with this medium. The core of agar, containing colonies, was then blown into an acid solution of bichloride of mercury (1:500). The presence of a clear zone around a colony indicated decomposition of the gelatin at least as far as the peptone stage. The width of the zone is indicative of the amount of liquefaction. Essentially the same procedure was repeated, substituting 1 percent tannic acid for the bichloride solution.

Gelatinolysis was also determined by incubating cultures of the organism at 55°C. in beef heart infusion containing 12 percent gelatin. Cultures were removed at intervals and cooled to determine liquefaction, but none was observed during one month’s incubation.

Upon Loeffler’s blood serum, inoculated, covered with paraffin oil and incubated at 55°C., colonies developed, but there was no observable digestion of the medium.

Upon von Hibler’s (10) brain medium the colonies were small, round and black. Indol was not produced as determined by
growth in pea medium or beef heart infusion using the para-
dimethylaminobenzaldehyde test.

Reduction of nitrates. Nitrates were not reduced to nitrites. Beef heart infusion and pea medium with 0.1 percent potassium nitrate were inoculated, incubated at 55°C. for one, two and three days and tested for the presence of nitrates.

Growth temperature relationships. The organism is a thermophile growing at an optimum temperature of 55°C., a maximum of 65°C. to 70°C. and a minimum of 31°C. Tubes containing 30 cc. of beef heart infusion agar were inoculated with 5 cc. each of a stock culture of the organism. Six tubes were incubated at each of the temperatures. Growth at 50°C. and 55°C. occurred over night; at 37°C. and at 45°C. growth occurred after 48 hours; at 60°C. and 65°C. development was meager, while at 70°C. growth rarely occurred. At 30°C. and below no growth took place. The organism is a thermophile in that it prefers 55°C. and fails to grow at temperatures below 30°C. The organism is not thermoduric in the sense that it tolerates 55°C. but prefers to grow at lower temperatures.

Heat resistance. The ability of the organism to withstand the effects of heat determines in large measure the methods of control which may be applied. If the organism is destroyed at ordinary processing temperatures no further effort will be required. However, if it is resistant, control becomes a more difficult matter. Since the organism has a relatively low minimum growth temperature, its control takes on the aspect of prevention of contamination. The sulfid organism has proved not only to have a low minimum growth temperature for a thermophile, but to be quite resistant to the effects of heat. The thermal death times of the organism at different temperatures have been determined. A fixed number of spores of the organism was always employed so that the death time determinations would be comparable.

Clostridium nigrificans has proved to be one of the most heat resistant bacteria. Exposed to a temperature of 100°C. at pH 7.0, the spores remain viable for as long as eight hours. Young spores appear to be less resistant since young cultures of the organism die in less time than old cultures. This point has been difficult to determine since it has been impracticable to maintain conditions so as to obtain sufficient spores in young cultures for accurately controlled tests on thermal death time.

Thermal death times were determined in the following manner: One cubic centimeter quantities of the culture of Clostridium nigrificans adjusted to the desired pH and diluted with sterile distilled water so as to contain a fixed number of spores were sealed in thin glass tubes. These were brought to a temperature of 65°C. in order to reduce the time necessary to bring them to
the killing temperature, otherwise the time necessary for heat penetration thru the glass tube significantly increases the killing time. The tubes were then placed in a salt water or oil bath at temperatures desired, with motor stirrers to circulate the brine or oil. Tubes were removed after intervals, plunged into ice cold water and inoculations made into veal infusion medium containing a trace of ferric chloride. Incubation at 55°C. was continued for 10 days when final growth readings were recorded.

In the preliminary determinations of thermal death times considerable difficulty was experienced in obtaining readings at high temperatures (118°C. and 121°C.) to show agreement with the time at 100°C. when the results were judged by the logarithmic curves of Bigelow (4). The assumption was made that the protection afforded the bacteria by the suspended particles in the medium significantly prolonged the thermal death times at these high temperatures. An attempt to avoid this was made by centrifuging the pea medium sufficiently to throw down the particles without affecting the suspended spores; this procedure reduced the death times but still they were theoretically too long. Finally we succeeded in obtaining sporulation in a clear veal infusion-cystine broth (pH 7.4) which had been inoculated hot and layered with paraffin.

Thermal death times obtained with this medium are plotted in fig. 2 on semi-logarithmic paper against temperature. These results are plotted for three typical experiments and show the spores at 100°C. (pH 7.0) killed only after exposures of from 450 to 480 minutes. At 118°C. from 11 to 13 minutes and at 120°C. from 7 to 10 minutes have sufficed to destroy them. The
graph for pH 6.3 is given in the same figure and shows death times reduced by the acidity but in general agreement with those at pH 7.0.

In fig. 2 the logarithms of the least and the greatest thermal death time in minutes are plotted as abscissae against the temperature in degrees centigrade as ordinates. The best straight line is drawn thru the different ranges of time. The use of semilog paper permits of a more accurate reading of intermediate points than does coordinate paper, since the curve on the former is a straight line. The use of the logarithmic graph to determine death time after any two points have been determined is permissible only between limits. Bigelow set these at 105° and 125°C. The cans of the pack upon which the present work was carried out were processed for 70 minutes at 118°C. They entered the retort at 71°C. (160°F.) If we assume that the processing was carried out in an approved manner, we may conclude that the organisms are not killed during such a process. In processing at 118°C for 70 minutes, the interior of a No. 2 can reaches a maximum temperature of approximately 115°C. (239°F.). Fig. 3 was kindly furnished us by W. H. Harrison, Director of Research of the Continental Can Company. It shows the heat penetration of a No. 2 can of sweet corn. In the light of our present experience, it seems desirable to attack the problem of control of sulfid spoilage from the standpoint of elimination of foci of infection rather than to depend on the process to destroy the spores of the organism. In this discussion the heat resistance of Clostridium nigrificans has been dealt with.

![Fig. 3. Graph showing the heat penetration of a No. 2 can of sweet corn.](image)
and the results do not apply to any other thermophilic (or thermoduric) hydrogen sulfid-liberating anaerobes (or facultative forms).

**pH range of growth.** The optimum growth of the organism occurred between pH 7.2 and 7.4 with the minimum pH 5.8 and the maximum pH 7.6. Beef heart infusion agar adjusted at intervals of 0.2 pH value from pH 5.0 to pH 8.0 was heavily inoculated at 45°C, and drawn into 1 mm. bore glass tubes, allowed to harden and incubated at 55°C.

It is apparent from the pH range of growth that the acid canned foods are not susceptible to sulfid spoilage. This is shown by the results obtained with experimentally inoculated canned foods. In no case did blackening result in the acid products having an acidity greater than pH 5.8.

**Pathogenicity.** The organism was not pathogenic when ingested by man, guinea pig, mouse, rat or rabbit. Introduced intraperitoneally into the guinea pig, mouse, rat or rabbit the organism produced no general symptoms or cutaneous lesions. As much as 5 cc. of pea medium cultures were introduced intraperitoneally into rabbits, guinea pigs and rats; mice received 1 c.c. injections.

**Production of agglutinins.** In a study of anaerobes Le Clainche and Morel (13) found that injection of the bacilli into animals generally led to the production of agglutinins. Since then the agglutination reaction has been found of value in the differentiation of anaerobes. *Clostridium welchii* and *Clostridium tertium* are possibly exceptions in that the use of their sera has not given satisfactory results.

Preparation of a satisfactory antigen for the macroscopic test did not prove practicable. Resort to the microscopic test was made. Rabbits were injected intraperitoneally every fourteenth day with 1 cc. of pea medium culture until four such injections had been made. The animals were bled 10 days after the last injection. Agglutination was observed in a 1 to 500 serum dilution. Specificity tests were not attempted.

**CLASSIFICATION AND NOMENCLATURE**

Perhaps no group of microorganisms has been less adequately studied than the thermophilic anaerobes. Oprescu (15) described three species of thermophilic or thermoduric anaerobes altho there is considerable doubt that he was working with anaerobes. His organisms probably were facultative forms. Benignetti (2) isolated an organism from water which was thermophilic and could be cultivated anaerobically. Bardou (1) reported the isolation of four strains of facultative anaerobes from sewage which grew at 18°C. and 60°C. Veillon (16) has des-
scribed three species of thermophilic anaerobes. The organisms isolated and described by Veillon are unlike the sulfid form in that they are strongly saccharolytic and differ in other respects. Damon and Feirer (7) describe and name four species of thermophilic anaerobes. The description of one of these, *Clostridium thermoputrificum*, as given by Damon and Feirer, sufficiently resembles that of the sulfid spoilage organism that two cultures were obtained for comparison. One culture was that kept in the American Type Culture Collection, the other was obtained thru the courtesy of Dr. F. W. Tanner of the University of Illinois, along with his description. The two cultures agreed in cultural and physiological characters. Furthermore, our description of the organism agreed with that kindly sent to us by Dr. Tanner. We did not find them to be strict anaerobes as reported by Damon and Feirer.

*Clostridium thermoputrificum* differs in several respects from the sulfid spoilage organism. It is described as “uniform short rods with homogeneous protoplasm.” The sulfid organism has a markedly granular protoplasm as stained by the usual anilin dyes and varies in length from 3 to 6 microns. Altho motility is often a difficult point to decide in the case of certain organisms, it appears that the sulfid organism also differs from *Cl. thermoputrificum* in that it is motile. Sporulation is described as terminal by Damon and Feirer; we would class the sulfid organism as of the sub-terminal sporulating type. Fermentation of maltose, glycerol, sucrose, mannitol and inulin with a marked gas production but no acid is reported for *OZ. thennoputrificum*. No saccharolytic properties of the sulfid organism have been observed. Damon and Feirer offer the explanation to account for the absence of acid in their cultures, that possibly the medium was sufficiently well buffered to prevent the lowering of the pH. The sulfid organism is a true anaerobe. Damon and Feirer were apparently unaware of the work of Veillon.

Von Hibler (11) describes a group of sporulating anaerobes with oval terminal spores. He designated these forms as group IX but made no exhaustive study of them. McIntosh (14) describes several strains which conformed in general to von Hibler’s group IX. His type III-C most closely resembles the sulfid spoilage organism. Douglass, Fleming and Colebrook (8) in an unpublished report to the Medical Research Council, gave the name *Bacillus cochlearius* to McIntosh’s type III-C organism.

According to McIntosh, *Bacillus cochlearius* is a sporulating bacillus frequently found in war wounds. It is an actively motile, slender rod, variable in length and having a tendency to give up the gram stain. The spores are strictly terminal and
Fig. 4. Growth of the sulfid spoilage organism on peas.

oval when fully developed, giving the organism a spoon-shaped appearance.

From the description of *Bacillus cochlearius* available, it appears that it is a mesophile. Furthermore, the inability of *B. cochlearius* to produce hydrogen sulfid or blackening of brain media serves to differentiate *B. cochlearius* from the sulfid spoilage organism. Neither organism has saccharolytic properties and neither liquifies gelatin.

Cultural and morphological characteristics of the sulfid organism place it in the genus *Clostridium*. Bergey (3) characterizes the genus as "anaerobes or microaerophiles, often parasitic rods, commonly enlarged at sporulation and producing clostridial or plectridial forms." Considerable confusion exists in the literature regarding the classification of the anaerobes, and it is not the intention to add to it. On the other hand, it is desirable to fix the organism in a classification for reference purposes. It seems that the differences pointed out are sufficiently basic in character to warrant giving specific rank to the organ-
ism. The name *Clostridium nigrificans* has been proposed for this organism by Werkman and Weaver (17) in a preliminary paper.

**Diagnosis:** Conforming to the diagnosis of the genus *Clostridium*. Gram positive, thermophilic rods, 3 to 6 microns long and from 0.2 to 0.3 of a micron in diameter. Sporulation is subterminal. Carbohydrates, glucosides and alcohols not attacked with the production of acid or gas. Nitrates not reduced to nitrites. \( \text{H}_2\text{S} \) liberated from proteins. Indol not formed. Gelatin not liquefied. Optimum growth occurs at \( 55^\circ \text{C} \). Anaerobic.

Ability of *Clostridium nigrificans* to produce spoilage in various canned vegetables. It was deemed of value to determine the effect of *Cl. nigrificans* when introduced in pure culture into various canned vegetables.

The ability of the organism to produce the condition in both canned peas and sweet corn has been noted. Peas are uniformly blackened if the seed covering is broken; if the covering is not broken, blackening is slow to develop. Relatively few crushed peas in a can will allow darkening of the contents. The blackening of peas is more marked than in the case of sweet corn.

In table I are given the results of inoculating pure cultures of *Clostridium nigrificans* into various canned vegetables as they are purchased in the market and with the pH adjusted to 7.0. The effect of adding an iron salt is also shown. From these results it would seem that canned peas and sweet corn offer the only opportunities for the development of spoilage. The acidity is the controlling factor in the case of many canned vegetables. Bean Hole Beans occasionally produce a colony growth in which a few black colonies develop with no tendency to spoilage.

![Fig. 5. Growth of the sulfid spoilage organism on hominy.](image)
TABLE I. EFFECT OF *CL. NIGRIFICANS* ON VARIOUS CANNED VEGETABLES.

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>pH</th>
<th>Original cans</th>
<th>0.1% FeCl₃ pH 7.0</th>
<th>Reaction adjusted to pH 7.0</th>
<th>0.1% FeCl₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagus Beans</td>
<td>5.7</td>
<td>---</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>Wax</td>
<td>6.3</td>
<td>---</td>
<td>B</td>
<td>---</td>
<td>B</td>
</tr>
<tr>
<td>Green Beans</td>
<td>6.4</td>
<td>---</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Bean Hole</td>
<td>6.8</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Beets</td>
<td>5.4</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Carrots</td>
<td>5.2</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Corn (Sweet)</td>
<td>6.3</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Hominy</td>
<td>6.8</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Lima Beans</td>
<td>5.8</td>
<td>---</td>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>Peas</td>
<td>6.3</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>5.2</td>
<td>---</td>
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<tr>
<td>Spinach</td>
<td>5.2</td>
<td>---</td>
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</tr>
<tr>
<td>Sweet Potato</td>
<td>5.2</td>
<td>---</td>
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<td>---</td>
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</tr>
</tbody>
</table>

B, blackening.

spread or cause general blackening. Hominy may occasionally develop sulfid spoilage following heavy inoculation. It is doubtful whether spontaneous spoilage would ever cause loss. The methods of preparing these two vegetables would probably preclude sulfid spoilage by *Cl. nigrificans*.

**Habitat of Clostridium nigrificans.** Repeated attempts have been made to determine the source of the organism with the point in mind that possible contamination in the cannery could be prevented. If the source of contamination were shown to be restricted to the sugar used in canning or to certain types of soil, the probability of avoiding contamination by suitable precautions would be enhanced.

Numerous samples of sugar, soil and manure were collected and experiments run to determine whether or not the sulfid spoilage organism was present. The difficulty of always isolating *Cl. nigrificans* from a sample of material is to be appreciated. Each sample was suspended in distilled water and boiled for four hours in order to reduce the number of other organisms present. Heavy inoculations of each sample were then made into pea medium and beef heart infusion agar. The latter was drawn into capillary tubes, allowed to cool and incubated at 55°C for long periods. Blackened colonies were isolated and their identity determined. *Cl. nigrifi*

Fig. 6. Agar plate showing colony of *Clostridium nigrificans*. 
cans has been in this manner isolated from field and horse manure. Sixteen isolations have been made out of 70 attempts, 32 on soil, 24 on manure and 14 on samples of sugar. Five of the isolations were made from soil and eleven from horse manure.

James (12) in a note on the distribution of thermophilic spoilage bacteria has shown the presence of very few organisms of the Clostridium nigrificans type in two series of samples obtained from eight different sources from a cannery which had experienced practically no spoilage for 10 years.

He did not obtain Clostridium nigrificans from processed corn. The process temperature was not stated. However, of 20 samples of sugar examined for the presence of Cl. nigrificans it was found in 10, or 50 percent. It is possible that included as Cl. nigrificans were different organisms having the common property of producing H$_2$S but differing in heat resistance and other respects. Our results with canned corn show that spores of Cl. nigrificans in cans of spoiled corn may remain viable after entering the autoclave at 71°C. and processing at 118°C. for 70 minutes. It is also probable that strains vary markedly in their heat resistance.

Of 9 soil samples, James found 6 to contain Clostridium nigrificans; manure contained the organism in large numbers. His method of isolation was as follows: "Fifty gram sub-samples were thoroly washed in sterile water, and this solution and dilutions thereof were inoculated into different test media. All media were incubated at 55°C." Using a similar technique, i.e., omitting the boiling, we secured results comparable to those of James, but upon identification of the organism we did not find it to be Cl. nigrificans in most cases. The boiling was then introduced to eliminate as many of the irrelevant forms as possible. It is our belief as previously implied in our definition of sulfid spoilage, that the condition may be caused by different species of bacteria. The condition in sweet corn known as sulfur stinker spoilage is caused by Cl. nigrificans. On the other we know that manure and soil are abundant sources of the organism. Cameron and Williams (6) have recently succeeded in isolating Cl. nigrificans from sugar.
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