Obligately Anaerobic Strains of *Corynebacterium vaginale* (*Haemophilus vaginalis*)

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Six obligately anaerobic strains of *Corynebacterium vaginale* (*Haemophilus vaginalis*) have been isolated and their characteristics studied. The reactions of the anaerobic strains, as well as of facultative strains tested under anaerobic conditions, are similar to the reactions previously reported for this species.

In 1955 Gardner and Dukes (10) proposed the name *Haemophilus vaginalis* for an organism they found to be gram-negative, requiring blood, and a cause of "nonspecific" vaginitis. The overtly pathogenic role of this species is still open to question, but it has been thought to be a cause of or closely associated with vaginitis, nongonococcal urethritis, cystitis, and abortion (1, 14, 15, 16, 19). Subsequent investigations have determined that this species is not a member of *Haemophilus* because, although blood may greatly enhance growth, neither X or V factors are required (5, 9, 18, 21); moreover, the organism is gram positive when grown on optimal media (22). Zinnemann and Turner (22) suggest that the species could possibly be included in the genus *Corynebacterium*, although other investigators (20) think that it should be excluded from *Corynebacterium* as well, apparently because of the composition of the cell wall and because it does not produce catalase. In *Bergey's Manual of Determinative Bacteriology*, 8th edition (13), the species was included as species *incertae sedis* B under *Haemophilus*, with the note that it is placed here because most readers will probably look for it under *Haemophilus*. Clearly, the taxonomic position of this species has not yet been settled. For an excellent and critical bibliographic review of *C. vaginale* (*H. vaginalis*), the reader is referred to Dunkelberg (4).

C. vaginale has been considered difficult to culture. Many workers use a blood-containing medium and incubate in a candle extinction jar or CO₂ incubator. A fermentable carbohydrate greatly enhances growth. Casman agar (2), chocolate agar, and peptone-starch-dextrose agar (6) have been commonly used in studies to specifically isolate this species. Dukes and

Gardner (3) reported that strains grew well under anaerobic conditions established with hydrogen, but did not grow in anaerobic conditions established with phosphorus or alkaline pyrogallate. Although the species is considered facultatively anaerobic, no obligately anaerobic strains have been previously reported.

In 1973, we isolated from a vaginal abscess an organism that appeared identical with *C. vaginale* except that it was an obligate anaerobe. The strain was isolated on TYB (brain heart infusion agar with 6% added agar plus 0.1% Tween-80, 0.5% yeast extract, 5% human blood) plates in a GasPak jar. No colonies grew on plates incubated in a CO₂ incubator. A lipase reaction on egg yolk agar (11), similar to that we had noted with facultative strains of *C. vaginale*, was produced. Although production of lipase by strains of *C. vaginale* has not been reported previously, we find that most of our strains do produce the pearly layer on egg yolk agar plates incubated anaerobically. Although the reaction is usually discernible after anaerobic incubation for 48 h, it occasionally (10% of positive strains) is seen only after the plates have been reincubated (anaerobically or at room temperature aerobically) for a few days. The lipase reaction, when present, is a helpful hint of the presence of this organism, particularly in mixed cultures. Unfortunately, not all strains uniformly give this reaction (Table 1), and there is some variation in results from laboratory to laboratory, for which we have no explanation.

We have now studied six strains of *C. vaginale* that were obligately anaerobic on original isolation. Four were isolated on routine anaerobic blood (sheep) agar plates (brucella agar or brain heart infusion agar supplemented with

TABLE 1. Reactions of strains of *C. vaginale*^a

Culture no. ^b	Source	Lipase ^c	Hemo- lysis ^d	Carbohydrate ^e								Growth on BAP ^f			Fermentation products	
				Arab- inose	Fruc- tose	Lac- tose	Man- nose	Starch	Suc- crose	Tre- halose	Xy- lose	Anaer- obic	Candle jar	Aer- obic	Growth ^g medium	Acids ^h
F-609-72	Abdominal inci- sion	+	Diffuse β	A	A	—	—	—	—	—	A	G	D	nt	PYG serum	A, l, s
F-624-72	Abdominal fluid	+	Diffuse β	A	A	A	—	A	A	—	A	G	D	nt	PYG serum	A, l, s
F-2733-72	Incision	+	Diffuse β	—	w	—	—	A	—	—	—	G	D	nt	PYG serum	A, l, s
F-3167-72	Bartholin cyst	—	Diffuse β	nt	nt	—	—	nt	—	—	—	G	D	nt	PYG serum	A, l, s
F-713-73	Unknown	+	Diffuse β	A	w	—	—	A	—	—	A	G	D	nt	PYG serum	A, l, s
F-974-73	Vaginal cuff	+	Diffuse β	A	A	—	—	w	—	—	A	G	D	nt	PYG serum	A, l, s
F-2683-73	Blood	+	Diffuse β	A	A	—	—	A	—	—	—	G	D	nt	PYG serum	a, s
F-3855-73	Vaginal abscess	+	Diffuse β	—	w	—	—	A	—	—	—	G	—	nt	PYG serum	a, l, s
F-233-74	Unknown	nt	Diffuse β	A	A	—	—	A	—	—	—	G	D	nt	PYG serum	a, l, s
F-434-74	Endometrium	+	Diffuse β	—	A	—	—	A	—	—	—	G	D	nt	PYG serum	a, l, s
F-707-74	Intra-uterine	+	Diffuse β	A	w	—	—	A	—	—	—	M	D	nt	PYG serum	A, L, s
F-1187-74	Vaginal	+	Diffuse β	—	A	—	—	A	—	—	—	G	—	—	PYG serum	A, L, S
F-1708-74	Throat	+	Diffuse β	A	A	w	—	A	—	—	A	M	D	nt	PYG serum	A, L
F-1848-74	Unknown	+	Diffuse β	A	w	—	—	A	—	—	A	G	M	P	PYG Tween	A, F, L
V-7274	Vaginal cuff	—	nt	—	A	—	—	A	—	—	—	M	—	—	PYG Tween	A, l
V-7380B	Urine	w	Diffuse β	—	A	—	w	A	—	—	w	G	—	—	PYG Tween	A, f, l, s
V-7644	Cervix	+	nt	—	A	—	w	A	—	—	—	M	G	—	PYG Tween	A, L
V-7854	Bartholin gland abscess	+	Diffuse β	—	w	—	w	A	w	—	nt	G	—	—	PYG Tween	A, L, f
V-8094	Cervix	w	nt	—	A	—	w	A	—	—	—	G	P	P	PYG Tween	A, l
V-8821	Bartholin cyst	w	Diffuse β	—	A	—	w	A	w	—	—	G	G	G	PYG Tween	A, f, l
V-9477	Unknown	+	nt	A	A	A	A	A	—	A	A	G	M	M	PYG serum	A, F
V-9660	Puerperal ab- scess	—	nt	—	A	—	w	A	—	A	—	P	—	—	PYG Tween	A, F, l
V-9949	ATCC 14018	+	Diffuse β	—	A	—	—	A	—	—	—	G	G	G	PYG	A, l
V-9950	ATCC 14019	+	Diffuse β	A	A	—	—	A	—	A	—	G	G	M	PYG	A, F, l

^a All strains fermented dextrose and maltose. None of the strains fermented amygdalin, cellobiose, esculin, mannitol, melezitose, raffinose, rhamnose, salicin, or sorbitol. None of the strains hydrolyzed starch or esculin, produced indole or catalase, reduced nitrate, or was motile.

^b F denotes strains from Florida. The biochemical reactions given were determined in fluid thioglycollate (without dextrose or indicator)-0.5% yeast extract-10% (vol/vol) horse serum base; cultures were incubated from 1 to 5 days. V denotes strains from Virginia. The biochemical reactions given were determined in prerduced media (11) after incubation for 3 to 5 days.

^c +, Positive reaction; —, negative reaction; nt, not tested; w, weak reaction.

^d Beta hemolysis on human blood (F) or sheep blood (V).

^e The pH of cultures was determined with a pH meter and a combination electrode. A, acid from carbohydrate (pH below 5.5); w, weak acid from carbohydrate (pH 5.5 to 6.0); —, negative fermentation (pH above 6.0); nt, not tested.

^f G, Good growth; D, delayed growth; M, moderate growth; P, poor growth; —, no growth.

^g Prerduced peptone-yeast extract-glucose (PYG) (11) with serum (10% sterile horse serum, vol/vol) or Tween-80 (0.02%) added as indicated.

^h A, Acetic acid; F, formic acid; L, lactic acid; S, succinic acid. Capital letters indicate major amounts of acid (greater than 1 milliequivalent/100 ml) and lower case letters indicate less than 1 meq of culture per 100 ml. Acids were determined by gas chromatographic analyses (11) with a Dohrmann Anaerobic Bacteriology Analyzer.

vitamin K and Heme) incubated for 48 to 72 h in an anaerobe jar. The cellular and colonial morphological characteristics of the anaerobic strains were similar to those of the facultative and reference strains. Reactions of these anaerobic strains, compared with reactions of the type strains (ATCC 14018), with another reference strain (ATCC 14019), and with facultatively anaerobic strains are given in Table 1. With the exception of mannose fermentation (often very weak) noted when strains were tested in prereduced Tween media, there was no difference in biochemical reactions among strains tested in serum-thioglycollate medium, prereduced medium plus Tween-80, and the results previously reported for this species (3, 7). Also, acetic acid was the major fermentation acid that accumulated in anaerobic cultures as has been previously reported in aerobically incubated cultures (8, 17). Addition of 0.02% Tween-80 (final concentration) to the prereduced peptone-yeast extract media (11) enhanced the growth of the majority of the strains, and in peptone-yeast-glucose-Tween cultures lactic and formic acids, in amounts less than the acetic acid, often were detected. The addition of Tween-80 may compensate for some nutritional deficiency in the basal peptone used in the prereduced medium. Dunkelberg and McVeigh (5) have noted that, of all the peptones they tested, only two commercial peptones (Difco Proteose peptone no. 3 and possibly BBL Myosate) contained all the required growth factors for *C. vaginale*. Dunkelberg et al. (6) also used Proteose peptone no. 3 in 0.5% agar as basal medium for determination of biochemical reactions.

Contrary to the findings of Dukes and Gardner (3), 24 of the 25 strains we have tested grew better under our anaerobic conditions than in candle extinction jars, probably because more recent anaerobic culture conditions also provide at least 5 to 10% CO₂ in the gaseous atmosphere. Of the six strains that we have studied that were obligately anaerobic on initial isolation, all six continued to be obligately anaerobic after four to six laboratory transfers. Of three of the anaerobic strains that were available for retesting after lyophilization, two were still obligately anaerobic and one grew on the surface of plates incubated in a candle extinction jar when a heavy inoculum of a culture in the early stationary phase of growth was used for inoculum.

Although a medium containing starch is probably preferable for selective isolation of *C. vaginale* strains (5, 6, 18), strains of the species do grow on routine blood agar commonly used in bacteriology laboratories. The presence of

small amounts of glucose in many of these media also probably enhances growth. We hope that this report will alert bacteriologists to the fact that *C. vaginale* can grow on routine media and to the fact that some strains may be obligately anaerobic. Also, incubation under anaerobic conditions would protect *C. vaginale* in mixed culture from the inhibitory effects of H₂O₂ that accumulates around colonies of streptococci and pneumococci grown aerobically (18, I. A. Miller, Ph.D. thesis, Univ. of West Virginia, 1970).

The anaerobic strains of *C. vaginale* can be differentiated from strains of other obligate anaerobes by the characteristics previously described and as shown in Table 1. The usual diffuse beta hemolysis, colonial morphology, cellular shape (diphtheroid-like), and lipase reaction are key characteristics for presumptive recognition of the species. Identity can be confirmed by demonstration of fermentation of starch, maltose, and dextrose, no reduction of nitrate or production of indole or catalase, and production of acetic acid, often with lactic and formic acids.

This elucidation of the anaerobic nature of some strains of *C. vaginale* should not, and is not intended to, further confuse or complicate the question of the taxonomic position of this species. There is already ample precedent for having obligately anaerobic and "aerotolerant" or facultatively anaerobic organisms in one genus, or in one species. *Clostridium tertium*, *C. carnis*, and *C. histolyticum*, all aerotolerant, are included with the obligately anaerobic species of sporeforming rods in the genus *Clostridium*. We are accustomed to accepting variation in aerotolerance in strains of actinomycetes and lactobacilli. It was recently suggested (12) that some species formerly considered in the genera *Peptostreptococcus* and *Peptococcus* be transferred to *Streptococcus* because it was recognized that both obligately anaerobic and facultatively anaerobic strains of these species, that make lactic acid as the sole major fermentation acid, belong to the same species. And, as with these species recently placed in *Streptococcus*, some of the *C. vaginale* strains that were obligately anaerobic on initial isolation could later be grown in a candle extinction jar on blood agar plates when heavy inoculum from a pure culture was used. Some of the strains, however, are still obligately anaerobic, even after multiple laboratory transfers.

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