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Sheath and Cellular Irregularities of *Sphaerotilus natans* Caused by Ferric Ammonium Citrate and Glucose

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Abstract. Growth in citrate broth was turbid, rusty brown in color with some slime formation. The glucose broth had a milky white color with pronounced slime accumulation. Cells grown on the citrate medium were uniformly spaced within the sheath and ranged from 1 to 4 μ in length and 1 to 2 μ in width. The sheath was uniform in shape and ranged from 2.0 to 2.5 μ in width in young cultures. Sheath irregularities began to appear by the third day and were the greatest, 1 to 12 μ, by the fifth day when they gave the appearance of disintegrating. Cells grown on the glucose medium had clear intracellular structures and ranged from 2.5 to 6.0 μ in length, 1.0 to 2.5 μ in width with the sheath ranging from 1.5 to 3.5 μ.

Some confusion has existed in the nomenclature of *Sphaerotilus natans*. One reason for this is that *S. natans* can assume two or possibly three different physiological forms, which are classified as *S. natans, Cladothrix dichotoma,* and *Leptothrix ochracea* (8). These three organisms can give rise to similar cultures by appropriate treatment (4, 9). Deoxyribonucleic acid base composition, estimated from the buoyant density in ceasium chloride, of *S. natans* and *S. discophorus* indicate that these two organisms are closely related (7).

Identification of *S. natans* is based on its most characteristic structure, the filaments. These consist of chains of rod-shaped cells with rounded ends which are enclosed in closely-fitting sheaths (11). The sheath is slimy and optically invisible but can be demonstrated in India ink mounts (6). Various stains have been used in studying the sheath but all seem to be ineffective unless stainable material is present in the culture and is absorbed by the sheath (2).

The diameter of the cells has been reported to vary from 1.2-1.8 μ (11) and 2-3 μ (3). The differences probably are due to difficulty in differentiating between the cell and surrounding sheath. The length of the cell varies with age, habitat and nutrients. Butcher (1) reported cells ranging from 3-6 μ in length but Lackey and Wattie (6) indicated 2.5-16 μ and Hohnl (4) reported lengths of 5-11 μ.

S. natans displays two basic colony shapes when cultured on a solid medium. Fresh isolates from natural habitats normally grow with a filamentous colonial form which is compared to the R-type of colony found among some bacteria (11). With continued laboratory culturing, the R-type dissociates and an S-type develops.

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The colonies are strikingly different in that they are smooth, glistening, and often hemispherical with a regular edge. Intermediate forms between the R- and S-colony types occur. The factors that determine colony type in *Sphaerotilus* are not clear, but glucose can stimulate S-colony formation of *S. discophorus.*

Glucose and citrate are among the carbon sources that can be utilized by *S. natans* (3, 11). When *S. discophorus* and *S. natans* are grown in a medium containing glucose, they exhibit a very limited capacity to oxidize sugars, amino acids, and compounds of the tricarboxylic acid cycle (12). However, if grown in a medium deficient in glucose, they oxidize these compounds rapidly and extensively.

A review of the literature indicates that *S. natans* is a highly variable organism. Possible explanation for the discrepancies obtained by different investigators may be found in the use of different basal media, different substrate concentrations or genetic variants. The object of this study was to compare the growth and morphology of the cells and sheath of *S. natans* when glucose is substituted for ferric ammonium citrate in the medium.

Procedures

Sphaerotilus natans was isolated from a riffle area in the Yellow River at Volney, Iowa, using the method of Stokes (11). Stock cultures were maintained on a base medium consisting of 5 g peptone, 0.5 g ferric ammonium citrate, 0.2 g magnesium sulfate, 0.5 g calcium chloride, 0.01 g ferric chloride and 12.5 g agar in 1 liter of distilled water. The pH was adjusted to 6.5-7.0 with 0.1 N sodium hydroxide. The glucose medium was made by eliminating the ferric ammonium citrate from the base medium and substituting 0.5 g glucose.

Two hundred fifty ml of the base broth were put into 500 ml, 4-baffle bottom flasks and sterilized. The flasks were inoculated with agar plugs cut with a sterile #4 cork borer from 48 hour cultures grown at ambient conditions. The flasks were incubated at 35°C in a water bath shaker.

Dry weights were determined by placing the contents of the flask in a sterile blender and blending for two minutes. Two, 50 ml samples were centrifuged for 20 minutes, washed with distilled water and centrifuged a second time. The pellets were resuspended in 25 ml of distilled water and dried at 105°C for 24 hours.

Microscopic slides were made by transferring the organism to a drop of 50% India ink located at one end of the slide. The material was mixed and a thin film prepared by drawing the mixture across the slide with another slide held at a 45° angle. Slides were

air dried and stained with 1% aqueous crystal violet for 2 minutes. The stain was rinsed off and the slides air dried.

Discussion

Citrate medium changes from a clear, light yellow color to a turbid rusty brown. This color remained constant after 24 hours. The glucose medium changed from a clear, light yellow to a milky white. With increased incubation the glucose medium took on a light brown color. Differences in color of the two media probably were due to the amount of ferric oxide produced or the presence of glucose. The citrate medium had a greater potential for formation of ferric oxide, either chemically or by *S. natans*. The method of formation and deposition of ferric oxide by *S. natans* is not definitely known but appears to be enzymatic (5). On the other hand, glucose represses synthesis of many enzymes (12).

No visible flocs formed in the citrate medium. There was an even dispersion of small, suspended masses of material at all times after 24 hours. Slight slime accumulation occurred on the sides of the flasks. The glucose medium contained large amounts of white flocculent material after 24 hours. The slime characteristic was most pronounced after 24 hours and then decreased with increased incubation.

A *t*-test was used to test differences between the mean dry weights of the two cultures. The amount of growth for the first day was significantly greater in the glucose medium, Table 1. The difference between the means were not significant on days 3, 4, and 6. On days 5 and 7 the growth on the citrate medium was significantly greater.

Table 1: Mean dry weight comparisons of *S. natans* grown on citrate and glucose media ($p=.01$, $t>3.169$).

	MEAN DRY WEIGHT (mg)		
DAY	glucose	citrate	*t*-VALUE
1	26.9	17.3	5.30
2	32.3	27.2	3.76
3	30.2	31.2	.31
4	24.5	38.9	1.48
5	24.5	32.0	5.77
6	28.4	27.7	.94
7	21.2	30.0	8.54

Cells grown on the glucose medium appeared to have nonstaining vacuoles or granular material (Fig. 1). *S. natans* contains

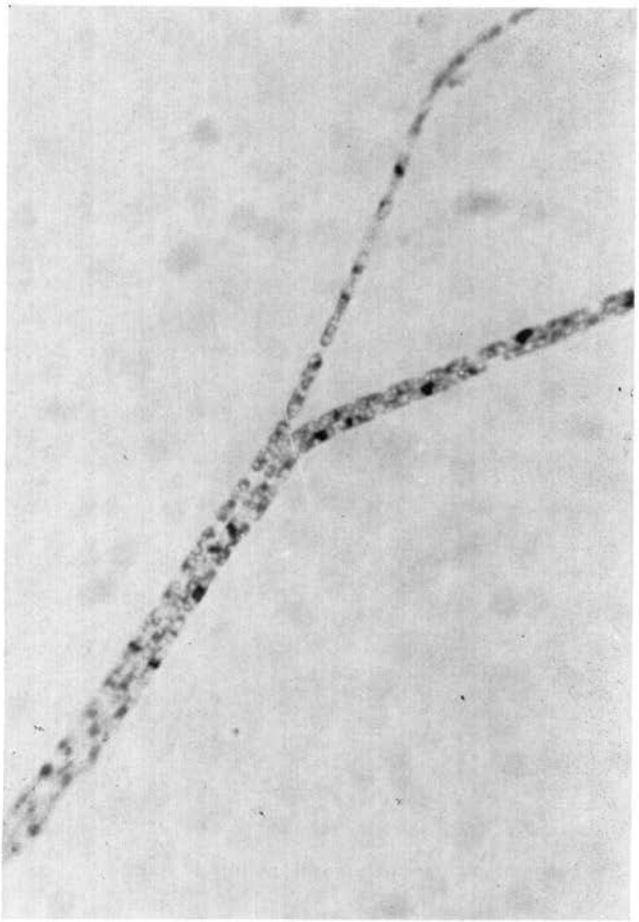

Figure 1: Glucose grown cells with vacuoles and granules.

prominent and numerous refractive bodies of various sizes. These bodies are present in young and old cultures (11) and are poly-β-hydroxybutryic acid (10). Some of the cells contained two larger, terminal nonstaining spores. Cells were difficult to distinguish in 24 hour cultures and appeared to be packed very close together and completely filled the sheath, but as the culture aged it became easier to distinguish individual cells. The cells ranged from 2.5-6.0 μ in length, depending on age, with the longest cells appearing on day three. The width of the cells ranged from 1.0-2.5 μ with the width of the sheath ranging from 1.5-3.5 μ.

Cells grown on the lactate medium were uniformly spaced with-

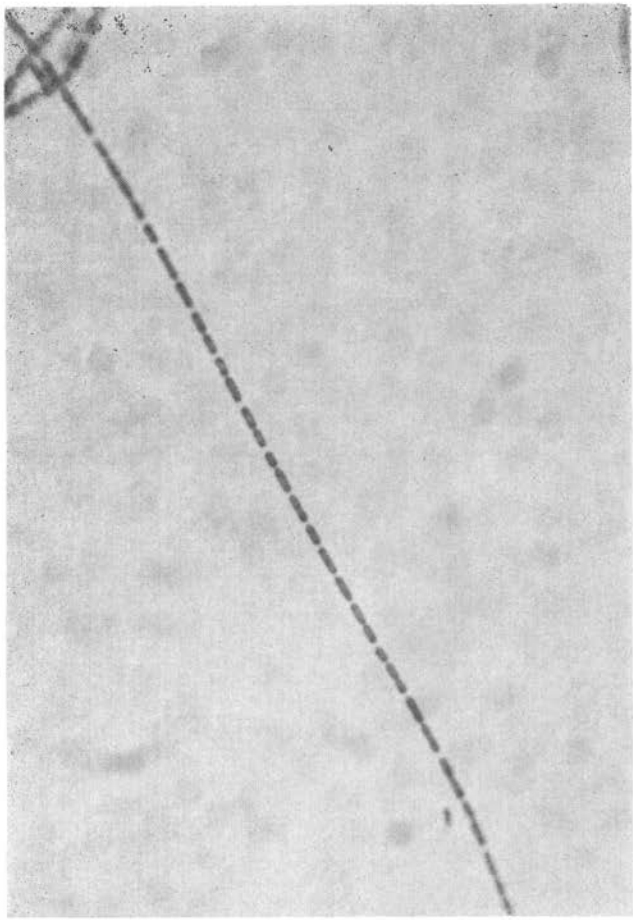

Figure 2: Ferric ammonium citrate cells uniformly stained.

in the sheath, did not develop intracellular bodies and stained evenly (Fig. 2). The cells ranged from 1-4 μ in length and 1-2 μ in width. The sheath, in young cultures, was uniform in shape and ranged from 2.0-2.5 μ in width. Sheath irregularities began to appear by day 3 and were the greatest, 1 to 12 μ, by day 5 when they gave the appearance of disintegrating. The cells within the sheath became smaller and took on a coccus-shape. Some false dichotomous branching was observed.

Large sample techniques were used to test for differences between the mean cell size produced on the two media. Differences were significant at the 0.01 level depending on the age of the culture (Table 2). The length of the glucose grown cells was

Table 2: Z-values for cell and sheath sizes obtained from growth on citrate and glucose media ($p=.01$, $Z>2.58$).

DAY	Cell LENGTH	Cell WIDTH	Sheath WIDTH
1	11.30*	8.70*	4.69*
3	9.75*	13.16*	2.44**
5	4.04*	1.01*	6.55**
7	10.80*	3.43*	1.03**

* glucose medium
** citrate medium

significantly greater at all ages than the citrate cells and the width of the glucose cells was significantly greater for days 1, 3, and 7. The sheath of the glucose cells was significantly wider for the first day, but by day five the citrate sheaths were significantly wider.

Summary

Various aspects of the growth of *S. natans* on glucose medium and citrate medium differ. Glucose caused greater cell length than citrate at all stages of growth tested. Cell width is greater with glucose in early stages (days 1 and 3) and late stages (day 7) of incubation with the width being approximately the same during the intermediate stage (day 5). The glucose medium produced greater width and abnormalities from days 3 to 7. Glucose stimulated slime formation.

Glucose stimulated greater total growth than citrate for days 1 and 2. Dry weight determinations indicated greater growth on the citrate medium as the cultures aged. Further study is needed to determine whether the citrate actually stimulated greater total growth in older cultures or if the increase is a result of chemical reactions with the medium.

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