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Autoradiographic Studies of [*methyl*-^{3}H]Thymidine Incorporation in a Cyanobacterium (*Microcystis wesenbergii*)-Bacterium Association and in Selected Algae and Bacteria

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The present investigation showed by means of autoradiography that the cyanobacterium *Microcystis wesenbergii* did not incorporate [^{3}H]thymidine at nanomolar concentrations, whereas its associated heterotrophic bacteria appearing in the gelatinous cover of the cyanobacterium became labeled. Several other tested cyaobacteria and algae did not incorporate [^{3}H]thymidine.

The use of [^{3}H]thymidine has lately become a versatile tool in ecological research partly as a means of estimating bacterial production (2) but also as a convenient way of labeling bacteria for use in zooplankton grazing studies (5, 9; Bern, manuscript in preparation). One of the great advantages of thymidine in this respect is its extremely low uptake rate in algae as opposed to bacteria (3, 4, 7). Bell et al. (2) made in situ estimates of thymidine incorporation to measure heterotrophic bacterial production. At times, a great number of cyanobacteria were present in the lake studied, and simultaneously, a high ^{3}H activity was found in the >3-μm size fraction. The authors stated that "this phenomenon was most likely due to *Microcystis* spp., their associated bacteria, or both." The aim of the present work was to elucidate by means of autoradiography the fate of ^{3}H-labeled thymidine in the cyanobacterium *Microcystis wesenbergii* and some associated bacteria. In addition, a number of other cyanobacterial and eucaryotic algal species were investigated both in culture and in situ.

The cultured algae were grown in test tubes with 2xL16 as a nutrient medium (6). [^{3}H]Thymidine (40 to 50 Ci mmol^{-1}; Radiochemical Centre, Amersham, England) was added to actively growing cells for between 12 and 20 h (5 nM, 0.24 μCi ml^{-1}; final concentrations). In situ experiments were done in eutrophic Lake Norrviken, situated 15 km north of Stockholm, Sweden (for a detailed description of the lake, see reference 1). Jena glass bottles (250 ml) were filled with lake water filtered through a 150-μm-pore nylon net to remove larger zooplankters. The bottles were incubated with [^{3}H]thymidine at a depth of about 0.5 m for 3 to 4 h (the same concentrations as above).

To avoid isotope losses (10) or autoradiographic artifacts (8), no chemical preservatives were used. The algae were, after incubation, filtered onto a 3-μm or greater porosity Nuclepore filter, which was rinsed in sterile nutrient medium or sterile filtered lake water. The filter, still damp, was placed upside down on a glass slide, which was previously cleaned with ethanol, dried, and covered with subbing solution (3). After a few minutes, the filter was gently pulled off, and the cell preparation was allowed to dry. Some samples containing *M. wesenbergii* were sonicated in a Rapidis disintegrator (Ultrasonics, Ltd.) at about 100 W for 1 min to help separate *M. wesenbergii* cells from their attached bacteria and thus facilitate the investigation of a possible difference in labeling between the two.

In the darkroom (equipped with a Wratten OC filter), the algal preparations were dipped in Ilford K2 emulsion (diluted 1:1; 42°C) with 1 drop of glycerine added (8). The slides were dried in a vertical position for 30 min, placed in light-tight boxes with silica gel desiccant, and then exposed for about 1 week at 4°C. Development was in Kodak D19 (diluted 1:1) for 3 min with continuous agitation, stopped in 1% acetic acid, and fixed in Kodak Unifix 30% for 8 min. The preparations were then rinsed for 30 min in tap water with a final short rinse in distilled water. All solutions were kept at 20°C.

Unlabeled controls were run to check for chemography, and some preparations were exposed to artificial light to reveal potential latent image fading. None of these phenomena occurred.

No attempt was made to count grains. Nevertheless, a great difference in silver grain density could be seen between algae and cyanobacteria on the one hand and bacteria other than cyanobacteria on the other. The latter (either free solitary or filamentous or attached to *M. wesenbergii*) were

TABLE 1. Uptake of [*methyl*-^{3}H]thymidine in various algae and bacteria as detected by autoradiography

Species	Incubation conditions	Detectable label
Green algae		
Pediastrum duplex Meyen	Laboratory culture	−
Dichtyosphaerium sp.	Laboratory culture	−
Ankistrodesmus cf. *bibraianus* (Reinsch) Korš	Laboratory culture	−
Ankistrodesmus falcatus (Corda) Ralfs	Laboratory culture	−
Chlamydomonas sp.	Laboratory culture	−
Pandorina morum (Müll.) Bory	Laboratory culture	−
Diatoms[a]	In situ	−
Cyanobacteria		
M. wesenbergii Kom.	In situ	−
Oscillatoria agardhii Gom.	In situ	−
Anabaena sp.	Laboratory culture	−
Bacteria		
Solitary, planktonic	In situ	+
Filamentous, planktonic	In situ	+
Attached to *M. wesenbergii*	In situ	+

[a] Centric diatoms dominated by *Stephanodiscus hantschii* var. *pusillus* Grun.

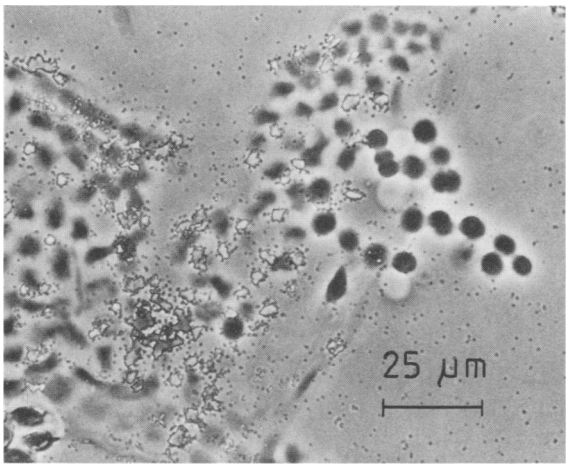

FIG. 1. Autoradiogram of a labeled colony of *M. wesenbergii*, wherein clusters of silver grains (light or dark sharp-edged, irregular spots) appeared on the gelatinous cover. *M. wesenbergii* cells squeezed out of the cover (to the right) caused no exposure of the emulsion. Single, small, black dots were background exposure. Phase-contrast illumination.

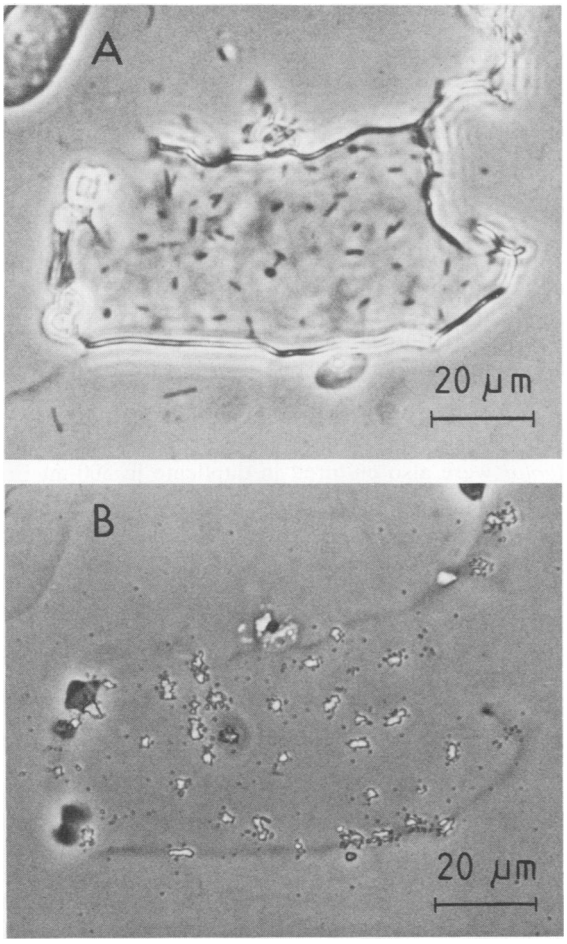

FIG. 2. (A) Fragment of a gelatinous cover with attached bacteria from a sonicated sample before application of photographic emulsion. (B) Same preparation as in (A) but with applied photographic emulsion, exposed and developed. Grain clusters (light, irregular spots) appeared over bacteria positions. Both A and B are phase contrast.

nearly always covered by a dense cluster of silver grains. Such clusters were not seen covering algal or cyanobacterial cells (Table 1 and Fig. 1 and 2).

Thus, the algae and cyanobacteria tested seemed not to incorporate detectable amounts of thymidine with short incubation times and nanomolar concentrations, whereas bacteria other than cyanobacteria did incorporate thymidine. These other bacteria are, in aerobic waters, most likely dominated by heterotrophic bacteria but might as well include nitrifiers, sulfur-oxidizing bacteria, and other specialized taxa which are potential incorporators of thymidine. They have not yet, however, been specifically studied in this respect.

The principal results obtained in this limnetic investigation were in agreement with those of earlier studies (3, 7, 9), all of which were performed in marine waters. This probable specificity of thymidine incorporation gives further support for the idea that production assessments employing [^{3}H]thymidine should at first hand measure a production of bacteria other than cyanobacteria and that zooplankton grazing experiments with [^{3}H]thymidine-labeled natural particle assemblages (9; in preparation) should mainly reflect a consumption of corresponding organisms. Finally, it can also be concluded that the potential [^{3}H]thymidine labeling of *M. wesenbergii* considered by Bell et al. (2) largely reflects incorporation by heterotrophic bacteria attached to the gelatinous cover of this cyanobacterium.

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