Electron Microscopic Study of Succession in the Periphyton Community of Lake Washington

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Abstract

Microbial succession has been observed on electron microscope grids immersed in lake water for 1, 3, 6, and 10 days. As predicted by ecological theory, the biomass, numbers, and diversity of attached microorganisms increased as succession proceeded. The diversity index of Shannon showed a marked increase from 3.1 at day 1 to 4.2 at day 3. It continued to rise at day 6 and attained the maximum value calculated on day 10 of 4.8. Bacteria were the major pioneer colonizers in this mesotrophic community. Based on these results and the results of other microbiologists who have found bacteria to be the dominant component during the pioneer stage of succession in periphyton communities of varying trophic status, we suggest that microbial heterotrophs may commonly, and perhaps always, be the major component of the early pioneer community of autogenic successions.

Introduction

According to ecological theory, an autogenic succession proceeds from a simple pioneer community having low species diversity toward an increasingly complex community with greater species diversity as it approaches the climax state [11]. Two types of autogenic succession are currently recognized based on the amount of organic material initially present in a system. *Autotrophic successions* are initiated by photoautotrophic pioneers in habitats containing low concentrations of organic matter. The growth of the photoautotrophs results in the formation of biomass and dissolved organic materials by primary productivity. This increase in organic matter, in turn, is thought to lead to the development of heterotophs and eventually the attainment of a climax state in which community respiration and production approach unity. In contrast, *heterotrophic successions* proceed in habitats containing high concentrations of organic material. The pioneers in this type of succession are microbial heterotrophs (primarily

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bacteria) whose role has been construed as one in which they deplete the concentration of organic materials before the photoautotrophic and subsequent stages of succession ensue.

Our studies were undertaken to assess the validity of these predictions about ecological succession with respect to the microbial periphyton community in freshwaters. To accomplish this we utilized the transmission electron microscope to examine grids which had been incubated in Lake Washington.

Materials and Methods

Sorption to Electron Microscope Grids. Clean glass slides were dipped into a solution of 0.25% Formvar in ethylene dichloride (Ernest F. Fullam, Schenectady, N. Y.) and allowed to air dry. The edges of a slide were scraped with a razorblade, and a square film of Formvar was floated onto tap water. Five to 10 300-mesh stainless steel microscope grids were placed on the floating film and the film and grids were inverted onto a clean glass slide. The edges of the film were taped with white Time tape according to the procedure of Hirsch and Pankratz [7]. The slides were placed in glass staining slide holders and immersed in the littoral zone of Lake Washington at a depth of 1 m on 2 July 1973. Slides were removed at 1, 3, 6, 10, and 14 days and fixed by air-drying. Grids were removed from the slides, stained with 2% phosphotungstic acid, pH 7.0, and examined in a JEM-100B electron microscope at 60 KV to determine the morphological types present at each sampling time.

Estimates of bacterial and algal biomass were made by measuring cell diameter and length and calculating volumes. Most organisms were rod-shaped so the formula for cylinders was used; however, for cocci, the formula for a sphere was used.

Diversity Index. Electron micrographs of individual sectors of grids from each sampling time were analyzed to determine the species composition of the periphyton community. Individual microcolonies were identified on the basis of the morphology of the cells that comprised them. The first 100 microcolonies encountered in transects across the grid surfaces were counted at each time interval and used to determine the diversity index by the Shannon formula [13]:

$$(H' \text{ Diversity index}) = -\sum_{i=1}^{S} n_i/N \; 3.3 \log_{10} n_i/N$$

where N is the total number of microcolonies counted (100), n_i is the number of colonies of one morphological type (each type was assumed to be a species), and S is the number of types (species) in the sample.

Results

When examined 24 hr after immersion in the lake the grids were sparsely colonized and organisms which were attached were unevenly distributed. Some sectors were entirely devoid of microbes. Of the five grids examined at day 1, the sector shown in Fig. 1 was the most densely

Fig. 1. Most densely populated grid sector located after incubation for 1 day in Lake Washington. The predominant organism is a coccobacillus. Note also that two cells of a *Caulobacter* are present. Bar represents 10 μm.

populated. The size and shape of organisms at this early period of colonization suggested that the vast majority of the pioneer community were unicellular bacteria. What we have interpreted as a microcolony of a coccobacillus is shown in this sector. Though the cells of this form are not closely spaced and other types of microbes (i.e., *Caulobacter*) are present, there is considerable evidence that this coccobacillus comprises a clone. First, all the cells are of the same morphological type, and second, this morphological type was not found on contiguous sectors of the grid. Considering the uneven distribution of cells on the grid surface, it is highly improbable that the cells of this type coincidentally attached at this same location. Thus, we conclude they most likely have arisen from a single mother cell.

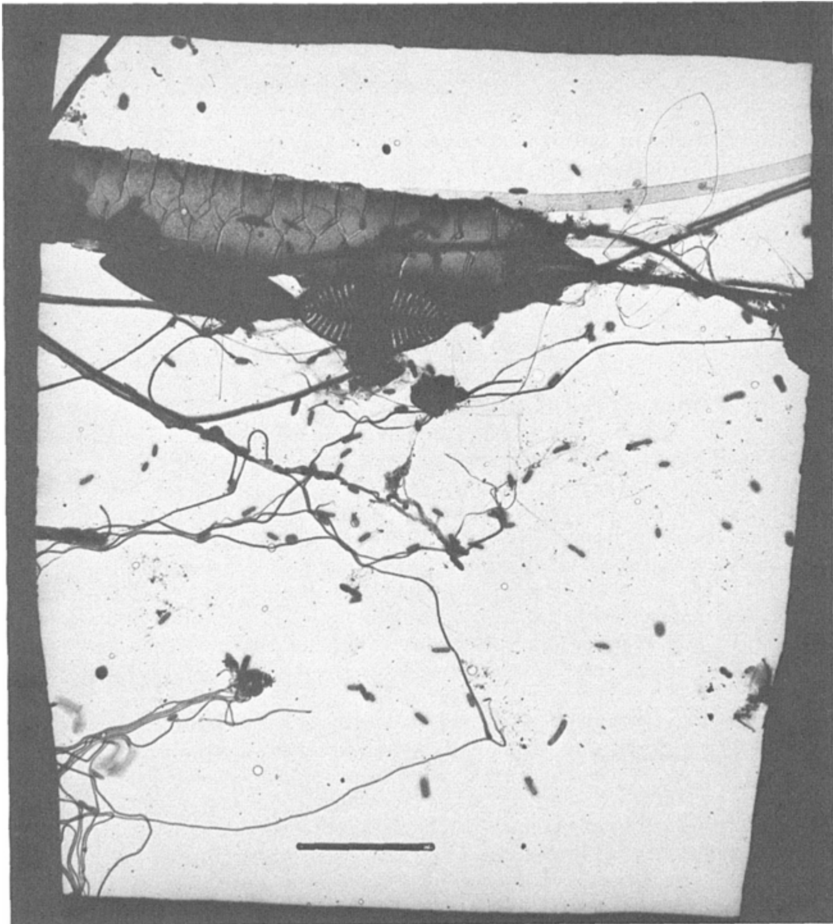

Fig. 2. Typical grid sector at day 10. Note the diatom and diffuse bacterial microcolony. Bar represents 10 μm.

In contrast to the grids incubated for 1 day, those incubated for 10 days were more densely populated and contained a greater variety of microorganisms including diatoms and filamentous algae (Figs. 2 and 3). Even at this time, however, there were entire sectors that were uncolonized and the distribution of organisms was uneven. Note that the two typical sectors shown contain different types of microbes. One portion of one grid contained a large microcolony of fusiform caulobacters which dominated one sector and extended into all adjoining sectors (Fig. 4). The total number of cells in this microcolony could not be accurately censused, but on the illustrated sector

Fig. 3. Another grid sector at day 10. Several types of rod-shaped bacteria can be differentiated. Compare with Fig. 2. Bar represents 10 μm.

alone there are in excess of 100 cells of this organism compared to 25 cells of other species.

Although many microorganisms attached to the grids, 8 predominant morphological types were located on the grids during the course of the investigation (Fig. 5). Four types including a coccobacillus (Type 1), a subvibrioid *Caulobacter* (Type 2), an encrusted rod (Type 3), and a wide rod (Type 4) were the most numerous organisms at day 1 (Table 1). Though the proportion of coccobacillus and *Caulobacter* microcolonies were approximately the same, there were more cells in the microcolonies of the

Fig. 4. Large microcolony of fusiform caulobacters found in another sector at day 10. Again note how diffuse the microcolony is. Bar represents 10 μm.

coccobacillus at this time. The calculated generation times for these two organisms are about the same, so this large difference in microcolony size cannot be explained by a difference in growth rate. A likely explanation is that many of the motile progeny of *Caulobacter* dispersed from the grids thereby reducing colony size.

At three days the percentage of Types 1, 2, and 4 had decreased and Type 3 was no longer detected. The most abundant organism was a rod (Type 5) which was not found at day 1. Type 5 remained dominant at 6 days while the percentages of Types 1 and 2 decreased further and both Types 3 and 4 were absent. Type 6, a fusiform caulobacter, was first detected in low numbers on day 6. At 10 days a large number of species were present with no one particular organism dominating. Two new rods were present at this

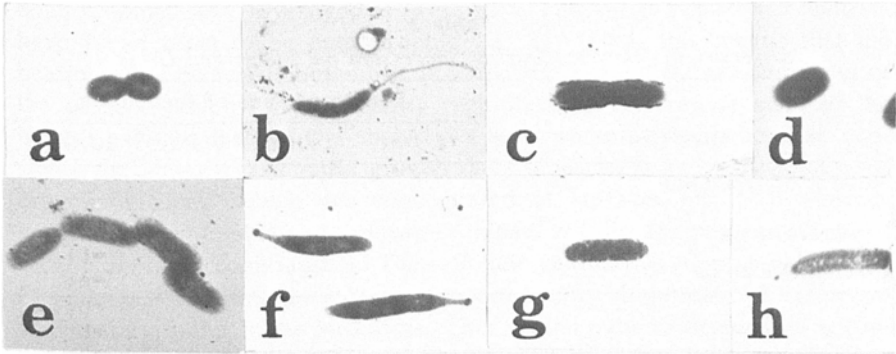

Fig. 5. The eight predominant morphological types of bacteria detected on the grid throughout the course of the investigation. (A) Type 1, (b) Type 2, (c) Type 3, (d) Type 4, (e) Type 5, (f) Type 6, (g) Type 7, (h) Type 8.

time: Type 7, with a wrinkled surface; and Type 8, a long flexible microbe. Other important organisms at this time were Types 2, 5, and 6.

Electron micrographs of many sectors were analyzed to determine the species composition of the attached organisms at each sampling time. The first 100 microcolonies encountered on the 1, 3, 6, and 10 day grids were categorized according to organismal morphology (the microcolonies in the 14-day samples were too closely spaced to permit accurate identification). The total number of species encountered in the 100 microcolonies examined increased throughout the 10-day period from the low of 16 at day 1 to a high of 38 at day 10 (Table 2). This in itself suggests that the species diversity is increasing with increasing incubation time, but an actual calculation of the diversity index of Shannon was undertaken so that species evenness as well

Table 1

Succession of Predominant Microbes on Submerged Grids

Morphological type	% Total microcolonies			
	Day 1	Day 3	Day 6	Day 10
Type 1	26	13	6	—[1]
Type 2	24	8	5	3
Type 3	11	—	—	—
Type 4	10	9	—	—
Type 5	—	20	18	5
Type 6	—	—	2	4
Type 7	—	—	—	9
Type 8	—	—	—	6

[1]not encountered in the 100 microcolonies examined (less than 1 microcolony per 100).

Table 2

Diversity of Microbes During Succession on Submerged Grids

Diversity	Length of time of incubation			
	1 Day	3 Days	6 Days	10 Days
Number of types/100 microcolonies	16	33	35	38
Diversity index (H')	3.1	4.2	4.4	4.8

as heterogeneity could be considered [8]. Using this formula we discovered that the diversity did indeed increase with incubation time (Table 2).

Algae and cyanobacteria, though present at day 1, did not appear in significant numbers relative to the bacteria until day 3 (Table 3). The predominant one initially found on the grids was *Lyngbya limnetica*, an ensheathed filamentous cyanobacterium. At day 1 the biomass of the bacteria exceeded that of the algae and cyanobacteria. By day 3, however, algal and cyanobacterial biomass surpassed that of the bacteria, and the relative biomass estimate at day 6 was similar.

Table 3

Proportion of Bacteria to Algae in Terms of Numbers and Biomass during the Initial Stages of Succession

	Day 1	Day 3	Day 6
Numbers of bacteria/algae	58.5	9.7	5.5
Biomass of bacteria/algae	6.07	0.23	0.31

Discussion

The results of our investigation are similar to the findings of other microbiologists who have studied the initial stages of succession in periphyton communities. Both in freshwater and marine habitats of various trophic states, bacteria have been found to be numerically the dominant pioneer colonizers of the periphyton [6, 10, 15]. Furthermore, in our own studies we have found that not only are there greater numbers of bacteria, but their biomass is also greater than that of the algae during the early stage of colonization. Moreover, considering the greater surface area: volume ratios of the small bacteria observed in the Lake Washington periphyton, it seems probable that the bacterial:algal biomass relationship greatly underestimates the relative bacterial activity. It seems likely that most of the periphytic bacteria are heterotrophic in that those which can be identified directly, namely *Caulobacter* spp. and the fusiform caulobacter, are known to be

heterotrophic, and investigators who have cultivated periphytic bacteria have found them to be heterotrophic [2, 3]. If so, this means that the heterotrophic bacterial community is *a* major if not *the* major component of the pioneer periphyton community regardless of the trophic state of the natural habitat. Perhaps this should not seem so surprising when one considers the ubiquity and rapid growth rates of bacteria and the ubiquity of organic nutrients which are concentrated at surfaces [9, 16]. Indeed, heterotrophic microbes may play a dominant role in the pioneer stages of succession in all communities. The rationale behind this view is as follows: Organic material and microbial heterotrophs are ubiquitous wherever organisms are found in the biosphere. Thus, when either a primary or secondary succession is initiated rapid dispersal of microbes from neighboring communities will colonize the habitat very quickly [5]. Heterotrophic bacteria in aquatic habitats and heterotrophic bacteria and fungi in terrestrial habitats would utilize preexisting organic material [14], resulting in their domination of the pioneer stage. If heterotrophic microbes comprise the dominant pioneer community in successions then, not only is there a need to reevaluate current models of succession, but there would no longer be a basis for distinguishing between heterotrophic and autotrophic succession. The reasons heterotrophs may have been overlooked in "autotrophic" successsions are because of their small size and the technical difficulty of detecting their activities during the initial stages of succession.

Though there is strong evidence that microbial heterotrophs play a dominant role in the pioneer stage of succession it is not clear that they are required for succeeding stages or are there merely because they are ideally suited for rapid colonization. In laboratory microcosms, Gorden *et al.* [4] conclude that the bacteria which dominate in the pioneer stage provide growth factors and possibly nitrogenous nutrients for the primary producer that succeeds them. Further experiments will be needed in open systems to differentiate between these two possibilities.

In other respects the results of our studies follow the predictions of the ecological succession model by Odum [11]. The biomass and diversity index of the community continued to increase with time of incubation as would be expected as community maturation proceeds. These data are in agreement with those of Marshall *et al.* [9] in suggesting that many of the microorganisms in the planktonic habitat are able to adsorb onto grid surfaces despite the fact that most do not appear to have specialized holdfast structures.

Two assumptions were necessary to justify use of the Shannon formula for determining the diversity index [12]. One was that the distribution of organisms on the grid surface was random and not clumped or patchy. At the time of insertion of the grids into the habitat we would, *a priori*, expect

that the attachment to the grids would be random unless large clumps of detritus carrying bacteria were attaching to the grids. There was little evidence of this when the grids were examined following 24 hr incubation. After a period of incubation one might expect that patchiness would occur due to reproduction of attached organisms resulting in the formation of microcolonies. Such was the case, but this patchiness was taken into account by determining the diversity index for *clones* (including single-cell microcolonies) rather than *individuals*. The other assumption was that the total number of species on the grid was known at each time interval. The total number of species attached to the grids is impossible to determine. Thus, the absolute values for the diversity indices determined in this study may be in error. Though this may be true, it is doubtful that the values determined relative to one another would be affected. For example, if we assume that there were twice as many species at each interval than actually found in the 100 microcolonies counted and apply Basharin's estimator formula [1, 12]

$$E(H') = H' - (s-1)/2N$$

the new estimates of diversity for each time interval are 2.97, 3.90, 4.03, and 4.46 for the 1, 3, 6, and 10 day intervals, respectively. Therefore, the pattern of values relative to one another is unaffected.

The electron microscope serves as an excellent tool for distinguishing bacteria by this procedure. Accordingly, over 20 different types of rods were differentiated at one time interval. Clearly if there were some means of relating those that can be grown in the laboratory with those present on the grid, it would be possible to follow changes in species composition during succession.

We cannot explain why some organisms found initially on the grids are not found later, or conversely why others found later are not found initially. The simplest interpretation is that, as the autogenic succession proceeds, some of the pioneer colonizers (e.g., Types 1 and 4) are not able to compete in the changed environment whereas others can (Types 5–8). Though this interpretation might be correct, it is also plausible that seasonal succession is proceeding in the planktonic habitat of the lake and accounting for the changes. Thus, Types 1 and 4 may have been succeeded in the planktonic habitat due to other changes unrelated to the autogenic succession on the grid surface. Such changes in seasonal succession do not appear to have been very significant during this time interval as our periodic monitoring of temperature, oxygen concentration, and total viable counts of heterotrophic bacteria and caulobacters indicated that conditions did not vary significantly. Also the fact that the total percentage of caulobacters in the planktonic habitat remained constant while that in the periphytic com-

munity decreased with time suggests that the succession on the grid surface was more pronounced than in the plankton over this time period. Nonetheless, it would be advisable to control this aspect in future investigations by inserting new slides periodically to determine the rate at which seasonal succession is affecting the autogenic succession on the grids.

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