Growth Rate of *Sphaerotilus* in a Thermally Polluted Environment

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In situ growth of *Sphaerotilus* on microscope slides immersed in a thermally polluted stream was studied. Ultraviolet radiation was used to differentiate between passive attachment of organisms from the water and growth of the organisms on the slides. Colonization of the slides in this environment took place solely by means of swarmers. A generation time of 2.3 hr was obtained at a water temperature of 18–22 °C.

*Sphaerotilus* is a widespread organism in flowing waters, especially those receiving organic pollution, and has received considerable study in recent years (4, 6). In line with our general interest in studying the growth and function of microorganisms directly in the natural environment (1–3), we have capitalized on a continuous and predictable *Sphaerotilus* bloom in a small stream near our laboratory to study the mode and rate of growth of this organism. The stream originates as a series of cold hard water springs and receives thermal and organic pollution probably primarily in the form of steam condensate from the university heating system and from the cooling water of the system manufacturing distilled water for the building housing our laboratory. At the time the present observations were made the temperature of the stream varied from 18 to 22 °C, whereas the air temperature varied from 5 to 16 °C. Except in times of runoff immediately after heavy rains, the flow rate of the stream remains fairly constant.

We made use of the fact that the organism grows attached to submerged microscopic slides and is sufficiently large and morphologically distinct to be recognized directly microscopically. Microscopic quantification is considerably more sensitive than that involving dry weight measurements usually used for study of *Sphaerotilus* growth in nature and permits a study of the early stages of bacterial colonization and growth. By use of a simple technique involving ultraviolet irradiation (1), we were able to distinguish between passive attachment to slides of organisms from the environment and growth in situ.

MATERIALS AND METHODS

The techniques for slide placement, irradiation, and microscopy have been described elsewhere (1). In quantifying *Sphaerotilus*, values were obtained for (i) the total number of filaments, (ii) the total number of individual cells present singly or in filaments, and (iii) the number of cells in each individual filament. A filament was defined arbitrarily as any unit containing two or more cells. Because many slides were heavily colonized, the organisms in one-eighth of a microscopic field (rather than the entire field) were counted at 10 evenly spaced intervals on the slide and the individual counts were summed. Values for each slide represent numbers per $9.7 \times 10^4 \mu m^2$, the total area counted.

RESULTS AND DISCUSSION

Data for slides removed after various lengths of time are shown in Fig. 1. Initially most of the *Sphaerotilus* cells are present as unicellular organisms, probably deriving from swarm cells, but as time proceeds filaments appear. On the ultraviolet-treated slides, however, only unicellular *Sphaerotilus* cells are present no matter how long the incubation. Interestingly, after an initial period of increase, the number of unicellular forms on the irradiated slides levels off. Since the unicellular forms result from passive attachment of swarm cells from the water, this suggests either that after a certain density of swarmers is reached, further attachment is inhibited or, alternatively, release of swarmers from the *Sphaerotilus* population in the stream is not continuous. If the latter were the case, it may be that swarmers are released primarily during daylight hours since the ultraviolet treated slides were removed for counting after dark. (We might note that only one slide surface was irradiated and that germicidal ultraviolet irradiation does not penetrate the glass slides. Changes resembling those
GROWTH OF *SPHAEROTILUS*

on unirradiated slides took place on the bottoms of the irradiated slides, showing that handling or other disturbances attendant on the irradiation process did not affect growth.)

In Fig. 2, we present a frequency distribution of number of cells per filament as a function of time. It can be seen that, as time progresses, the filaments become longer, undoubtedly the result of growth.

From these data, the growth rate of *Sphaerotilus* in this habitat can be calculated. The cells appearing on the slide as a result of passive attachment (estimated from the data of the ultraviolet-irradiated slides) could be deducted from the total at each time, yielding a net increase in cell number due to growth. However, during the period of exponential growth (approximately 11 to 15 hr), there was probably little passive attachment, since the cell counts on the irradiated slides after immersion for longer periods did not increase. Thus it is not necessary to use any correction factor to calculate growth rate. We estimate the doubling time over the interval of exponential growth to be 2.3 hr. This rate is considerably faster than those calculated from dry weight measurements in experimental channels reported by Phaup and Gannon (7) and Ormerod et al. (5). The rate is even faster than that reported for growth of laboratory cultures under presumably more favorable conditions (8). The optimal temperature for growth of *Sphaerotilus* has been reported to be 25 to 30 C (4), a range slightly

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**Fig. 1.** Quantitative counts of total *Sphaerotilus* cells and of filaments per microscope field (9.7 X 10^4 um^2) on glass slides immersed in the Jordan Hall branch of the Jordan River, Ind. Zero time, 12:40 AM, 22 November 1968. Solid lines, unirradiated; dashed lines, irradiated. Ultraviolet irradiation was done approximately every 90 min beginning at 9:15 AM.

**Fig. 2.** Frequency distribution of number of cells per Sphaerotilus filament after various immersion times of glass substrates.
higher than that of our stream at the time of the experiment.

The results of our study confirm the idea that *Sphaerotilus* in nature colonizes new substrates by means of unicellular swarmer cells which attach and initiate the formation of filaments. The ultraviolet irradiation technique demonstrates clearly that on the substrate we used filaments arise only as a result of the growth of unicellular swarmer cells and not by passive attachment from the environment. It does not follow, however, that, in all habitats where *Sphaerotilus* lives, colonization of new substrates will occur only by unicellular swarmer cells. In a grossly polluted habitat, where there is much drift of filamentous *Sphaerotilus* masses, attachment and subsequent growth of filaments may occur. Further, glass slides may not be especially favorable for the colonization of filaments as opposed to swarmer cells. At any rate, the simplicity of our technique is such that it may find wider application in the study of the growth of *Sphaerotilus* directly in nature.

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