THE CLASSIFICATION OF ORGANISMS TERMED *LEPTOTRICHIA (LEPTOTHRIX) BUCCALIS*

III. Growth and Biochemical Characteristics of *Bacterionema matruchotii*[1]

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Because of the confusion which has existed in the classification of the large gram-positive oral filamentous organism characteristically having a filament attached to a bacillus-like body (11), a comprehensive description of those traits useful for identification is essential. Some of these traits have been reported by the following authors: Kligler (16), for anaerobic isolates which were named *Leptothrix buccalis*; Mendel (18), for an aerobically isolated organism which he termed *Cladothrix matruchoti*; Bulleid (7), for anaerobic isolates subsequently cultured aerobically which he designated *Leptothrix buccalis*; Bibby (3), and Bibby and Berry (4), for anaerobically isolated organisms which were termed *Leptotrichia buccalis*; Bartels (1), for an anaerobically isolated filamentous organism; Morris (19) for anaerobic isolates which he believed were *Leptotrichia*; Beck and Gilmour (2), for aerobic pour plate isolates of a filamentous organism; Davis and Baird-Parker (9), for the aerobically isolated organism which they termed *Leptotrichia dentium*; Richardson and Schmidt (22), for their aerobic pour plate isolates; and Gilmour *et al.* (11), in a literature review of previously described organisms which they termed *Bacterionema matruchotii*. As shown in tables 1 and 2, the various descriptions differ in fermentation patterns, gaseous growth requirements, and the occurrence of branching. These discrepancies may be attributable to the varying cultural conditions employed, to culture instability, or to a variety of biochemically different organisms having similar cellular morphologies. To test these possibilities, the physiological characteristics and environmental effects on morphology have been studied in 55 strains of *Bacterionema matruchotii*.

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Materials and Methods

Cultures and growth conditions. The isolation and morphology of the 55 strains studied has been previously described (10). All cultures were maintained on aerobically incubated slants of brain heart infusion agar (Difco) supplemented with 0.2 per cent yeast extract, and transferred at 3-week intervals. For comparison purposes, tests were also made on *Nocardia asteroides* strains 343 (isolated from a human subject) and ATCC 9970, *Nocardia polychromogenes* ATCC 3409, *Nocardia corallina* ATCC 4273, *Nocardia madurae* ATCC 6245, and *Nocardia brasiliensis* 9961 (isolated from a human subject), all of which were kindly supplied by Dr. A. Howell, Jr. *Nocardia* stocks were transferred as above, but incubated at room temperature.

Media employed included brain heart infusion (brain heart infusion broth (Difco), 37 g; yeast extract, 2 g; distilled water, 1000 ml; pH 7.4), half strength brain heart infusion (as above, but with 18.5 g brain heart infusion broth), and nutrient medium (nutrient broth, Difco, 8 g; yeast extract, 2 g; distilled water, 1000 ml; pH 7.4). Two per cent agar was added for solidification, and 5 per cent citrated sheep blood or sheep serum was included when required.

Because most of the strains exhibited granular growth, inocula for the majority of tests were lightly homogenized buffer or broth suspensions of log phase cells adjusted to standard optical densities ranging from scale readings of 50 to 70 on a Klett-Summerson colorimeter, with filter 54 and the menstruum as blank.

Cultures were incubated at 37 C aerobically, or anaerobically using the Pine and Howell (21) technique or anaerobic jars (McIntosh and Fildes, or Brewer's). The latter were evacuated and filled three times with 95 per cent H_2 and 5 per cent CO_2, and then attached to an electric circuit for 1 hr. Cultures of *N. asteroides* were used to detect deficient anaerobiosis. All tests on *Nocardia* cultures were incubated aerobically.

TABLE 1

Fermentative characteristics of organisms morphologically similar to Bacterionema matruchotii, reported by other authors

Carbohydrate	Author and Number of Strains Tested										
	Kligler (15)	Mendel (3)	Bulleid (10)	Bibby (53)	Bartels (1)	Morris (17)				Davis and Baird-Parker (?)	Richardson and Schmidt (12)
						Type 1	Type 2	Type 3	Type 4		
Glucose	+	+	slow +	+	+	−	slow +	+	+	+	+
Fructose		+		+						+	
Salicin						−	−	−	−	+ and − strains	+
Sucrose	+	+	−	98% +	+	−	slow +	+	+	+	+
Mannose										+	+
Maltose	+		slow +	88% +	+	−	−	+	+	+	+
Raffinose				+	−	−	−	−	−	+ and − strains	+ and − strains
Mannitol			−	−	−	−	slow +	+	+		−
Lactose	−	+	−	−	−	−	−	+	+	−	−
Arabinose						−	−	−	−		−
Xylose					−	−	−	−	+		−
Galactose			slow +	−						+ and − strains	−
Rhamnose					−	−	−	−	−		−
Melibiose											−
Cellobiose										−	
Trehalose										−	−
Melizitose										−	−
Inulin			−	−		−	−	−	+		−
Dextrin			−		+	−	−	−	−	+	+
Sorbitol											−
Dulcitol				−	−						−
Inositol											−

Gaseous conditions for growth. To determine the preferential gaseous environment, shake tubes containing 15 ml brain heart infusion and 2.5 per cent agar were inoculated with 0.1 ml from aerobic broth cultures of either *Bacterionema matruchotii* or *Nocardia* species and observed daily for a 2-week period.

Aerobically and anaerobically grown cell suspensions of all strains were each also tested for growth under both aerobic and anaerobic (jars) conditions. Suspensions of approximately 7×10^4 cells per ml were streaked onto duplicate plates using either a standard loop for inoculation and streaking, or 0.05 ml of a 1:10 dilution and a glass rod. Seventeen or more strains were tested in duplicate broth tubes each containing 5 ml of medium and inoculated with 0.05 ml of the above suspension. The *Nocardia* species were similarly tested on plates. Media employed were brain heart infusion agar with and without blood, and brain heart infusions and nutrient broths with and without serum. Observations and Gram stains were made after 1 or 2 weeks of incubation, or both.

To ascertain that anaerobic growth could not be attributed to large cell masses, small inocula of aerobically and anaerobically grown cells from each of eight strains were tested for aerobic and anaerobic growth. Each strain was incubated aerobically and anaerobically in brain heart infusion broth supplemented with 5 per cent sheep serum until a faintly turbid suspension (scale reading approximately 5 to 10 on the Klett-Summerson colorimeter) was obtained after the clumps had settled out. Aliquots of each supernatant were removed, counted in a hemocytometer, and diluted to 20 cells per ml in each of the 3 media employed: (a) brain heart infusion broth;

TABLE 2

Biochemical characteristics of organisms morphologically similar to Bacterionema matruchotii, reported by other authors

Characteristic	Author and Number of Strains Tested										
	Kligler (15)	Mendel (3)	Bulleid (10)	Bibby (53)	Bartels (1)	Morris (17)				Davis and Baird-Parker (?)	Richardson and Schmidt (12)
						Type 1	Type 2	Type 3	Type 4		
Esculin hydrolysis										+	
Hippurate hydrolysis										+	
Starch hydrolysis				19% +	+	−	slow +	−	−	+	+
NH_3 production	−									−	
Indole	−			−						−	−
CO_2	−*	−*		−*						−	−*
Acetoin						−		−	+	+	+
Acetoin by original Voges-Proskauer				−							
$NO_3 \rightarrow NO_2$				96% +		−		+	+	+	+
Gelatin liquefaction	−	−		−						−	
Catalase						−		−	−	+	+
Gaseous characterization†	An, F, A	A	F, A	Preferential An	An to F	Strict An, some strains become O_2 tolerant				Preferential A	Experiments aerobic
Branching	−	+	+	Rare	+	Rare	Rare	Rare	Rare	+	+

* By Durham tube test.

† An = anaerobe, F = facultative, A = aerobe.

(b) brain heart infusion broth supplemented with 5 per cent sheep serum; and (c) brain heart infusion broth supplemented with laked blood (5 ml sheep blood in 10 ml distilled water added to 100 ml medium). Eighty 1-ml aliquots of each of the resulting 6 dilutions from each strain were dispensed into 80 Kahn tubes, one-half of which were incubated aerobically, the other half anaerobically. Following 7 days of incubation, the numbers of tubes showing growth were recorded.

pH limitations on growth initiation. Fifteen strains were tested on brain heart infusion broths and agars adjusted at 0.5 pH unit intervals in the range pH 4.5 to 9.0. Broth tubes and duplicate plates were each inoculated with 0.1 ml buffer suspensions of aerobically grown cells. All tubes and one set of plates were incubated aerobically, while the other set of plates was incubated anaerobically in an atmosphere of H_2. Observations were made daily over a 1-week incubation period and Gram stains were prepared from all cultures with growth.

Fermentation tests. Aerobically incubated tests were performed on all stains shortly after purification and, using stock cultures which were transferred every 3 weeks during the interval, again after 1 year. Tests were also repeated twice on 11 strains incubated anaerobically and on the 5 *Nocardia* cultures.

Aqueous 20 per cent solutions of each of the carbohydrates at pH 6.5 were sterilized and added aseptically at a final concentration of 1 per cent to the basal media. Sucrose, fructose, lactose, arabinose, galactose, xylose, mannose, and maltose were sterilized with filtration; mannitol, dulcitol, inulin, raffinose, salicin, glycerol, rhamnose, glucose, trehalose, cellobiose, melibiose, sorbitol, and α-methyl-glucoside, by steaming for 20 min on each of 3 successive days. The basal medium for aerobic tests was nutrient medium broth with 0.001 per cent final concentration of bromothymol blue. Because this medium did not support anaerobic growth, nutrient medium serum broth was used for the anaerobically incubated tests, and aerobically incubated controls were performed to ascertain that the fermentation pattern was not affected by the change of medium.

Aerobically incubated tests were performed in Durham tubes inoculated with 0.1-ml aliquots of aerobically grown cell suspensions. Inoculated and uninoculated control tubes were observed daily for color change, and after 2 weeks of incubation the final pH was determined. Following inoculation with anaerobically grown cells, anaerobic tests were incubated for 2 weeks and the pH in both inoculated tubes and uninoculated controls of each sugar was measured with a pH meter.

The rates of pH change with aerobic and anaerobic growth were determined with 2 diffusely growing strains in nutrient medium serum broths with and without 1 per cent glucose. Each strain was tested by inoculating 30 tubes of each medium with 0.1-ml aliquots of an aerobically grown cell suspension giving a scale reading of 50 on the Klett-Summerson colorimeter. One half of the inoculated tubes and an equal number of uninoculated control tubes were incubated aerobically; the other half and their controls, anaerobically in jars. Following 2 hr incubation, and thereafter at 24-hr intervals over a period of 14 days, pH determinations were made on one tube of each of the inoculated and uninoculated media from each incubation condition, and the growth was estimated by visual comparison.

Additional biochemical tests. All tests were made on each strain simultaneously with its fermentation tests and using the same inoculum. Unless otherwise specified, the basal media employed were half strength brain heart infusion broth for aerobically incubated tests, and the same medium supplemented with serum for the 11 strains tested anaerobically. Serum was added to all media employed for anaerobic cultures. The majority of methods used are described in the *Manual of microbiological methods* (24) and the *Manual of methods for pure culture study of bacteria* (23) of the Society of American Bacteriologists. Esculin hydrolysis was tested in broth supplemented with 0.5 per cent esculin and 0.5 per cent ferric ammonium citrate, and in streak plates containing 2.5 per cent esculin, 2.5 per cent yeast extract, 2 per cent agar, and 0.05 per cent ferric ammonium citrate (G. Knaysi, *unpublished data*); daily observations were made over a 2-week incubation period. Hippurate hydrolysis was determined by mixing equal volumes of 50 per cent H_2SO_4 and the supernatant from a 2-week culture grown in nutrient medium broth containing 1 per cent sodium hippurate. Starch hydrolysis was tested by flooding 5-day cultures on 0.5 per cent starch plates with Lugol's iodine. Gelatin liquefaction was observed in chilled 2-week cultures in 12 per

cent gelatin. Ammonia production was determined by nesslerization of 1-week broth cultures containing 1 per cent arginine hydrochloride. Tests for indole production were made with Kovac's reagent on 2-week cultures; for nitrate reduction to nitrite on 1-week cultures; and for acetoin production from glucose in 2-week 2 per cent glucose cultures employing the method as originally described (8) and 0.3 per cent creatine in 40 per cent KOH. CO_2 production was tested in Durham tubes using 1 per cent glucose nutrient medium broth, in 2 per cent glucose agar shake cultures, and in Eldredge tubes using 25 strains grown aerobically in 2 per cent glucose nutrient medium broth. The production of catalase was assessed by suspending an aerobically grown colony in 3 per cent H_2O_2 and observing at 32× magnification.

Antibiotic sensitivities. Tests were made on 9 strains with aerobic incubation, on an additional 5 strains with aerobic and anaerobic incubation performed in parallel, and on 5 *Nocardia* species. Following uniform inoculation of brain heart infusion serum agar plates with suspensions of aerobically or anaerobically grown cells for the respective incubation condition, a Sensi-disc (BBL) was placed on each plate. Sensi-discs of each antibiotic were from a single series batch including high and low concentrations: penicillin and bacitracin, 2 and 10 units; dihydrostreptomycin, 10 and 50 μg; triple sulfonamide (sulfadiazine, sulfamethazine, and sulfamerazine), 0.25 and 1.0 mg; erythromycin and carbomycin, 2 and 15 μg; chlortetracycline, oxytetracycline, tetracycline, neomycin, chloramphenicol, and polymyxin B, 5 and 30 μg. After 5 days of incubation, the diameter of the zone without growth, including the disc, was measured.

RESULTS

Gaseous conditions for growth. Of the 34 strains tested in agar shake cultures, 30 grew throughout the tube, 2 did not grow at the top, and 2 did not grow at the bottom. The latter 4 strains did, however, subculture on both aerobically and anaerobically incubated streak plates. The majority of the first group had most abundant growth extending from ⅛ in. below the surface halfway to the bottom of the tube. Two of such strains exhibited a ring of heavy growth approximately ¾ in. below the medium surface. In contrast, growth of the *Nocardia* species was restricted to the top ¼ in. of medium.

TABLE 3

*Growth of Bacterionema matruchotii and Nocardia spp. under aerobic and strictly anaerobic conditions**

Medium	Ratio + Cultures *B. matruchotii*		Ratio + Cultures *Nocardia* spp.	
	Aerobic incubation	Anaerobic incubation	Aerobic incubation	Anaerobic incubation†
Brain heart infusion agar + 0.2% yeast extract	55/55	55/55	5/5	0
Blood agar	—‡	55/55	5/5	0
Brain heart infusion broth + 0.2% yeast extract	22/22	16/22	5/5	0
Brain heart infusion broth + 5% sheep serum	17/17	17/17	—	—
Nutrient broth + 0.2% yeast extract	17/17	2/17	—	—
Nutrient broth + 0.2% yeast extract + 5% sheep serum	17/17	16/17	—	—

* From aerobic inocula.

† All cultures grew when subsequently incubated aerobically.

‡ — = Not tested.

The results with aerobically and anaerobically incubated broth and agar cultures of *Bacterionema matruchotii* are compatible with those given above, providing that nutritional requirements, particularly in broths, were met (tables 3 and 4). Successful growth from anaerobically grown small inocula (20 cells per ml) was particularly dependent on the medium (table 4), and relatively few anaerobically grown cells of the two strains which grew only near the surface in shake cultures were able to reproduce (see strain WHB-9A, table 4). It should be noted that growth rates in non-serum or non-blood containing media were greater aerobically than anaerobically, whereas when serum or laked blood was added, anaerobic and aerobic growth rates of some strains were comparable as judged by visual comparison. Although *Bacterionema matruchotii* cultures were able to reproduce under aerobic or anaerobic conditions, the five *Nocardia* species grew only aerobically (table 3).

TABLE 4

Effect of media and aerobic and anaerobic incubation on growth of small inocula of Bacterionema matruchotii

Inoculum*	Subcultures		% Subcultures Yielding Growth‡				
	Incubation	Medium†	No. 2A31	No. 12	No. 7	No. WHB9A	No. 30
Aerobic	Aerobic	0	100	95	100	100	100
		Blood	100	100	100	100	100
		Serum	100	100	100	100	100
	Anaerobic	0	100	95	95	90	100
		Blood	100	97.5	100	92.5	100
		Serum	100	95	90	95	100
Anaerobic	Aerobic	0	77.5	87.5	95	30	100
		Blood	100	100	100	20	100
		Serum	100	100	100	52.5	100
	Anaerobic	0	97.5	15	10	5	25
		Blood	100	100	100	10	100
		Serum	97.5	80	100	32.5	32.5

* Grown on brain heart infusion medium supplemented with 5 per cent sheep serum.

† Brain heart infusion with no additions, or supplemented with 5 per cent final concentration sheep serum or laked blood, (5 ml. sterile sheep blood + 10 ml distilled water added to 100 ml medium).

‡ Each figure represents tests of growth in 40 tubes.

Cell morphologies in aerobic and anaerobic cultures were consistently different in the following respects. (a) Branching occurred very rarely in anaerobically grown cultures, whereas it was a common phenomenon in aerobic cultures. (b) The frequency of occurrence of long filamentous cells was greatly increased in anaerobic cultures as compared to aerobic cultures. (c) Cell size (both diameter and length) was larger in anaerobic cultures than in aerobic cultures.

pH limitations on growth initiation. As shown in table 5, the optimal pH range for initiation of growth on both solid and fluid media was 7.0 to 7.5. In general, growth was not initiated at a pH of 9.0, or at a pH of 5.5 or lower. Branching cells were consistently found more frequently in cultures grown at pH 6.0 than in cultures grown at higher pH values.

Fermentation tests. With a few exceptions, the 55 aerobically tested strains and the 11 anaerobically tested strains were remarkably similar and constant in their sugar fermentation patterns. One strain differed from all others in being a "slow" fermenter of all fermentable substrates (*i.e.*, requiring 1 to 2 weeks to give an acid reaction). All strains consistently fermented glucose, fructose, sucrose, and maltose to a final pH of 4.7 to 5.2. Including 3 "slow" fermenters, mannose was fermented by 54 and 10 of the respective aerobically and anaerobically tested strains, the final pH being 5.0 to 5.5. Fifty-one

TABLE 5

Effect of pH on growth initiation of Bacterionema matruchotii in broth and agar media

pH of Medium	Ratio Cultures with Growth		
	Broth (aerobic)	Agar	
		Aerobic	Anaerobic
4.5	0/15	—*	—
5.0	0/15	0/15	0/15
5.5	0/15	1/15	0/15
6.0	11/15	9/15	9/15
6.5	15/15	13/15	15/15
7.0	15/15	15/15	15/15
7.5	15/15	15/15	15/15
8.0	12/15	13/15	15/15
8.5	7/15	1/15	4/15
9.0	0/15	—	—

* — = Not tested.

of the aerobically tested and 9 of the anaerobically tested strains fermented salicin to yield a final pH of 5.3 to 5.4. However, salicin fermentation was not a reliable characteristic; 15 strains were "slow" fermenters and 3 strains changed from negative to fast fermenters between the two test times. Raffinose was slowly fermented by 19 of the 55 aerobically and 5 of the 11 anaerobically tested strains. One strain changed from raffinose ± to raffinose + between the two test times. Inocula from late positive tubes did not cause a rapid fermentation when subsequently inoculated into fresh raffinose tubes, indicating that the "slow" fermentation was not attributable to a major population shift to raffinose positive cells. Mannitol was fermented by 7 of the aerobically tested strains and 2 of those tested anaerobically, yielding a final pH of 5.0 to 5.3, and one strain changed from negative to positive. Trehalose was fermented by 3 of the aerobically tested strains, and by none of those tested anaerobically. None of the strains fermented lactose, arabinose, xylose, galactose, α-methyl-glucoside, rhamnose, melibiose, cellobiose, inulin, glycerol, sorbitol, or dulcitol.

None of the 5 *Nocardia* species tested under these conditions produced acid or gas from any of the carbohydrates tested. Final pH values were either the same as the uninoculated control tubes, or higher.

Although the incubation condition (*i.e.*, aerobic or anaerobic) did not affect the sugar fermentation pattern, it appeared to influence the final pH values obtained. In the absence of a fermentable substrate, the final pH increased as compared to the uninoculated control tubes by an average of 0.5 unit with aerobic incubation, but exhibited little change anaerobically. In the presence of a fermentable substrate, the final pH as compared to the controls decreased an average of 2.6 units with aerobiosis, but only 1.6 units with anaerobiosis. These differences in pH decrease could be attributable, for the majority of strains tested, to the slower growth rates found with anaerobiosis than with aerobiosis.

However, to determine if the gaseous growth environment *per se* could affect the final pH values attained, the rates of pH changes were measured with growth conditions and strains such that aerobic and anaerobic growth rates were comparable. The results of the rates of pH changes given in figure 1 are from a typical experiment in which the stationary growth phase was initiated after 5 or 6 days. In the presence of the fermentable substrate glucose there was a greater initial rate of pH decrease and a lower final pH with anaerobic incubation than with aerobiosis (see figure 1*a*). When corrected for pH changes in the anaerobically incubated uninoculated control tubes, the rates of pH decrease due to fermentation differed aerobically and anaerobically until at the initiation of stationary phase the net pH change was similar in both incubation conditions (figure 1*b*). Subsequent to this period, further acid production occurred aerobically to yield a net pH decrease greater aerobically than anaerobically. It is probable that the low pH found in the anaerobic cultures prevented further acid production; cells from 14-day cultures did not stain well, and yielded few positive subcultures as compared to cells from 14-day aerobically incubated cultures. In the absence of glucose and under aerobic conditions there was a steady slow increase in pH until a plateau region was established at pH 8.3, whereas under anaerobic conditions no pH change occurred.

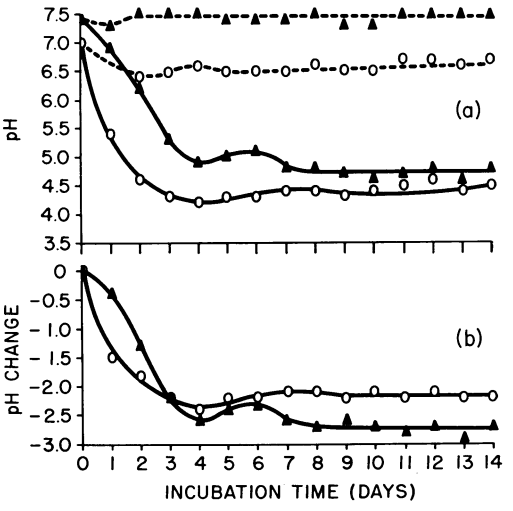

Figure 1. Effect of aerobic and anaerobic growth of *Bacterionema matruchotii* on pH change in the presence of the fermentable sugar, glucose. (*a*) Absolute pH measurements of inoculated tubes and their uninoculated controls. (*b*) pH changes calculated from the difference of pH between uninoculated controls and inoculated experimental tubes. ▲——▲ aerobic growth; ▲-----▲ aerobic uninoculated control; O——O anaerobic growth; O-----O anaerobic uninoculated control.

Additional tests. No changes were noted between the two testing times in the following traits. Esculin was hydrolyzed by 48 of the aerobically tested strains and 11 of those tested anaerobically. All strains hydrolyzed sodium hippurate and starch. None liquefied gelatin, produced indole from tryptophan, or produced ammonia from arginine. All reduced nitrate to nitrite. All strains produced acetoin from glucose, but not enough to give a positive reaction by the original test. The 25 aerobically tested strains were found to produce CO_2 by Eldredge tube technique but not by the Durham tube or agar shake culture methods. All strains were catalase positive.

Antibiotic sensitivities. Among the 14 strains tested with aerobic incubation, one was insensitive to all antibiotics tested, and all of the remaining 13 were sensitive to penicillin, dihydrostreptomycin, erythromycin, carbomycin, neomycin, oxytetracycline, tetracycline, and bacitracin. The 13 were sensitive to high concentrations of chloramphenicol and chlortetracycline but 2 were insensitive to the low concentration of chloramphenicol and 5 to the low concentration of chlortetracycline. Twelve were sensitive to the high concentrations of polymyxin and triple sulfonamide, and 4 to the low concentrations of each antibiotic; the strains insensitive to each compound were not necessarily the same. Zones without growth for the respective high and low concentrations ranged from 4.5 to 6.0 cm and 1.0 to 5.5 cm for the first group of compounds; 2.5 to 5.0 cm and 0.0 to 3.0 cm for chloramphenicol and triple sulfonamide; and 0.0 to 3.0 cm and 0.0 to 2.0 cm for chlortetracycline and polymyxin. Comparison of the 5 strains tested both aerobically and anaerobically showed that the above sensitivity patterns were essentially the same for both incubation conditions, although more strains were sensitive to chloramphenicol and triple sulfonamide and fewer to polymyxin when incubated anaerobically than aerobically. Since the pH of the medium after anaerobic incubation was 6.6 as compared to pH 6.9 after aerobic incubation, these differences might be attributable to differing rates of antibiotic diffusion.

The 5 *Nocardia* species tested were less sensitive to the antibiotics. *N. brasiliensis* was insensitive to all the antibiotics. Among the 4 remaining cultures, 1 was sensitive to penicillin, 3 to streptomycin, 3 to oxytetracycline, 3 to tetracycline, 1 to triple sulfonamide, and 2 to polymyxin B. With the exceptions of *N. polychromogenes* on dihydrostreptomycin, neomycin, oxytetracycline, and triple sulfonamide, and *N. asteroides* on dihydrostreptomycin, the inhibition zones were 3 cm or smaller in diameter.

Discussion

Although one strain was characteristically a "slow" fermenter, and a second was resistant to all antibiotics tested, the 55 strains of *Bacterionema matruchotii* studied herein comprise a reasonably homogeneous group similar to many previously reported isolates (see tables 1 and 2). The finding that at least two growth conditions, pH and gaseous environment, govern the frequency of branching in this organism reconciles the data presented here with those descriptions reporting the rareness of branching (3, 16, 19) and with those reporting its regular occurrence in this microorganism (1, 7, 9, 14, 18, 22). It seems, therefore, that the controversy over whether or not this organism branches arose because of differences in the growth conditions employed in the earlier studies. The ability of our strains to grow both aerobically and anaerobically, (although growth rates were generally greater with aerobiosis than with anaerobiosis) also differs from the aerobic (9, 14, 18) or more strictly anaerobic (3, 16, 19) habits previously attributed to similar organisms. This discrepancy may be attributable to two factors: characterization as aerobic could result from failure to make experimental allowances for the media requirements and slower growth rates reported here for anaerobic growth; characterization as more strictly anaerobic could result from anaerobic isolates being more strictly anaerobic than the aerobic pour plate isolates studied herein. There are, therefore, probably three classes of isolates: facultative aerobes, similar to those described here and by others (7, 9, 18, 22), facultative anaerobes as described by Kligler (16), Bibby (3), and Morris (19), and strict anaerobes (19). The aerobic and anaerobic pH limitations on growth are similar, and are the same as those previously given for both anaerobic (3) and aerobic (22) isolates. Additionally, biochemical characteristics, including sugar fermentation patterns, are similar to the majority of earlier descriptions (1, 3, 4, 9, 16, 22). Comparison with Bibby's

anaerobic isolates (3), the largest number of strains previously studied, shows differences only in the percentages of maltose-, raffinose-, and starch-positive strains (respectively, 88, 100, and 19 per cent as compared to 100, 35, and 100 per cent reported here). There is no real discrepancy in the results for CO_2 production, since assessment with the more sensitive Eldredge tube technique yields positive results whereas Durham tubes used here and by others give negative results. There are, however, apparent radical differences from the essentially asacharolytic strains described by Bulleid (7) and the 4 anaerobic catalase-negative groups noted by Morris (19). Although the use of aerobiosis or anaerobiosis during either isolation or testing cannot solely account for these differences, it is possible that the asacharolytic strains are actually "slow" fermenters, since relatively short test incubation times were employed by both authors. Thus, the majority of isolated strains are both morphologically and biochemically similar, the biochemical characteristics being relatively stable despite long culture periods and changes in media or gaseous environment during testing. However, as indicated by Morris' (19) work, other probably rarer morphologically similar but biochemically different forms exist.

Because of its ability to branch, its reproduction by fragmentation (12), its sensitivity to acid and antibiotics, and its size variations, this microorganism has characteristics of both the higher fungi and the bacteria. Gilmour *et al.* (11) have therefore classified this type of organism in the family *Actinomycetaceae*, but because of differences from the two existing genera, *Nocardia* and *Actinomyces*, have recommended that a new genus, *Bacterionema*, be formed. The type species suggested, *Bacterionema matruchotii*, comprises organisms identical to those described here. Further differences from the former two genera are reported in this paper. *Bacterionema matruchotii* differs markedly from the *Nocardia* species tested in its gaseous growth characteristics, fermentative capacities, and antibiotic sensitivities (see also (17) and (25)). It differs in gaseous growth characteristics, fermentative pattern, reactions in additional tests, and inability to grow on the Howell and Pine (13) casitone medium for *Actinomyces* (Gilmour, *unpublished data*), from both the anaerobic *Actinomyces* and the facultative oral form termed *Actinomyces naeslundii* by Thompson and Lovestedt (26) and also described by Naeslund (20), Bibby and Knighton (5), Bowen (6), and Howell *et al.* (15).

Summary

A number of characteristics of 55 strains of *Bacterionema matruchotii* have been studied. Fifty-three of the strains form a homogeneous group. They are facultative aerobes; initiate growth optimally in the pH range 7.0 to 7.5; ferment glucose, fructose, sucrose, mannose, and maltose to yield a final pH in the range 4.7 to 5.2 in a nutrient broth yeast extract medium; hydrolyze starch and sodium hippurate; reduce nitrate to nitrite; produce acetoin and CO_2 from glucose; are catalase positive and sensitive to many antibiotics. Two strains differed from the others: one required 1 to 2 weeks to give an acid reaction on fermentable substrates, and one was insensitive to all antibiotics tested. With the exceptions of reactions to salicin, raffinose, and mannitol, all strains were stable in all characteristics tested. Morphologically and biochemically these strains resemble organisms described in the majority of previous studies (1, 3, 4, 7, 9, 14, 16, 18), but differ from the four anaerobic types reported by Morris (19).

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