THREE NEW SPECIES OF THE GENUS CLOSTRIDIUM

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At the last meeting of our society in Philadelphia a brief description was presented of three interesting species of the genus *Clostridium* (Spray, 1947) that had apparently not been previously identified. To these were assigned names presumed to be suitable to their morphologic and physiologic characters. Time did not permit their full description, and it is the intention to give here more detail, following the form used in *Bergey's Manual of Determinative Bacteriology*. In addition to the routine description certain reactions reported by the author (Spray, 1936, 1937) are included.

These three species were isolated from the sources indicated below, by methods given in the previously mentioned publications, including various procedures of enrichment, with subsequent use of the anaerobic dish proposed by the author (Spray, 1930).

CLOSTRIDIUM NAUSEUM N. SP.

This species was isolated thrice from topsoil of the University campus. Soil samples were shaken in tubes of sterile tap water and heated for 10 minutes at 80 C, then inoculated into freshly boiled and cooled tubes of Difco brain liver heart (semisolid) medium covered with a heavy oil. After some 48 hours' incubation at 37 C several tubes yielded a most nauseating odor, far beyond that of ordinary putrefaction.

Stains from these cultures revealed several morphologic types. The cultures were then plated in Difco liver veal agar and incubated at 37 C in the anaerobic dishes. The same nauseous odor was observed upon opening several dishes, and a variety of well-isolated colonies were fished, with the aid of a wide-field binocular, to the semisolid medium under oil seal. Microscopic and cultural studies later revealed some of these cultures to be *Clostridium perfringens* and *Clostridium tertium*, but several fished from minute, lenticular, creamy colonies proved to be the organism in question, beyond doubt, from its characteristic odor.

C. nauseum n. sp.: Rods, 0.8 to 1.1 by 6.0 to 12.0 microns, with rounded ends, occurring singly, in pairs, and in short chains of 4 to 6 cells. Actively motile, especially in young cultures in semisolid medium, with numerous peritrichous flagella. Spores ellipsoid to elongate, subterminal, distinctly swelling the rods, often becoming apparently terminal at maturation. Gram-positive in early vegetative stage, but gram-negative at sporulation.

Gelatin (or iron-gelatin): Very slowly liquefied; softened at 14 days; completely liquefied at 30 days; not blackened even in the presence of an iron strip.

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Agar surface colonies (anaerobic): Minute, transparent, flat, slightly lobate.

Agar deep colonies: Minute, lenticular, entire, whitish to creamy.

Blood agar not hemolyzed.

Milk (with iron strip): Solidly coagulated at 4 to 5 days; clot shrinks slowly, but without gas, blackening, or digestion. Evidently a rennet curdling, since the whey reaction was neutral to litmus.

Indole: Questionably formed; if so, obscured by an abundance of skatol. Mercaptan is formed, together with other aromatic, putrid nitrogenous compounds not yet identified.

Acid and gas from glucose, fructose, and maltose. Sucrose, lactose, inulin, mannitol, sorbitol, glycerol, and inositol are not fermented.

Nitrates are not reduced to nitrites.

Coagulated albumin not liquefied.

Blood serum not liquefied.

Brain medium (Hibler): Blackened but not visibly digested.

Lead acetate agar or peptone iron agar (Difco): Blackened in 24 hours.

Nonpathogenic for white mice, guinea pigs, and rabbits.

Optimum temperature: Not determined, but grows well at both 37 C and at room temperature.

Outstanding character: The extremely nauseous, fecal odor, due apparently to some presently unidentified aromatic nitrogenous compound.

Strictly anaerobic.

Source: Thrice isolated from soil.

Habitat: Not known other than in soil.

CLOSTRIDIUM MICROSPORUM N. SP.

A tiny navicular organism isolated with considerable difficulty from a fatal case of peritonitis. The peritoneal fluid, at autopsy, was peculiarly foul. Stains showed the usual mixed flora, but also a few tiny navicular rods with sharply pointed ends and tiny spherical, central to eccentric, spores that slightly swelled the rods. Spores of other anaerobes were more abundant, together with the usual mixed nonsporulating aerobic flora. By selective heating the latter was eliminated, leaving apparently only three forms, later identified as *C. perfringens*, *C. tertium*, and the tiny navicular organism in question. By passage through milk (followed by heating) *C. perfringens* was eliminated. After plating the milk and incubating it at 37 C for 72 hours, tiny pin-point colonies were observed among the larger colonies of *C. tertium*, and finally after some 32 days' effort pure cultures of the desired organism were isolated.

C. microsporum n. sp.: Rods, 0.8 by 2.0 to 4.0 microns; occasional long, pleomorphic filaments, distinctly vacuolate, especially in old cultures; occurring singly and in pairs but not in long chains. Organisms navicular and sharply pointed at both ends. Spores tiny, spherical, central to slightly eccentric, and slightly swelling the rods. Actively motile particularly by a spinning movement with little progressive motion. Presence, number, or position of flagella not detected.

Gelatin (or iron-gelatin): Not liquefied nor blackened.

Agar surface colonies (anaerobic): Tiny, almost imperceptible, transparent dewdrop colonies, very slightly raised, with entire edge; visible only after some 48 hours' incubation.

Agar deep colonies: Tiny, 0.5 to 1.0 mm, lenticular, with smooth entire edges; whitish-translucent (smaller and less opaque than *C. tertium*). Growth perceptible only after some 72 hours' incubation.

Blood agar not hemolyzed.

Milk (with iron strip): Fine and constant evolution of gas bubbles for many days, but no coagulation after 22 days' incubation. Medium slowly grayed but not blackened.

Indole is not formed.

Acid and gas from glucose, maltose, and galactose. Lactose, trehalose, rhamnose, raffinose, dulcitol, and inositol are not fermented.

Nitrates are not reduced to nitrites.

Coagulated albumin not liquefied.

Blood serum not liquefied.

Brain medium (Hibler): Not blackened nor digested, even in the presence of an iron strip.

Lead acetate agar or peptone iron agar: Not blackened.

Nonpathogenic for white mice, guinea pigs, and rabbits.

Optimum temperature: Not determined, but grows better at 37 C than at room temperature.

Outstanding characters: The minute size, navicular pointed form, and the tiny spherical, central to eccentric spore.

Strictly anaerobic.

Source: Isolated only once from the abdominal contents of a fatal case of peritonitis.

Habitat: Not known other than this single source.

CLOSTRIDIUM GUMMOSUM N. SP.

A large, blunt rod, evidently a member of the "butyric group" from its later description. It was isolated without great difficulty, once from a case of gaseous gangrene, once from adult normal human feces, and once from feces of a normal infant of some 9 months' age. The colonies simulated those of *C. perfringens*, but further study quickly revealed active motility, abundant and early sporulation, and the absence of a capsule. Its fermentation pattern soon distinguished it as differing entirely from that of *C. perfringens*; nor did this pattern check with that of any of the described species of the "butyric" anaerobes.

It did display the typical "stormy fermentation," especially in iron-milk (Spray), but so also do many other of the "butyrics," particularly when heavily inoculated. However, it was soon observed that both the surface and, especially, the subsurface colonies were extremely mucoid in contrast to those of other members of the group. On the basis of these observations of the markedly gummatous character, and the failure of the fermentation pattern to fit into that of any known species, it is presented as:

C. gummosum n. sp.: Large rods, 0.8 to 1.0 by 4.0 to 8.0 microns; single and in

pairs, not in chains. Sporulation active at 24 to 48 hours; spores eccentric to chiefly subterminal; large ovoid to elongate, markedly swelling the rods. Moderate motility, increasing in activity up to 48 hours.

Gelatin (or iron-gelatin): Not liquefied nor blackened at 19 days.

Agar surface colonies (anaerobic): Large, round, convex, edge entire; very glistening and mucoid.

Agar deep colonies: Large, lenticular to buckwheat (*C. perfringens* type). White to creamy; very viscid to rubbery mucoid; entire colony dissected from the medium, or dragged unbroken by needle through 2 per cent agar (subsurface colonies).

Blood agar not hemolyzed.

Milk (with iron strip): Slow fermentation, with a stream of fine gas bubbles; coagulation at 18 to 20 hours, with the coagulum torn and forced to the surface. No digestion or blackening even upon prolonged incubation.

Indole is not formed.

Acid and gas from glucose, maltose, galactose, and mannitol. Lactose more slowly fermented. Sucrose, salicin, dulcitol, and inositol not fermented.

Nitrates are not reduced to nitrites.

Coagulated albumin not liquefied.

Blood serum not liquefied.

Brain medium (Hibler): Not blackened nor digested, even in the presence of an iron strip.

Lead acetate agar or peptone iron agar: Not blackened.

Nonpathogenic for white mice, guinea pigs, and rabbits.

Optimum temperature: Not determined; grows well at both 37 C and at room temperature.

Outstanding character: The extremely gummatous character of the submerged colonies.

Strictly anaerobic.

Source: Isolated once from gaseous gangrene and twice from normal human feces (adult and infant).

Habitat: Not determined other than from these sources.

SUMMARY

A detailed description is presented of three anaerobic species that are believed not to have been previously isolated or identified. To these there have been assigned names appropriate to their morphologic and physiologic characteristics, namely, *Clostridium nauseum*, *Clostridium microsporum*, and *Clostridium gummosum*.

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