

CLOSTRIDIUM STICKLANDII NOV. SPEC.

THRESSA C. STADTMAN AND L. S. McCLUNG

Laboratory of Cellular Physiology and Metabolism, National Heart Institute, Bethesda 14, Maryland, and Department of Bacteriology, Indiana University, Bloomington, Indiana

Received for publication August 9, 1956

A clostridium, strain HF, originally associated with *Methanococcus vannielii* in formate enrichment cultures (Stadtman and Barker, 1951) was subsequently isolated in pure culture and found to be an amino acid fermenting organism (Stadtman, 1954). Biochemical studies revealed that a characteristic property of the organism is its ability to obtain its energy for growth from coupled oxidation-reduction reactions between certain amino acid pairs, i.e., a "Stickland reaction" (Stadtman and White, 1954). It is distinctly different, both morphologically and in various of its biochemical characteristics, from the other clostridia known to catalyze these reactions; viz., *Clostridium sporogenes*, the organism investigated by Stickland (1934). The most closely related organism on the basis of standard bacteriological tests is *Clostridium difficile*. However, here also there are a number of readily discernible differences among which are size of cell, colony type, hydrogen sulfide production and ability to ferment maltose and salicin. Authentic strains of *C. difficile*, studied for comparison, do not produce hydrogen sulfide. They ferment salicin but not maltose. Little is known about *C. difficile* with respect to its biochemical activities except that it ferments various amino acids. Like strain HF, it reduces proline to δ -amino valerate but it differs in being able to utilize alanine and apparently does not utilize arginine, at least for growth. The two organisms are strikingly different with respect to the volatile unpleasant smelling compounds produced during growth on a tryptone-yeast extract medium: whereas strain HF forms fatty acids together with small amounts of volatile amines such as putrescine and cadaverine, the odor of *C. difficile* cultures is similar to that of horse urine. Since the amino acid fermenting clostridium, strain HF, can be distinguished from other recognized species of clostridia, it is proposed to name it *Clostridium sticklandii* after Dr. L. H. Stickland who first described the type of fermentation carried out by

this organism. A culture has been deposited in the American Type Culture Collection.

The characteristics on which the new species is based are included in the description and the following media and methods were used in this study. Deep agar colonies were observed in deep tubes of a basal mineral-agar medium (Stadtman, 1954) supplemented with 0.2 per cent L-arginine·HCl, 0.2 per cent L-lysine·HCl, 0.2 per cent sodium formate and 0.1 per cent yeast extract (Difco) or 2.0 per cent tryptone, 0.5 per cent yeast extract and 0.2 per cent sodium formate. Colonies on egg yolk agar were observed on plates of the medium of McClung and Toabe (1947) incubated 48 hr in a Brewer jar. The gelatin medium and iron milk used were prepared according to the method of Spray (1936) and the basal medium for carbohydrate fermentation was that of Spray (1936) with acid production determined by spot plate-indicator tests at 24 hr, 48 hr and 7 days. Hydrogen sulfide determinations were made in trypticase-lactose-iron agar (Baltimore Biological Laboratory) and in lead acetate agar (Difco) prepared according to method of Spray (1936). Nitrate reduction and acrolein production were tested by the methods of Reed and Orr (1941). Indole and skatole production were determined by the method of Roessler and McClung (1943) using trypticase nitrate broth (Baltimore Biological Laboratory). Action on coagulated albumin was determined by observation of a cube of coagulated egg in thio-glycolate broth.

The characteristics of *C. sticklandii* are as follows:

Morphology: Slender rods, with rounded ends, 1 to 2 μ in length (occasionally up to 4 μ), occurring singly, in pairs and sometimes in short chains. Spore formation rare and seen only in old cultures: oval spores are located centrally or subterminally and bulge the cells slightly. Gram positive and very active motility.

Deep agar colonies: 1 to 2 mm in size, lens shaped, becoming lobate and soft.

Surface colonies on egg yolk agar: Moist colonies, somewhat punctiform to irregular in larger colonies and no precipitate or metallic sheen is laid down.

Blood agar plates: Grayish-white colonies similar to those on egg yolk agar; no hemolysis.

Gelatin: Slight blackening but no liquefaction within two weeks.

Coagulated albumin: No digestion.

Acrolein production: Negative.

Indole not produced; slight production of skatole.

Nitrates not reduced.

Carbohydrates: Slight fermentation of glucose, galactose, and maltose. Lactose, sucrose, salicin and glycerol not fermented.

Blood serum not liquefied.

Litmus milk not changed.

Hydrogen sulfide produced in trypticase-lactose-iron agar (B.B.L.) and lead acetate agar (Difco).

Not pathogenic for guinea pigs.

Anaerobic.

Grows well from 30 to 38 C.

Source—originally isolated from San Francisco Bay black mud.

SUMMARY

Clostridium sticklandii nov. spec. has been described and its relationship to other clostridia discussed.

REFERENCES

- McCLUNG, L. S. AND TOABE, R. 1947 The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. *J. Bacteriol.*, **53**, 139-147.
- REED, G. B. AND ORR, J. H. 1941 Rapid identification of gas gangrene anaerobes. *War Med.*, **1**, 493-510.
- ROESSLER, W. G. AND McCLUNG, L. S. 1943 Suggested method for use of vanillin as a test reagent for indole and skatole production by bacteria. *J. Bacteriol.*, **45**, 413.
- SPRAY, R. S. 1936 Semisolid media for cultivation and identification of the sporulating anaerobes. *J. Bacteriol.*, **32**, 135-155.
- STADTMAN, T. C. 1954 On the metabolism of an amino acid fermenting clostridium. *J. Bacteriol.*, **67**, 314-320.
- STADTMAN, T. C. AND BARKER, H. A. 1951 Studies on the methane fermentation. X. A new formate-decomposing bacterium, *Methanococcus vannielii*. *J. Bacteriol.*, **62**, 269-280.
- STADTMAN, T. C. AND WHITE, F. H., JR. 1954 Tracer studies on ornithine, lysine, and formate metabolism in an amino acid fermenting clostridium. *J. Bacteriol.*, **67**, 651-657.
- STRICKLAND, L. H. 1934 Studies in the metabolism of the strict anaerobes (genus *Clostridium*). I. The chemical reactions by which *C. sporogenes* obtains its energy. *Biochem. J. (London)*, **28**, 1746-1759.