CLOSTRIDIUM OROTICUM COMB. NOV.
AMENDED DESCRIPTION

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ABSTRACT. The organism formerly designated Zymobacterium oroticum, Wachsman and Barker (1954), has been shown to produce heat resistant spores, and is therefore a member of the genus Clostridium. The characteristics of C. oroticum differ significantly from those of other species of the genus Clostridium that have been described.

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A single strain of an orotic acid-fermenting organism was isolated from San Francisco Bay mud by Kornberg (Lieberman and Kornberg 1953) and characterized by Wachsman and Barker (1954). It was designated Zymobacterium oroticum, the type species of a new genus which was tentatively placed in the family Lactobacillaceae. The holotype strain was deposited in the American Type Culture Collection as strain 13619. The species Zymobacterium oroticum was placed in the family Propionibacteriaceae in the 7th edition of Bergey's Manual of Determinative Bacteriology (Breed et al. 1957).

Using the anaerobic techniques described by Moore (1966) and the methods of Moore and Cato (1965) modified by Moore et al. (1966) and Holdeman et al. (1967), characteristics of the holotype strain, obtained from Dr. H.A. Barker, were determined in this laboratory. Results were in excellent agreement with those published in the original descriptions. However, we observed spore formation microscopically (Fig. 1) in cultures grown in peptone-yeast extract broth (PY) and in peptone-yeast extract-glucose broth (PYG) and demonstrated that the organisms survived heating in starch broth for 10 minutes at 90°C. Therefore the species is a member of the genus Clostridium. It retains the specific epithet oroticum Wachsman and Barker (1954).

The characteristics of this species differ significantly from those of the other species of the genus Clostridium that have been described. Characteristics in addition to those reported by Wachsman and Barker are listed in Table 1 and in the text following.

Figure 1. Photomicrographs of Gram's stains of cultures of Clostridium oroticum, holotype strain:

- a. 48-hour culture in peptone yeast extract broth.
- b. 24-hour culture in peptone yeast extract-glucose broth.

Other characteristics:

Broth cultures, after incubation for 1 to 2 days, contain long chains of Gram-positive rods with rounded or tapering ends, 0.3 to 0.6 by 1.3 to 4.1 μ . Spores are round to ellipsoidal, subterminal, and do not distend the cells. The organism is anaerobic and nonmotile. Growth is equally abundant at 30 and 37°C. Ammonia is produced from the fermentation of orotic acid and threonine, but not from basal media without either substrate. These results are in agreement with the original description. No lecithinase, lipase, or lytic reaction is produced on modified McClung-Toabe egg yolk agar. Using the orotic acid medium of Moore and Cato (1965), crystals of orotic acid have disappeared after 3 weeks of incubation at 37°C and the optical density of the medium permits 50-60% T as compared with 0% T by an uninoculated blank when measured at 280 m μ using a Beckman DU spectrophotometer.

Distinguishing characteristics:

C. oroticum is most readily distinguished by its occurrence as oval Gram-positive cells in chains of 100 or more units. In this respect it may be confused with some species of Peptostreptococcus that occur as oval elongated cells.

TABLE 1. CHARACTERISTICS OF *CLOSTRIDIUM OROTICUM*

Characteristic	Result	Characteristic	Result
Hemolysis	slight beta	Lactate utilization	- [nvg]
Broth growth	"cottony" sediment [granular sediment]	Lactate conversion	- [nvg]
Growth in:		Pyruvate conversion	+
bile	+	Threonine (DL) con-	
6.5% NaCl	vw	version to propionate	-
pH 9.6	+	Milk curd, digestion	- [-]
Gas (glucose agar)	+	Digestion of:	
Reduction of:		gelatin	w [-]
nitrate	+	coag. egg albumin	- [-]
neutral red	+	chopped beef	-
resazurin	+	NH ₃ production from:	
Terminal pH in:		arginine	-
basal medium	- 6.1-6.4 [-6.4]	threonine-free medium	-
adonitol	- 6.0-6.4	threonine (DL)	+
amygdalin	- 6.2-6.4	chopped beef	-
L-arabinose	+ 5.0-5.7 [+5.6]	orotic acid	+
cellobiose	+ 5.2-5.5	PY-glucose	+
dextrin	v 5.7-6.2	NO ₃	-
dulcitol	+ 5.5-5.6	Hydrolysis of:	
DL-erythritol	v 5.9-6.2	esculin	-
esculin	- 6.4-6.5	starch	- [-]
D-fructose	+ 4.9-5.1 [+5.6]	hippurate	-
D-galactose	+ 5.1-5.8 [+5.5]	Production of:	
glucose	+ 4.9-5.2 [+5.7]	indol	- [-]
glycerol	- 6.4-6.5 [nvg]	catalase	- [-]
glycogen	- 6.1-6.3	acetoin (AMC)	- [trace]
inositol	+ 5.2-5.6	H ₂ S	-
inulin	+ 5.3-5.6	Acids from glucose: (mM/100 ml)	
lactose	+ 5.4-5.6	acetic	2.4-2.7 [1.15]
D(+)-maltose	v 5.0-6.1 [+5.7]	formic	0.6-1.8 [0.10]
D-mannitol	- 6.3-7.4 [+6.1]	succinic	trace possible*
D(+)-mannose	+ 5.8-5.8	lactic	trace [0.21]
melezitose	v 5.3-6.0	other 3,4,5,6,7,	
melibiose	v 5.8-6.6	and 8-carbon acids	absent
pectin	+ 5.5-5.9	Acids from basal medium:	
raffinose	+ 5.2-5.4	acetic	2.5 [0.46]
rhamnose	+ 4.9-5.1	formic	0.0 [0.10]
D(-)-ribose	+ 5.1-5.4	succinic	0.0
salicin	+ 5.6-5.9	lactic	0.0 [0.11]
D-sorbitol	v 5.6-6.2	other 3,4,5,6,7,	
L-sorbose	v 5.8-6.1	and 8-carbon acids	absent
starch	v 5.8-6.2	Ethanol	major amt. [2.50]
sucrose	+ 5.1-5.5 [+5.6]	Other alcohols	-
trehalose	+ 5.3-5.7	Other products:	
D-xylose	+ 5.0-5.1	gas	+
		glycerol	- [2.52 CO ₂]
		2,3 butylene glycol	- [-]

* Lieberman and Kornberg (1954) reported the possible presence of a dicarboxylic acid (or acids) from the fermentation of orotic acid.

TABLE 1. Cont.

Legend:

[] = reactions reported by Wachsmann and Barker (1954); numbers in brackets = culture pH after incubation for 10 days (column 1) and mM of product per 100 ml of medium (column 2).

numbers = culture pH after incubation for three weeks (column 1) and mM of product per 100 ml of medium determined on 3-week cultures with silicic acid and gas chromatography (Moore et al. 1966) (column 2).

+

 = positive reaction

-

 = negative reaction

v

 = variable results

w

 = weak reaction

nvg

 = no visible growth

Our results are based on analyses of duplicate or triplicate (rarely single) cultures of the strain. Excellent growth was obtained in all media except where otherwise indicated.

Of more than a hundred strains of anaerobic streptococci with similar morphology tested, none was able to attack orotic acid. C. oroticum is mesophilic and nonmotile; it produces large quantities of gas, ethanol, and acetic acid, but does not produce lecithinase. The above characteristics alone differentiate it from previously described species of the genus. However, the other characteristics that are described are useful for positive identification.

REFERENCES

- Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's Manual of Determinative Bacteriology, 7th ed. The Williams and Wilkins Co., Baltimore, Md.
- Holdeman, Lillian V., Elizabeth P. Cato, and W. E. C. Moore. 1967. Amended description of Ramibacterium alactolyticum. Int. J. Syst. Bacteriol. 17(4):323-341.
- Lieberman, I. and A. Kornberg. 1953. Enzymic synthesis and breakdown of a pyrimidine, orotic acid. I. Dihydro-orotic dehydrogenase. Biochem. et Biophys. Acta 12: 223-234.
- _____ and _____. 1954. Enzymatic synthesis and breakdown of a pyrimidine, orotic acid. II. Dihydro-orotic acid, ureidosuccinic acid and 5-carboxymethylhydantoin. J. Biol. Chem. 207:911-924.

- Moore, W.E.C. 1966. Techniques for routine culture of fastidious anaerobes. *Int. J. Syst. Bacteriol.* 16:173-190.
- _____ and Elizabeth P. Cato. 1965. Synonymy of Eubacterium limosum and Butyribacterium rettgeri: Butyribacterium limosum comb. nov. *Int. Bull. Bacteriol. Nomen. and Taxon.* 15:69-80.
- _____, _____ and Lillian V. Holdeman. 1966. Fermentation patterns of some Clostridium species. *Int. J. Syst. Bacteriol.* 16:383-415.
- Wachsman, J. T. and H. A. Barker. 1954. Characterization of an orotic acid fermenting bacterium, Zymobacterium oroticum, nov. gen., nov. spec. *J. Bacteriol.* 68:400-404.

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