CYTOPHAGA SUCCINICANS SP. N., A FACULTATIVELY ANAEROBIC, AQUATIC MYXOBACTERIUM[1-3]

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During the course of investigations relating to myxobacterial fish diseases, a number of nonpathogenic cytophagas were found which could grow anaerobically at the expense of carbohydrate fermentation. Myxobacteria which are able to carry out fermentations have rarely been encountered in the past (Bachmann, 1955). The present isolates invoked special interest because their fermentation appeared to be a CO_2-consuming, perhaps even a CO_2-requiring, process. They were, therefore, subjected to further study.

This paper concerns the isolation and description of some fresh-water fermentative myxobacteria. The three strains included in this study are considered to be members of a new species, *Cytophaga succinicans* sp. n.

MATERIALS AND METHODS

Media. Cytophaga agar, a medium used for the isolation and purification of strains, and for routine plating, consists of tryptone (Difco), 0.05 per cent; yeast extract, 0.05 per cent; beef extract, 0.02 per cent; sodium acetate, 0.02 per cent; and agar (Difco), 0.9 per cent; adjusted to pH 7.2 to 7.4. Stock cultures were maintained in tubes of cytophaga medium containing 0.4 per cent agar (Difco). Nutrient broth, a medium consisting of peptone, 0.5 per cent, and beef extract, 0.3 per cent, adjusted to pH 7.0, was used for growing cells for physiological studies. The composition of fermentation broth varied, but the base always contained peptone, 0.1 to 0.2 per cent; yeast extract, 0.05 to 0.1 per cent; and beef extract, 0.05 to 0.1 per cent; adjusted to pH 7.0. In some cases, phosphate buffer was included in a concentration of 0.016 to 0.05 M and at a pH of 7.1 to 7.2. Filter-sterilized sodium bicarbonate was added aseptically to autoclaved basal medium in concentrations ranging up to 0.025 M. Filter-sterilized glucose or other compounds capable of serving as an energy source were added aseptically in a concentration of 0.25 to 0.9 per cent. This medium was used for anaerobic cultures.

Compounds that could serve as energy sources for anaerobic growth were determined by adding the test compounds in a concentration of 0.4 per cent to fermentation broth. Cellulose was added in the form of a filter paper strip. All compounds tested were sterilized by filtration except for the cellulose and starch, which were autoclaved. After inoculation the tubes were sealed with vaspar and were incubated at room temperature. The cultures containing lactose, cellobiose, and cellulose were observed for six weeks; all others were discarded after 1 week.

Analytical methods. Volatile acids were identified by chromatography on paper (Kennedy and Barker, 1951) after steam distillation. The presence of formate in the volatile acid fraction was confirmed by the colorimetric method of Grant (1948); the presence of acetate in the volatile acid fraction was confirmed by chromatography on paper after distilling to dryness over magnesium sulfate and mercuric oxide to destroy the formate (Friedemann, 1938). Nonvolatile acids were identified by chromatography on paper (Brown, 1951) after extraction with ether. Lactate was tested for by the colorimetric method of Barker and Summerson (1941). Common neutral products were tested for by the methods of Neish (1952). Residual glucose was determined

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by the method of Folin (1928) and Kolmer and Boerner (1938). Quantitative determinations of succinic, acetic, and formic acids were made by titration following separation by chromatography on cellulose columns. Chloroform with increasing concentrations of butanol was used as the solvent (Eaton and Klein, 1957). Manometry was performed by conventional techniques (Umbreit, Burris, and Stauffer, 1945). Turbidity measurements in the quantitative growth experiment were determined with a Coleman Nepho-Colorimeter equipped with a green filter. The absorption spectrum of the pigment was determined with a Beckman model DU spectrophotometer.

RESULTS

Isolation. Myxobacteria are frequently encountered when material from the gills or lesions of diseased fish is streaked on cytophaga agar. While many of the myxobacteria isolated in this manner prove to be pathogenic for fish, many others are found to be avirulent and so are considered to be saprophytes or secondary invaders. A significant percentage of these secondary invaders have been found to be fermentative, thus obviating the need for artificial enrichment for the isolation of *C. succinicans.*

To obtain quantitative data on the number of fermentative myxobacteria in certain water samples, the following operations were performed: water samples were collected from various fish tanks[5] at the University of Washington School of Fisheries. Aliquots (0.1 ml) of 10-fold dilutions of the samples were plated on cytophaga agar and were incubated aerobically at room temperature. After 3 days, the colonies of eubacteria and myxobacteria were counted. Most aquatic myxobacteria form distinctive colonies on cytophaga agar and so can readily be distinguished from eubacteria. Cytophaga colonies on this medium are translucent, iridescent, and contain patterns of light and dark areas due to diffraction of light. *Chondrococcus columnaris*, a common aquatic fruiting myxobacterium, also forms an easily recognized type of colony on cytophaga agar (Anacker and Ordal, 1959). The counts listed in table 1 show that myxobacteria were present in every sample of water, and in some samples to an appreciable extent. Sample no. 12, for example, yielded 19 times as many myxobacteria as eubacteria. Most of the myxobacteria appeared to be cytophagas, although a few were identified as *Chondrococcus columnaris.*

[5] The tanks contained species of trout and salmon and were fed continuously with water from a well and from Lake Washington. Water temperatures were usually maintained at 10 to 16 C.

TABLE 1

Colony counts of eubacteria and myxobacteria per ml of water plated on cytophaga agar

Sample No.	Eubacteria	Myxobacteria
1-1	2,100	1,800
1-2a	2,600	500
1-2b	1,400	20
1-5	3,000	2,600
1	17,300	1,100
2	5,200	30
3	6,600	200
4	75,000	40,000
5	6,700	1,400
6	40,000	8,000
7	32,000	30,000
8	308,000	77,000
9	280,000	110,000
10	9,200	200
11	46,000	23,000
12	4,900	94,000
13	64,000	1,200

To test for fermentative ability, 70 cytophaga-type colonies were picked from the plates, purified, and inoculated into tubes containing peptone, 0.18 per cent; yeast extract, 0.09 per cent; beef extract, 0.09 per cent; glucose, 0.9 per cent; and sodium bicarbonate, 0.18 per cent. The tubes were layered with sterile vaspar to exclude air and were incubated at room temperature. Turbidity, determined visually, was used as an indication of anaerobic growth and fermentation. It was found that 23 of the cytophagas tested, or 33 per cent, could grow to maximal density overnight, indicating that fermentative myxobacteria represented a significant part of the natural bacterial population of the aquaria.

The three strains of fermentative myxobacteria selected for further study represent isolations from a fairly broad geographical area over a span of 3 years. Strain 16 was obtained from the survey described above (water sample no. 1-2a) on December 3, 1957. Strain 8 was isolated on December 23, 1954 from the eroded caudal fin of a fingerling chinook salmon inflicted with

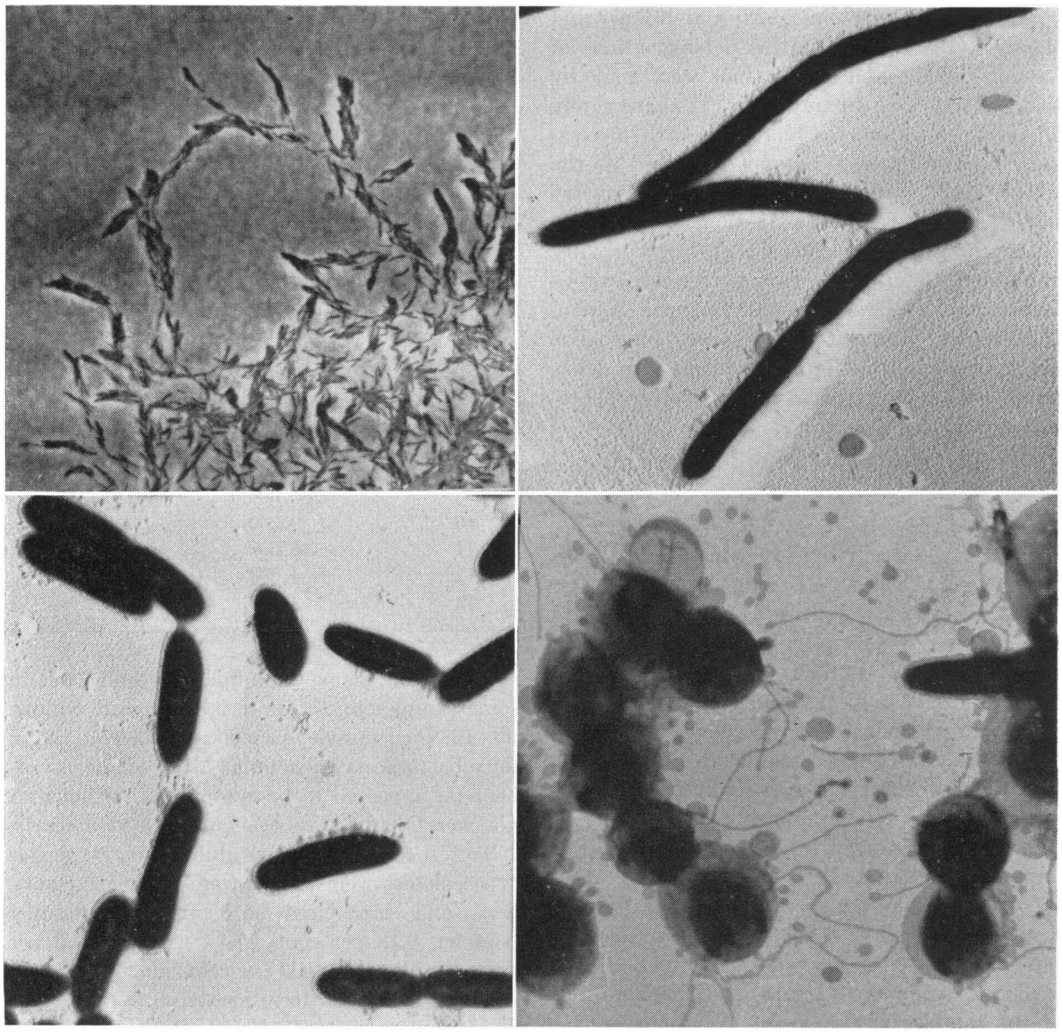

Figure 1. Top left. Phase contrast micrograph of the margin of a colony of *Cytophaga succinicans* strain 14 on cytophaga agar. Magnification 420×.

Figure 2. Top right. Electron micrograph of a 5-hr nutrient broth culture of *Cytophaga succinicans* strain 8. The preparation was fixed with osmium vapor and shadowed with palladium. Magnification 10,000×.

Figure 3. Bottom left. Electron micrograph of a 2-day nutrient broth culture of *Cytophaga succinicans* strain 8. The preparation was fixed with osmium vapor and shadowed with palladium. Magnification 10,000×.

Figure 4. Bottom right. Electron micrograph of involution forms found in an aged culture of *Cytophaga succinicans* strain 8. Magnification 10,000×.

furunculosis disease at the University of Washington hatchery. Strain 14 was isolated on September 16, 1957 from a lesion on an adult chinook salmon taken from the Snake River (Idaho) at Brownlee Dam during an investigation of columnaris disease.

Morphological characteristics. Colonies of *C. succinicans* on cytophaga agar are similar in appearance to those formed by many other aquatic cytophagas: they are translucent, thin, and spreading, often reaching a diameter of 10 to 15 mm in 2 days. When viewed by reflected

light, they appear iridescent and show a kaleidoscopic pattern. At the margins, swarms of cells project out at many points, often converging at the extremities. A photograph of the advancing edge of a representative colony is shown in figure 1.

Although the colony described may be considered typical, variations may occur with respect to the type of margin, the type of diffraction pattern, and the relative tendency to spread. That these characters are variable is evidenced by the observation that several colony types may be found in a culture originally derived from a single cell. Colony morphology also depends on environmental factors, such as temperature, concentration of agar, concentration of nutrients, and the amount of moisture present. No attempt was made to define specifically the effects of these parameters on the appearance of the colony.

Fruiting body formation by *C. succinicans* has never been observed either on cytophaga agar or on pieces of fish tissue submerged in water. The latter method was tried because it has been used successfully in demonstrating fruiting body formation by the aquatic myxobacterium *Chondrococcus columnaris* (Ordal, 1946; Borg, 1948; Rucker, Earp, and Ordal, 1953).

Cells of *C. succinicans* are rod-shaped with rounded ends. Actively growing cells of strain 8 in nutrient broth or on cytophaga agar are 4 to 6 μ long and about 0.5 μ in diameter (figure 2). As the culture ages, the cells become shorter and slightly thicker, the dimensions being about 2 by 0.7 μ (figure 3). Further aging usually results in protoplastlike involution forms (figure 4). Young cells of the other two strains investigated are in many respects similar to those of strain 8, but may differ in average length; some cells (or chains of cellular units?) of strain 14 grown in nutrient broth, for instance, may be more than 40 μ long, although the average length is much less. Strains 14 and 16 do not manifest the tendency to form short cells as does strain 8. All of the strains are gram-negative.

Although flexing and bending of the cells is reputed to be a distinguishing feature of the myxobacteria, none of the cells of *C. succinicans* appear to be flexible; indeed they appear to be as rigid as those of many eubacteria. This may be due in part to the relative shortness of the cells, but even the long cells have not been seen to flex. Stanier (1947), in his admirable discussion of the problems involved in distinguishing between myxobacteria and eubacteria, also noted that flexibility of the former is not invariably conspicuous.

The cultures have been examined under a variety of conditions for microcysts. Although the long viability (several months) of cultures in cytophaga agar deeps argues for the formation of some sort of resting stage by these myxobacteria, nothing which could indisputably be called a microcyst has been found. The very short cells formed by strain 8 (figure 3) cannot be considered microcysts because they are at times motile, and they usually eventually degenerate by lysing or by forming the spherical involution forms. Moreover, very short, coccoid-type cells have been noted in other *Cytophaga* species as the predominant vegetative form even in very young cultures (Stanier, 1947). The protoplast-like involution forms (figure 4) are also excluded as resting stages: they do not resemble microcysts in appearance, and cultures consisting primarily of these forms are often nonviable. This type of involution form has been noted in cultures of a number of other myxobacteria, including a marine fermentative species, *Cytophaga fermentans* (Bachmann, 1955).

Although *C. succinicans* does not possess flagella, the cells exhibit gliding motility when attached to a surface. Gliding motility can be discerned by examining the edge of a colony on cytophaga agar with a high dry objective, preferably phase contrast. If the colony edge has spread into a monolayer (as in figure 1), the cells can be seen to glide slowly back and forth across the surface of the agar. The motility ceases if the petri plate is left open to allow the film of surface water on the agar to evaporate. If a drop of water is placed on a colony margin, the cells become actively motile and form writhing aggregates of about 10 to 100 cells each.

An alternate method for observing motility is by the use of wet mounts; the cells adherent to the slide or cover slip can be seen to glide along on these surfaces. Motility can be in either direction, and the cells can frequently be seen to glide back and forth repeatedly in a distance of only two or three cell lengths. It is of interest to note that although this gliding type of motility is so characteristic of the myxobacteria, its nature is not understood.

Pigmentation. Anaerobically grown cells of *C.*

succinicans are white, but cells grown aerobically in nutrient broth or on cytophaga agar are yellow-orange. As with *Myxococcus xanthus* (Mason and Powelson, 1958), much or all of the pigment resides in the cell walls.[6] To extract the pigment, cells were mixed with anhydrous sodium sulfate, placed in a Soxhlet apparatus, and extracted continuously with diethyl ether for about 45 min. The absorption spectrum of the ethereal solution is shown in figure 5. When the ethereal solution was treated with sulfuric acid, a blue color characteristic of carotenoids (Karrer and Jucker, 1950) developed.

Nutrition. C. succinicans was grown aerobically in nutrient broth or on cytophaga agar. Strain 8 was found to survive serial transfer on 0.2 per cent vitamin-free casein hydrolyzate, but growth was always sparse. Attempts to grow strains 14 and 16 on casein hydrolyzate were not successful even when the medium was supplemented with a mixture of vitamins.

During attempts to grow the cells anaerobically, yeast extract was added to the peptone-beef extract medium. Although *C. succinicans* can grow readily on this complex medium in the presence of air, anaerobic growth requires the addition of a fermentable carbohydrate and CO_2 (supplied as bicarbonate). Phosphate buffer could not replace the bicarbonate, indicating that the bicarbonate effect was not merely due to a buffering action.

To determine quantitatively the effect of CO_2 on anaerobic growth and the amount of glucose fermented, strain 8 was inoculated into tubes of fermentation broth containing varying amounts of sodium bicarbonate. As can be seen in figure 6, both the amount of growth and the amount of glucose fermented are functions of the amount of CO_2 available. It can also be seen that a lowering of the pH by the production of acidic end products could not have been a limiting factor for either growth or fermentation, except possibly in the tube with the highest concentration of bicarbonate. These findings indicate that CO_2 is required in substrate amounts for anaerobic growth. The data suggest also that CO_2 is required for fermentation; otherwise, the cells would be expected to dissimilate glucose even after the cessation of growth. Although a quantitative determination of the viable cells was not made, the broth was found to contain live cells 1 week after maximal turbidity had been reached.

[6] Cell walls of *Cytophaga succinicans* can be obtained by sonic disintegration of the cells. When the disrupted cells are centrifuged at high speeds, a translucent, yellow pellet is obtained which can be seen by phase contrast and electron microscopy to consist of cell walls.

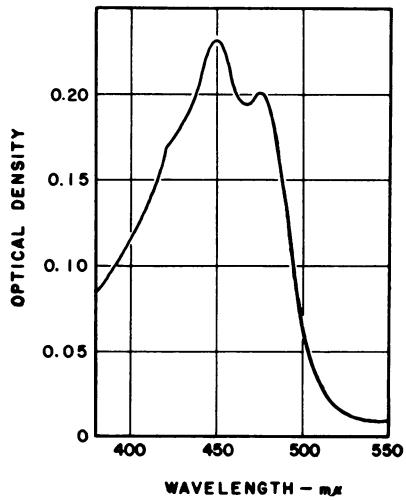

Figure 5. Absorption spectrum of the ether-extractable pigment of *Cytophaga succinicans* strain 8.

Compounds that could serve as energy sources for anaerobic growth are listed in table 2. Growth, which was determined visually, always reached a maximum within 24 hr after turbidity was first detected.

CO_2 as a requirement for fermentation. The data shown in figure 6 suggest that CO_2 is required for the fermentation of glucose by *C. succinicans*. To test this hypothesis, resting cell suspensions of each of the strains were compared with respect to their ability to ferment glucose in the presence and absence of CO_2. One series of Warburg vessels contained 60 μmoles of phosphate buffer at pH 7.1, 21 μmoles of sodium bicarbonate, and washed cells (30 mg by dry weight) in 1.8 ml in the main compartment, and 5.0 μmoles of glucose in 0.2 ml in the side arm. The gas phase was 5 per cent CO_2 in nitrogen and the temperature was 31 C. A second series of vessels was minus the bicarbonate, had KOH in the center well, and had an atmosphere of nitrogen. A second side arm on each vessel contained 0.1 ml of 5 N H_2SO_4 which was tipped in to stop the reaction at the end of the incubation period. The vessel contents were analyzed for

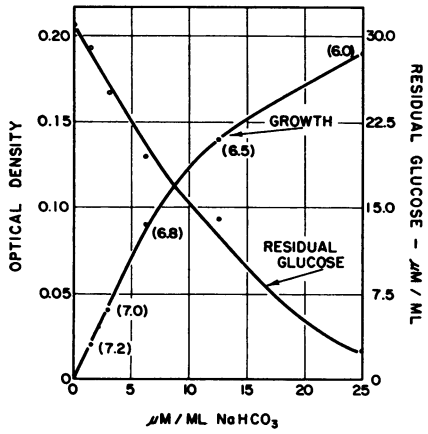

Figure 6. Effect of bicarbonate concentration on anaerobic growth and glucose utilization. Each tube contained 8 ml of a medium consisting of peptone, 0.2 per cent; yeast extract, 0.1 per cent; beef extract, 0.1 per cent; glucose, 0.5 per cent; phosphate buffer, 0.05 M, pH 7.2; and $NaHCO_3$ in the concentrations shown in the figure. The tubes were inoculated with one loopful of a 20-hr nutrient broth culture of *Cytophaga succinicans* strain 8, sealed with vaspar, and incubated at room temperature for several days until turbidity had reached a maximum in each tube. The numbers in parentheses denote the final pH in each tube.

residual glucose after removal of cells by centrifugation. Fermentation of the glucose in the vessels containing bicarbonate was found to be rapid and complete. In the vessels lacking bicarbonate, however, no utilization of the glucose could be detected. These results confirm the hypothesis that CO_2 is required for the fermentation of glucose by *C. succinicans*. The data depicted in figure 7 show that there is a net fixation of CO_2 during the fermentation of glucose by a cell suspension of *C. succinicans*.

Fermentation balance. For initial work on the identity of the end products of glucose fermentation, washed cells harvested from aerated nutrient broth were allowed to ferment glucose in the presence of bicarbonate in glass-stoppered bottles. Fermentations for quantitative analyses were carried out in Warburg vessels. Triplicate vessels contained washed cells (about 20 mg by dry weight) and 21 μmoles of $NaHCO_3$ in 1.7 ml in the main compartment, 5.0 μmoles of glucose in 0.2 ml in one side arm, and 0.1 ml of 5 N H_2SO_4 in the other side arm. The gas phase was 5 per cent CO_2 in nitrogen and the temperature was 31 C. After tipping in the substrate, readings were made until the CO_2 release due to metabolic acid production had stopped. The sulfuric acid was then tipped in to kill the cells and to release the bound CO_2. Appropriate controls were included to determine the initial bound CO_2 so that the amount of CO_2 fixed during fermentation could be calculated. The contents of the three experimental vessels were pooled and centrifuged to remove the cells, and a portion of the supernatant fluid was analyzed for residual glucose. The fermentation acids in a 3-ml aliquot of the supernatant fluid were titrated with a microburette after separation by chromatography on a cellulose column.

No residual glucose could be detected in the experimental vessels at the end of the fermentation. Gas evolution in control vessels without glucose was negligible. The only products that could be detected were succinic, acetic, and formic acids. Fermentation balances for strains 8 and 14 are shown in table 3. Although a complete fermentation balance was not determined for strain 16, the amount of CO_2 fixed during fermentation by a cell suspension was measured. It was found that 3.2 μmoles of CO_2 were consumed during the fermentation of 5.0 μmoles of glucose. Thus it would seem that strain 16

TABLE 2

Compounds fermented by Cytophaga succinicans

Substrate	Days Required for Visible Growth		
	Strain number		
	8	14	16
D-Galactose	1	1	1
Maltose	1	1	2
D-Glucose	1	2	2
D-Mannose	1	2	2
Starch	2	2	2
Lactose	38	16	6
Sucrose	—*	2	—
L-Arabinose	—	2	—
Cellobiose	—	—	2
Trehalose	—	—	2

* — = No growth.

Compounds not fermented: cellulose, D-xylose, D-fructose, D-sorbose, raffinose, melezitose, inulin, salicin, glycerol, mannitol, sorbitol, dulcitol, inositol, malate, and lactate.

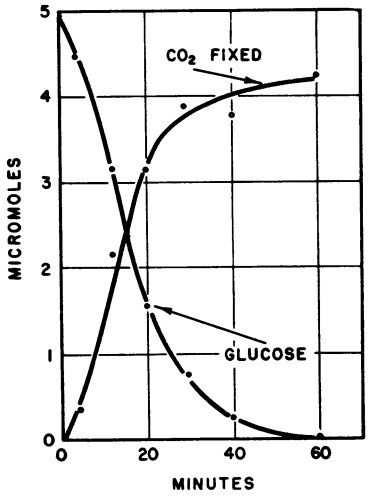

Figure 7. Fixation of CO_2 during the fermentation of glucose by a cell suspension of *Cytophaga succinicans*. Each Warburg vessel contained washed cells of *C. succinicans* strain 8 and 18 μmoles of $NaHCO_3$ in 1.6 ml in the main compartment; 5.0 μmoles of glucose in 0.2 ml in one side arm; and 0.1 ml of 5 N H_2SO_4 in the other side arm. The temperature was 31 C and the gas phase 5 per cent CO_2 in nitrogen. After tipping in the glucose, the action in the vessels was stopped at various times by tipping in the H_2SO_4. The amount of CO_2 released from the bicarbonate by this operation was used to calculate the amount of CO_2 fixed. The vessel contents were analyzed for glucose after removal of the cells by centrifugation.

ferments glucose in a manner similar to that of the other two strains.

TAXONOMY

Cytophaga succinicans has been assigned to the order Myxobacterales because of its unicellular form and its gliding type of motility on surfaces. Because of its apparent inability to form microcysts or fruiting bodies, it has been placed in the family Cytophagaceae (Breed, Murray, and Smith, 1957). In the past, all members of this family have been grouped into one genus, *Cytophaga*, because of the limited knowledge of their physiology. Such a policy, in our opinion, is a wise one and should be continued until much more information is available for assessment. The three strains described in this report, then, are considered to be members of the genus *Cytophaga*. Moreover, they are considered to belong to one species, *C. succinicans* sp. n. The main distinguishing features of this species are its CO_2 requirement for fermentation and its ability to ferment glucose plus CO_2 to succinic, acetic, and formic acids. The specific epithet *succinicans* was chosen because succinic acid is the predominant end product of the fermentation. The description is as follows:

TABLE 3

Fermentation balance for the fermentation of glucose by Cytophaga succinicans

	Strain 8	Strain 14
	μmoles	*μmoles*
Substrates:		
Glucose	5.0	5.0
CO_2	3.8	3.9
Products:		
Succinic acid	5.6	5.9
Acetic acid	4.3	3.9
Formic acid	1.8	2.1
Carbon recovery:	96.8%	99.0%
Redox balance:	0.98	1.02

Morphology: Cells are rod-shaped with round ends. Average size of young, living cells is about 0.6 by 5 μ. Cells may vary in length depending on the strain, age of the culture, and conditions of growth. The flexibility that is characteristic of the cells of some myxobacteria is not readily discernible in this species. The cells are nonflagellated but exhibit gliding motility on surfaces.

Gram reaction: Negative.

Colonies on cytophaga agar are thin, spreading, translucent, iridescent, and show a kaleidoscopic diffraction pattern when viewed by reflected light. Nonspreading variants may occur.

Pigmentation: Cells grown anaerobically are white, but cells grown aerobically are yellow-orange. The pigment is carotenoid in nature and resides primarily in the cell wall.

Facultatively anaerobic. Grows well aerobically in nutrient broth or on cytophaga agar. Anaerobic growth can be obtained if a fermentable carbohydrate and bicarbonate are provided.

Fermentable carbohydrates include D-glucose, D-galactose, D-mannose, maltose, lactose, and starch (three strains); sucrose and L-arabinose (one strain); cellobiose and trehalose (one strain).

Compounds not fermented by any of the three strains tested are cellulose, D-xylose, D-fructose, D-sorbose, raffinose, melezitose, cellobiose, inulin,

salicin, glycerol, mannitol, sorbitol, dulcitol, inositol, malate, and lactate.

Carbon dioxide is required in substrate amounts for fermentation. Glucose plus carbon dioxide is fermented to succinic, acetic, and formic acids.

Nitrate reduction is variable. One of the three strains tested reduced nitrate to nitrite.

Gelatin was liquefied slowly by each of the three strains tested.

Catalase is produced.

Optimal temperature is around 25 C; slow growth at 2 C; no growth at 37 C.

Optimal pH is around 7 to 7.5.

Source: Migratory adult salmon in the Snake River (Idaho) and water from aquaria containing species of trout and salmon.

Habitat: Fresh water.

DISCUSSION

Until comparatively recently, all known myxobacteria were obligate aerobes. The first report of a myxobacterium capable of anaerobic growth was by Stanier (1947), who described a soil form which could carry out an anaerobic oxidation by means of denitrification. The only published description of a fermentative myxobacterium is by Bachmann (1955), who described a marine species which fermented glucose with the production of propionic, succinic, acetic, and lactic acids. Borg (1948) had earlier reported isolating some fresh-water fermentative myxobacteria from young salmon inflicted with gill disease, but the nature of the fermentation was not determined and the organisms were not named. The survey described in the present report demonstrated that fermentative myxobacteria are common in certain waters, with *C. succinicans* in particular being a well established representative. It is known that other kinds of fermentative myxobacteria exist also. Borg (1948), for example, noted that some strains could grow anaerobically in tryptone broth, indicating that they fermented some compound other than a carbohydrate. Also, some anaerobic organisms have been described which have certain of the characteristics of myxobacteria, but which were not classified as such. Thus Hungate (1950), referring to *Bacteroides succinogenes*, an organism isolated from the rumen of cattle, noted that "... the cells migrate through the cellulose agar in a manner resembling the cytophagas"; *Fusobacterium girans* (*Fusocillus girans*), a nonflagellated anaerobe isolated from the human intestine and oral cavity, has been reported to exhibit swinging and flexing movements as well as gliding motility (Prevot, 1940; MacDonald, 1953; Berger, 1956).

The main distinguishing features of *C. succinicans* are its CO_2 requirement for fermentation and its ability to ferment glucose plus CO_2 to succinic, acetic, and formic acids. A number of other bacteria which yield these fermentation products from carbohydrates have been reported during the past few years. All of these bacteria, which include strains of *Bacteroides* (Hungate, 1950; Bryant and Doetsch, 1954; Hamlin and Hungate, 1956; Bryant et al., 1958), *Ruminococcus* (Sijpesteijn, 1951), *Succinimonas* (Bryant et al., 1958), and *Succinivibrio* (Bryant and Small, 1956), are obligate anaerobes which have been isolated only from the rumen of cattle and sheep. Succinate, acetate, and in some cases, formate, are the only compounds that have been detected as fermentation products from carbohydrates. In some cases, a net uptake of CO_2 was observed. Although the fermentative mechanisms in these bacteria have not been investigated, it seems likely that they would be similar to that in *C. succinicans*. The pathway of fermentation in *C. succinicans* will be dealt with in a subsequent paper (Anderson and Ordal, 1961).

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SUMMARY

Cytophaga succinicans sp. n., a facultatively anaerobic myxobacterium that occurs in fresh water, is described. The organism can grow anaerobically on a medium containing peptone, yeast extract, beef extract, and glucose, only if a substrate amount of CO_2 is provided. Both the amount of growth and the amount of glucose fermented are proportional to the amount of CO_2 available. CO_2 is also required for the fermentation of glucose by resting cell suspensions. In a typical fermentation by a cell suspension, 5.0 μmoles of glucose yielded 5.9 μmoles of succinate, 3.9 μmoles of acetate, and 2.1 μmoles of formate, with a net uptake of 3.9 μmoles of CO_2.

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