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

IV. Physiological and Biochemical Characteristics of *Bacterionema matruchotii*

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Gilmour, Howell, and Bibby (11) proposed the name *Bacterionema matruchotii* for the branching filamentous organism originally described by Kligler (17) and Bulleid (5) as *Leptothrix buccalis* and by others as *Leptotrichia buccalis* (3, 14) or *Leptotrichia dentium* (8). The physiological and biochemical data presented for this organism by Kligler (17), Bulleid (5), Bibby and Berry (3), Davis and Baird-Parker (8), Richardson and Schmidt (23), and Beck and Gilmour (2) are, in general, of a limited nature. The following study was undertaken, therefore, in an attempt to substantiate previous findings, to characterize this species more adequately, and to relate it on a physiological level with those species to which it bears close morphological relationships.

Materials and Methods

A total of 37 strains of *Bacterionema matruchotii* isolated by the senior author from 29 oral samples and 2 saliva samples from 13 individuals, and 6 isolated by Dr. Marion Gilmour, Eastman Dental Dispensary, Rochester, New York, were used in these studies although not all strains were used in all experiments.

Procedures for the preparation of inoculum, measurements of growth, pH determinations, and general physiological studies were the same as those used in previous experiments (12), except that in most instances cultures were grown in air with increased carbon dioxide tension at 37 C on a rotary shaker (19). In addition, it was found that the liquid starch medium with either casein hydrolyzate (Nutritional Biochemicals Corporation, vitamin-free, acid hydrolyzed, 10 per cent solution) or Casitone (Difco) with the pH adjusted to 6.5 or 7.0, used for the cultivation of *Actinomyces* spp. (13, 19), was unsatisfactory for the continued cultivation of strains of *Bacterionema matruchotii*. Preliminary experiments indicated that the *Actinomyces* maintenance medium described by Pine and Watson (20) would support continued transfers and excellent growth of these organisms. The latter medium, modified as follows, was the basal medium used in most of the experiments reported herein: casein hydrolyzate (40 ml/L) and L-tryptophan (40 mg/L) were substituted for Casitone, the concentration of yeast extract was reduced to 0.025 per cent, coenzyme A and agar were omitted, and the pH of the medium was adjusted to 6.5 or 7.0. The medium, containing carbohydrate or organic acid but not starch, was sterilized by filtration through a Selas 03 filter as in previous studies. Subsequently it was mixed with the starch solution (13). In most experiments the medium was dispensed in 5-ml amounts into matched colorimeter tubes (20 by 150 mm).

In a few experiments the liquid Casitone-starch medium described by Pine and Howell (19) supplemented with 0.1 per cent yeast extract, and Bacto nutrient broth medium (Difco) supplemented with 0.2 per cent yeast extract and adjusted to pH 7.0, were used for comparison. A model B Beckman spectrophotometer at 660 mμ was used for optical density (OD) measurements (12).

For acid-fast stains, cultures were grown on litmus milk or litmus milk containing calcium carbonate. For this purpose, a 10 per cent suspension of calcium carbonate was autoclaved at 121 C for 1 hr. The powder was then resuspended and 1 ml of suspension added to a tube containing

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10 ml litmus milk. After inoculation, cultures were incubated at 37 C.

For fermentation analyses, each of 4 strains (CS-552, CS-585, CS-611, and C-372) were grown on the modified casein hydrolyzate medium described above or on this medium with Casitone (4 g/L) in place of casein hydrolyzate. Each medium contained 0.5 or 1.0 per cent glucose. Generally, duplicate centrifuge tubes (30 by 150 mm), containing 25 to 50 ml of medium, were inoculated with 1 to 5 ml of inoculum from cultures which had been grown in the same medium aerobically for 24 to 48 hr on a rotary shaker. One tube was incubated stationary at 37 C and shaken by hand twice daily; the second was incubated on the rotary shaker. In some instances analyses were made of cultures which had been grown in 200 ml of medium in cotton-stoppered 250-ml volumetric flasks, incubated stationary and shaken manually twice daily.

Residual glucose was determined by the method of Umbreit, Burris, and Stauffer (24). Volatile acids were isolated by steam distillation and were then identified by paper chromatography using the solvent of Kennedy and Barker (16) and using a 0.1 per cent alcoholic solution of ninhydrin to locate the ammonium salts immediately after the papers dried. The volatile acids were also converted to the hydroxamic acids according to the method of Wingerd as described in Block, Durrum, and Zweig (4). The hydroxamic acids were then separated and identified on paper chromatograms using the top layer of a mixture of amyl alcohol-formic acid-water (75:25:75) as the solvent. Volatile acids were determined quantitatively by the procedure of Friedemann (10) as modified by Rabinowitz and Barker (21). After oxidation of formic acid, acetic acid and propionic acid were determined by calculation from simultaneous equations based on the Duclaux values. Formic acid was determined by difference. Nonvolatile acids were isolated from the medium by continuous ether extraction for 48 hr. They were then separated by paper chromatography using Whatman no. 3MM paper and ethanol-ammonia-water solvent (30:5:15) (6) with bromocresol green as a spray indicator. Lactic acid was identified by its R_F and by a positive Feigl spot test when eluted from the paper (9). After elution from the paper, succinic acid was tentatively identified by its R_F in the ethanol-ammonia-water solvent and in ether-formic acid-water (5:2:1). This was confirmed in a two-dimensional chromatogram with these solvents, in which the unknown acid with and without known succinate gave identical spots. A sample of the unknown acid was converted to the hydroxamate and chromatographed using the amyl alcohol-formic acid-water solvent. In all cases, the respective hydroxamic acid had an R_F identical to that given by known hydroxamate of succinic acid.

Lactic acid was determined quantitatively by the method of Barker and Summerson (1); succinic acid was determined by either the succinoxidase procedure of Umbreit *et al.* (24) or by titration of the free acid after elution from the chromatograms. The eluted ammonium succinate was converted to the free acid prior to titration by treatment with Dowex 50. Carbon dioxide was determined qualitatively by growing the cultures in closed systems and trapping the carbon dioxide as barium carbonate; ammonia was determined by the Conway diffusion procedure (7). Amino acids present in the medium before and after the growth of the organism were determined by paper chromatography using the solvents of Redfield (22). Neutral volatile compounds were determined with the colorimetric dichromate method of Johnson (15).

A single strain of *Bacterionema matruchotii* was used for pathogenicity studies in hamsters. In two experiments, two tubes, each containing 5 ml of medium, were inoculated with the organism. After 24 hr incubation the cultures were combined and centrifuged. The cells were resuspended in 3 ml sterile distilled water. In the first experiment each of three 7-week-old male golden hamsters was injected with 0.2 ml of the aqueous suspension intradermally. Simultaneously, two of them were inoculated with 0.5 ml and one with 1 ml of the suspension intraperitoneally. In the second experiment each of three 8-week-old male golden hamsters received three injections intradermally: 0.2 ml of the aqueous suspension, 0.2 ml of a similar aqueous suspension which had been inactivated by heating for 1 hr at 60 C in a water bath, and 0.2 ml of the supernatant broth from the cultures which had been filtered through a sintered glass filter.

Results

Initially, general comparisons were made of growth under various conditions and in different

media. It soon became obvious that relatively high rates of growth and cell yields could be obtained with the modified casein hydrolyzate yeast extract medium, provided the cultures were shaken adequately during incubation. Under these conditions and with glucose as a substrate, maximal growth was usually reached in less than 48 hr. For example, in one experiment, the maximal growth of strains CS-552, CS-585, CS-611, and CS-612 was 3.95, 5.10, 6.80, and 5.50 growth units (= OD × 1/dilution), respectively. On the other hand, values for growth in duplicate tubes of nutrient broth fortified with 0.2 per cent yeast extract and 0.5 per cent glucose were 0.22, 0.18, 0.30, 0.21, respectively. From other experiments it was determined that by reducing the casein hydrolyzate to 0.1 per cent, the maximal growth of some strains was consistently below 1.5. Consequently, the concentration of the casein hydrolyzate was increased to the original 0.4 per cent. Likewise it was found that both cysteine and yeast extract were necessary for optimal growth in the modified casein hydrolyzate medium. Final satisfaction with the basal medium (modified casein hydrolyzate medium with yeast extract) used to test the effect of various substrates rested on the fact that the maximal rate of growth observed was obtained with this medium having 0.5 per cent glucose. It was subsequently found that 0.5 per cent sucrose gave equally good growth. To minimize experimental manipulations in further experiments, only direct optical density measurements were made, *i.e.*, adequate dilutions were not done to determine units of growth once an optical density of 1.5 was reached. Consequently the figures presented may not show the exact time when maximal growth was reached; or, in some instances in which the optical density was 1.5 or greater, the differences in amounts of growth on various substrates may have been greater than indicated by the figures presented.

The relative ability of strains of *Bacterionema matruchotii* to utilize various carbohydrates and organic acids, when these were incorporated into the modified casein hydrolyzate-yeast extract medium and incubated under increased carbon dioxide tension at 37 C, is shown in table 1. Of the 16 carbohydrates and 8 organic acids tested, a high cell yield (OD = 1.50 or greater) was obtained only with glucose, sucrose, acetate, and lactate. Somewhat lower, though moderately high, yields were obtained on maltose and pyruvate (OD = 1.11 and 1.28). Growth in medium 1, as determined by optical density readings, was poor with the remaining carbohydrates and organic acids tested and was not significantly different from that with the basal medium alone (table 1). Citrate was inhibitory for all 12 strains tested. Significant acid production (pH decreased 0.5 unit or more) was obtained only with glucose and sucrose, although a few strains produced a similar decrease in pH when they were grown on the nutrient broth-yeast extract medium containing maltose or salicin. Final pH determinations were not made on media containing organic acids.

Growth curves of a typical strain of these organisms obtained with the modified casein hydrolyzate-yeast extract medium alone or this medium containing specified carbohydrates or organic acids are shown in figures 1 and 2. Results with carbohydrates other than those shown were comparable to that shown for salicin. Growth on other organic acids was similar to that shown for succinate. Rates of growth were essentially similar in all instances, with the exception of citrate, with optical density reaching its maximum within approximately 20 to 30 hr, depending on the strain.

Ten strains were tested for their ability to grow in air at various initial pH levels using the modified casein hydrolyzate medium with yeast extract. No growth was obtained at an initial pH of 5.1 or 7.9 (mean OD = 0.04 ± 0.03[3] and 0.02 ± 0.02, respectively) and no significant growth at pH 5.6 (mean OD = 0.40 ± 0.36). Excellent growth was obtained at pH 6.1, 6.5, 7.0, and 7.4 to 7.5 (mean OD = 1.57 ± 0.29, 1.70 ± 0.20, 1.71 ± 0.25, and 1.49 ± 0.35, respectively), with the optimal initial pH being 6.5 to 7.0 under these conditions. Typical growth curves are shown in figure 3. Growth curves for initial pH values of 6.5 and 7.0 were essentially identical with that shown for pH 6.1 except that with many of the strains the maximal optical density was slightly higher at the two former pH levels. Individual strains varied somewhat in their rates of growth at the various pH levels, but these differences were not considered to be significant. Numerous strains showed a lag of 10 to 20 hr or more when grown at an initial pH of 7.5.

Oxygen tolerance studies on 33 strains, again using the modified casein hydrolyzate-yeast ex-

[3] Mean ± standard deviation.

TABLE 1

Maximal amount of growth[a] and final pH[a] obtained on specified substrates with strains of Bacterionema matruchotii incubated for 7 to 14 days at 37 C

Substrate	Medium 1[b]		Medium 2[b]	Medium 3[b]
	Number of strains tested			
	21		16	12
	OD	Final pH	Final pH	Final pH
Medium alone	0.58 ± 0.15[c]	—[d]	—	—
Glucose	1.85 ± 0.15	6.2 ± 0.1	6.6 ± 0.2	6.4 ± 0.2
Sucrose	1.79 ± 0.13	6.2 ± 0.2	6.5 ± 0.2[e]	6.3 ± 0.2
Maltose	1.11 ± 0.17[f]	6.9 ± 0.1	7.0 ± 0.0	6.8 ± 0.2
Lactose	0.56 ± 0.17	6.9 ± 0.1	7.1 ± 0.0	8.0 ± 0.2
Galactose	0.57 ± 0.17	6.9 ± 0.0	7.0 ± 0.0	7.1 ± 0.4
Rhamnose	0.39 ± 0.10	6.9 ± 0.0	7.0 ± 0.1	8.1 ± 0.1
Arabinose	0.59 ± 0.18	6.9 ± 0.1	7.0 ± 0.0	7.9 ± 0.3
Xylose	0.46 ± 0.17	6.9 ± 0.1	6.9 ± 0.0	7.5 ± 0.5
Raffinose	0.49 ± 0.14	6.9 ± 0.1	7.0 ± 0.0	7.4 ± 0.7
Glycerol	0.55 ± 0.16	6.9 ± 0.1	7.1 ± 0.0	8.2 ± 0.2
Inositol	0.59 ± 0.20	7.0 ± 0.0	7.1 ± 0.0	8.1 ± 0.1
Mannitol	0.41 ± 0.29	7.0 ± 0.1	7.0 ± 0.0	8.1 ± 0.2
Sorbitol	0.52 ± 0.11	6.9 ± 0.1	7.0 ± 0.1	8.1 ± 0.1
Inulin	0.46 ± 0.10	6.9 ± 0.1	7.0 ± 0.0	8.1 ± 0.1
Salicin	0.64 ± 0.14	6.9 ± 0.0	7.0 ± 0.0	6.6 ± 0.3
α-Methyl-D-glucoside	0.51 ± 0.14	6.9 ± 0.1	7.0 ± 0.0	7.9 ± 0.4
Acetate	1.57 ± 0.12[c]	—	—	—
Lactate	1.65 ± 0.22[c]	—	—	—
Pyruvate	1.28 ± 0.27[c]	—	—	—
Benzoate	0.58 ± 0.52[c]	—	—	—
Citrate	0.23 ± 0.12[c]	—	—	—
Gluconate	0.59 ± 0.23[c]	—	—	—
Succinate	0.77 ± 0.13[c]	—	—	—
Tartrate	0.47 ± 0.19[c]	—	—	—

[a] Readings taken daily and expressed as mean optical density (OD) ± standard deviation. Final pH expressed as mean ± standard deviation.

[b] *Medium 1:* Modified maintenance medium (20) containing casein hydrolyzate (Nutritional Biochemicals Corporation, vitamin-free, acid hydrolyzed, 10 per cent solution), 40 ml/L; yeast extract, 0.025 per cent; coenzyme A and agar omitted; initial pH 7.0. Tubes sealed with 10 per cent Na_2CO_3 + 1.0M KH_2PO_4 (13, 19); incubated on rotary shaker for 7 days.

Medium 2: Liquid Casitone-starch medium of Pine and Howell (19) supplemented with 0.1 per cent yeast extract; initial pH 7.0. Tubes sealed and incubated as with medium 1.

Medium 3: Nutrient broth with 0.2 per cent yeast extract; initial pH 7.3. Tubes incubated stationary 14 days in air.

[c] Only 12 strains tested.

[d] Determinations not made.

[e] Only 9 strains tested.

[f] Only 17 strains tested.

tract medium, indicated quite conclusively that, at least under these conditions, some of these organisms are strict aerobes, although most of the strains were capable of limited anaerobic growth for a single transfer under a pyrogallol seal. Maximal optical densities obtained and rates of growth were essentially similar when strains of *Bacterionema matruchotii* were grown in air or in air with increased carbon dioxide tension (OD = 1.87 ± 0.14 and 1.85 ± 0.13, respectively).

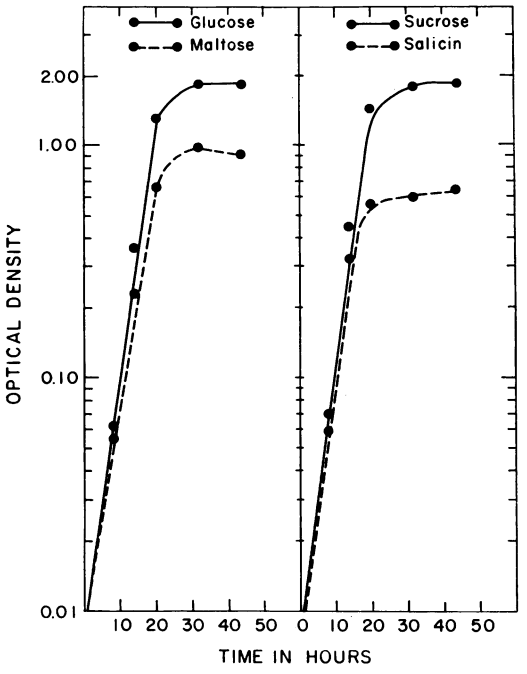

Figure 1. Rate of growth of strain CS-869 on the modified casein hydrolyzate-yeast extract basal medium containing specified carbohydrates (0.5 per cent).

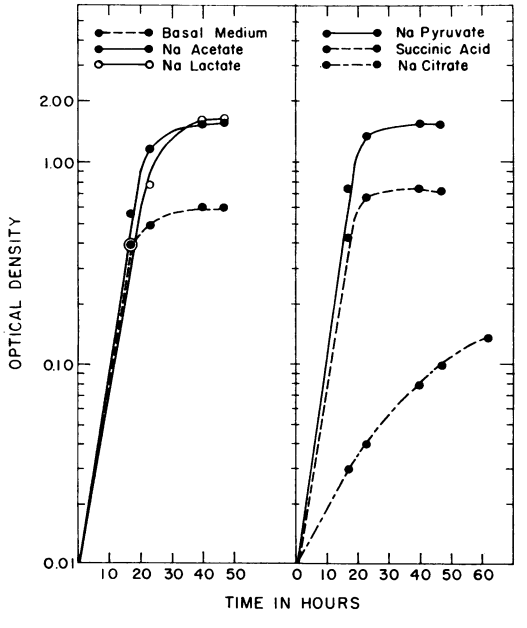

Figure 2. Rate of growth of strain CS-869 on the modified casein hydrolyzate-yeast extract basal medium alone and with specified organic acids (0.5 per cent).

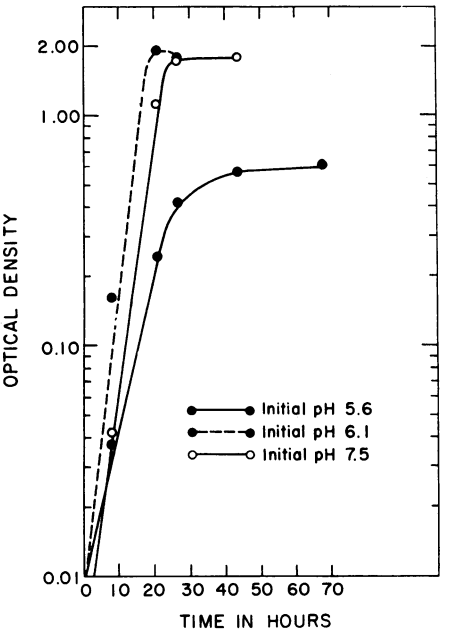

Figure 3. Rate of growth of strain CS-851 on the modified casein hydrolyzate-yeast extract medium with 0.5 per cent glucose adjusted to specified initial pH levels.

Although in most instances rates of growth were not significantly altered by growing the strains for one transfer under an anaerobic seal with increased carbon dioxide tension, the maximal amounts of growth were greatly reduced (mean OD = 0.66 ± 0.38). Four of the 33 strains tested under these conditions gave a maximal optical density of 1.28 to 1.49, whereas four failed to grow (OD < 0.15). Eight gave an OD between 0.15 and 0.50, and 17 an OD between 0.51 and 1.00. Typical growth curves for one strain are illustrated in figure 4. In this instance, as with most strains, growth in air with increased carbon dioxide tension was identical with that of the strain grown in air alone. In a few strains there was a slight lag when the organisms were grown in air alone. With most strains in which this occurred, increased carbon dioxide tension decreased this lag.

Other physiological tests performed included testing the ability of these organisms to grow in litmus milk, 10 to 40 per cent bile, and 4 and 6.5 per cent sodium chloride; to reduce nitrate; liquefy gelatin; produce indole, acetyl methyl carbinol, and catalase. None of 36 strains produced any reaction in litmus milk, grew in 10 per cent bile, liquefied gelatin, or produced indole.

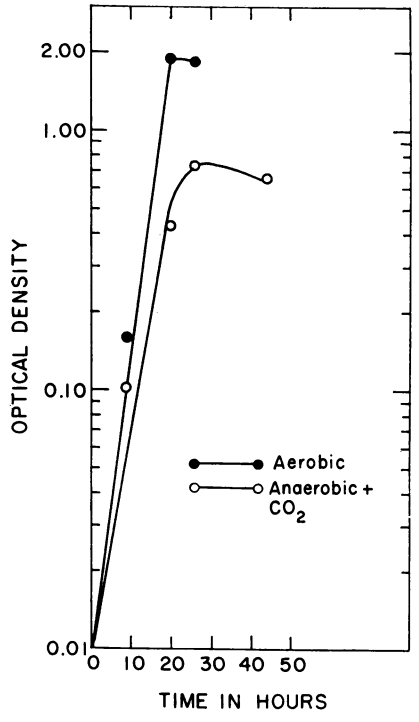

Figure 4. Rate of growth of strain CS-765 on the modified casein hydrolyzate-yeast extract medium with 0.5 per cent glucose in air and anaerobically with increased CO_2.

Eight grew in 4 per cent salt, two additional strains grew slightly in this concentration of salt, but none grew in 6.5 per cent. All 36 reduced nitrate to nitrite, 33 of 37 gave a positive Voges-Proskauer reaction, and all 37 were strongly catalase positive. Several strains, when grown on litmus milk containing calcium carbonate, showed acid fast granules within the filaments.

The products formed from the fermentation of glucose by four aerobic strains were primarily dependent on the availability of oxygen (table 2), although there were quantitative differences in the four strains tested and when Casitone was substituted for the casein hydrolyzate in the medium. When cultures were grown on the shaker the amounts of both volatile and nonvolatile acids formed were very small or negligible in two of the four strains tested. This was also indicated by the fact that there was no appreciable change in pH. However, when grown under stagnant conditions the pH dropped from the initial 6.5 to 5.5. Analyses of the broth under the latter conditions showed that varying amounts of formic, acetic, propionic, lactic, and succinic acids were formed (table 3). Two unidentified nonvolatile acids were also formed. Of these, one had an R_F in the water-ethanol-ammonia solvent intermediate between succinic acid and lactic acid; the second acid remained near the point of origin. Attempts to identify these acids were not successful. Of the products measured, lactic acid accounted for the greatest amount of glucose utilized. No significant amount of neutral volatile compounds were formed.

Under stagnant conditions, considerable amounts of carbon dioxide were formed as indicated by the formation of barium carbonate. It seems, therefore, that most of the unaccounted-for carbon resided in carbon dioxide and unidentified nonvolatile acids. There was also a general decrease in the amounts of amino acids in the medium as judged by the relative size of the ninhydrin spots on chromatograms before and after fermentation. However, no particular amino acid appeared to disappear preferentially during the growth of the organisms. With these results and since there was no increase in ammonia, it was concluded that no specific amino acid serves as a primary energy source under these conditions.

When lactic acid was substituted for glucose in stagnant cultures, growth was extremely limited and the formation of volatile products was negligible. The amount of lactate utilized by the three aerobic strains tested under these conditions ranged from 1.7 to 5.0 μmoles per ml as compared to 30.5 μmoles of glucose used during the same period in parallel fermentations.

This is in contrast to the growth and utilization of lactate when cultures were grown under continuous shaking (table 1 and figure 2). Consequently, it may be concluded that lactic acid itself cannot serve as a fermentable substrate. It also appears from these results that the accumulation of acids during stagnant growth represents an accumulation of intermediates by an aerobic organism and that these acids are not themselves the terminal products of glucose metabolism.

In each hamster inoculated intradermally with living cell suspensions, an abscess as large as 1 to 1.5 cm in diameter developed within 48 hr after inoculation. These ruptured and drained

TABLE 2

Effect of cultural conditions and media on the products of glucose utilization by strains of Bacterionema matruchotii

A. Modified maintenance broth with Casitone

	Strain[a] and Conditions of Growth							
	CS-552		CS-585		CS-611		C-372	
	Stationary	Shaken	Stationary	Shaken	Stationary	Shaken	Stationary	Shaken
	μmoles/ml		*μmoles/ml*		*μmoles/ml*		*μmoles/ml*	
Glucose used	28.9	28.8	28.2	29.4	28.4	28.1	—[b]	—
Products:								
Lactic acid	32.7	0.0	19.9	0.0	23.3	0.0	37.2	15.2
Volatile acids	14.0	2.9	18.6	3.2	29.2	22.5	17.9	22.0
Major volatile acids[c]	P	P	A, P	P	A, P	A, P	P[d]	A, P[e]
Final pH[f]	5.5	6.5	5.5	6.5	5.5	6.5	—	—

B. Modified maintenance broth with casein hydrolyzate

Glucose used	24.8	25.7	26.4	25.4	24.6	25.5	—	—
Products:								
Lactic acid	24.0	0.0	15.6	0.0	18.2	0.0	—	—
Volatile acids	9.7	2.8	21.2	8.1	21.9	10.3	—	—
Major volatile acids	A, P	P	A, P	A	A, P	A		

[a] Strains CS-552, CS-585, and CS-611 were incubated 5 days at 37 C; strain C-372 incubated 3 days.

[b] Determinations not made.

[c] A = acetic acid, P = propionic acid.

[d] Concentrations of volatile acids formed were formate, acetate, and propionate, 0.5, 0.0, and 17.4 μmoles, respectively.

[e] Concentrations of volatile acids formed were formate, acetate, and propionate, 1.0, 8.8, 12.2 μmoles, respectively.

[f] Initial pH 6.5.

TABLE 3

Analysis of glucose fermentation by specified strains of Bacterionema matruchotii in modified maintenance broth with casein hydrolyzate or Casitone under microaerophilic conditions[a]

	Strain and Experiment No.						
	CS-552		CS-585		CS-611		C-372
	No. 15[b]	No. 17[c]	No. 15[b]	No. 17[c]	No. 15[b]	No. 17[c]	No. 19[b]
	μmoles/ml		*μmoles/ml*		*μmoles/ml*		*μmoles/ml*
Glucose used	23.6	30.5	24.1	30.4	25.0	30.5	39.4
Formic acid	1.4	4.4	2.0	2.3	2.7	1.8	1.0
Acetic acid	1.0	9.6	4.2	4.2	4.6	5.5	3.3
Propionic acid	8.2	2.6	5.0	8.1	8.0	6.9	10.0
Lactic acid	24.9	14.3	26.1	13.3	26.7	13.8	31.0
Succinic acid		0.3		0.2		0.0	1.3
NH_3 utilized	1.6		2.7		1.3		

[a] Cultures for experiment nos. 15 and 17 incubated stationary at 37 C for 5 days; for experiment no. 19, 7 days.

[b] Casitone maintenance medium (1.0 per cent glucose).

[c] Casein hydrolyzate medium (0.5 per cent glucose).

in most animals within 3 to 5 days. Positive smears and cultures were obtained from unruptured nodules after 3 and 5 days. Each of the three animals injected intradermally with inactivated cells developed abscesses approximately half the size of those produced by living cells; all had ruptured by the third day. Animals injected with culture filtrate developed no lesions. The three animals injected intraperitoneally showed no internal lesions 3 days after inoculation.

Discussion

As indicated above, few extensive studies on the physiological and biochemical properties of the organisms described herein as *Bacterionema matruchotii* have been reported. Those of Kligler (17), Bulleid (5), Bibby and Berry (3), Davis and Baird-Parker (8), Richardson and Schmidt (23), and Beck and Gilmour (2) all indicate that this microorganism ferments only a limited number of carbohydrates, with the production of acid but no visible gas, reduces nitrate to nitrite, may produce acetyl methyl carbinol, fails to produce hydrogen sulfide or indole or to liquefy gelatin, and produces no change in litmus milk. In general the results of the present study confirm these findings.

Bibby and Berry (3), Richardson and Schmidt (23), and Gilmour and Beck (10*a*) reported that when these organisms were grown in glucose broth the terminal pH often dropped as low as 4.8. Such low pH values were not obtained in the present study, regardless of the medium employed. This might have been due to a more complete consumption of glycolytic products under increased oxygenation (table 2), or to a Pasteur effect (25).

Organisms described under other names but apparently identical to *Bacterionema matruchotii* have been described as aerobic or as growing under aerobic conditions (8, 14, 23), facultative (2) or anaerobic or facultative anaerobic (3, 5, 17). To date few experimental data have been offered to support any one of these conclusions. The data reported herein indicate that most strains are capable of limited anaerobic growth for at least one transfer although many strains are strict aerobes. Unlike strains of *Actinomyces* (19), carbon dioxide is not required for optimal growth.

The physiological and biochemical studies reported herein and those of others mentioned above support the conclusion (11, 23) that *Bacterionema matruchotii* should be placed in the family *Actinomycetaceae* and that it is probably intermediate between the genera *Actinomyces* and *Nocardia*. Like species of *Actinomyces*, *Bacterionema matruchotii* can utilize glucose with the formation of carbon dioxide and lactic, succinic, formic, and acetic acids. In addition, it can form large amounts of propionic acid. Although most strains of *Actinomyces israelii* which have been tested do not form propionic acid, one strain isolated from a case of lacrimal canaliculitis (18) does form this volatile acid. However, unlike most *Actinomyces* strains, which do not appear to degrade these products further, in adequate concentrations of oxygen these acids are either not formed or are used by *Bacterionema* strains for further growth. Unlike species of *Actinomyces* but like those of *Nocardia*, all strains of *B. matruchotii* are catalase positive. Like species of *Nocardia*, some strains of *B. matruchotii* are strict aerobes and some, as previously reported (23) may show acid-fast granules within the filaments. Unlike most species of *Nocardia*, strains of *B. matruchotii* do not grow at room temperature (23). *B. matruchotii* is much more demanding in its growth requirements than most species of *Nocardia* and most strains tested produced a significant amount of growth in one transfer under a pyrogallol seal.

Summary

Heavy growth (optical density of 1.00 or greater) of a representative group of strains of *Bacterionema matruchotii* was obtained consistently on only 3 of 16 carbohydrates tested (glucose, maltose, and sucrose) and 3 of 8 organic acids tested (acetate, lactate, and pyruvate). Under the conditions employed, all strains produced maximal growth in air or in air with increased carbon dioxide tension. Some strains were strictly aerobic although most were capable of limited anaerobic growth for at least one transfer. There was no change in litmus milk, no liquefaction of gelatin, no growth in 10 per cent bile or 6.5 per cent sodium chloride, and no production of indole or hydrogen sulfide. Catalase was produced by all strains tested; nitrate was reduced to nitrite; 33/37 gave a positive Voges-Proskauer reaction. Lactic acid was the chief

product of glucose fermentation when four strains were grown under microaerophilic conditions, although small amounts of formic, acetic, propionic, and succinic acids and carbon dioxide were also formed. Under aerobic conditions one of the four strains tested produced a small amount of lactic acid; two produced both acetic and propionic acid. In the remaining two strains the amounts of both volatile and nonvolatile acids were very small or negligible. Intradermal injections of aqueous suspensions of living and dead cells of a single strain into male golden hamsters produced abscesses which persisted for 3 to 5 days.

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