APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1985, p. 238–241
0099-2240/85/010238-04$02.00/0

Vol. 49, No. 1

Response of Bacteria Isolated from a Pristine Prairie Stream to Concentration and Source of Soluble Organic Carbon

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Received 19 April 1984/Accepted 2 October 1984

Responses of native aquatic bacteria to source and concentration of dissolved organic carbon was observed by using gradient plates. Dissolved organic carbon of prairie (grasses) or gallery forest (bur oak) origin was used as the carbon source in these observations. Bacteria isolated from sediments in the grassland reaches of a prairie stream showed growth only on grass leachates. Bacteria isolated from the gallery forest reaches of the same stream were able to grow on plates made from either source of leachate. The differing quality, quantity, and rate of supply of these dissolved organic carbon sources should select for variation in the bacterial assemblages.

The natural import of organic matter from riparian vegetation into streams and its subsequent decomposition by the microbiota are key processes in stream ecosystems (2, 4, 6). The organic matter provides a structural habitat for stream biota, and the combination of organic matter and the microbiota involved in its decomposition serves as a food resource for the stream fauna (4, 9). Organic compounds dissolved from both living and dead plants represent a significant fraction of the total organic import (3). Dissolved organic carbon (DOC) compounds vary widely in quality and are the most mobile and the most readily available to the microbiota. Microbial metabolism is thought to control the fate of DOC (1, 7, 8). Conversely, the nature of the organic substrates influence bacterial metabolism and other physiological aspects including the selection of mutants.

Streams in tallgrass prairie flow through distinct zones of riparian vegetation. Headwaters of tallgrass prairie watersheds are dominated by grasses. Grasses, shrubs, and small trees dominate the midreaches, and still lower reaches flow through a gallery forest dominated by oak trees (3).

Microbial uptake of the most biodegradable organic compounds in a vegetational zone should increase the relative concentration of refractory compounds being transported downstream (8). Such dissolved organic compounds may be refractory only to the local microbiota upstream, whereas they might serve as the carbon resource of the downstream microbiota. The combination of refractory compounds imported from upstream and leachates imported from local riparian vegetation yields a more diverse array of dissolved organic substrates that might interact to determine the assemblage of bacterial strains along the watercourse, the ability of native strains to use the imported substrates, or both.

The purpose of this study was to examine hypotheses that bacterial assemblages in the stream change along the watercourse and that these bacteria vary in their ability to grow on dissolved organic substrates from various terrestrial sources. This was done by evaluating the growth of bacteria from headwater reaches of a prairie stream compared with growth of bacteria from the lower reaches of the same stream when they were provided with the various dissolved organic substrates from the different riparian vegetation.

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Field observations and collections were made in the 1,060-hectare Kings Creek watershed on the Konza Prairie Research Natural Area. This tract of native bluestem prairie was purchased by The Nature Conservancy in 1977 and leased to Kansas State University for ecological research. The watershed was added to the network of benchmark watersheds of the U.S. Geological Survey in 1979. Collection sites for materials in this study were located in the grassland head waters (site 1), a grass-shrub riparian zone (site 2), and the gallery forest (sites 3 and 4) (Fig. 1).

Leachates used in these experiments were prepared from preabscission bur oak leaves collected just before leaf fall or from standing dead grass. Plant material was air dried, and 5 g of leaf material was leached in 500 ml of distilled water for 24 h. The resulting leachate was filtered (pore size, 0.2 μm) to remove particulate matter and bacteria. Bacto-Agar (15 g/liter; Difco Laboratories, Detroit, Mich.) was added to prepare pour plates. Water collected at the field sites in preashed bottles also was filtered to remove particulates and bacteria, agar was added, and pour plates were prepared.

Experiments to determine the possible toxicity of leachates were performed with pour plates of minimal salts-glucose medium (5) supplemented with 2 g of nutrient broth (Difco) per liter which were then inoculated by spreading. When colony growth had just begun, wells were cut into this media with a sterilized cork borer, and medium from bur oak leachate at various concentrations was placed in the wells.

DOC concentrations of leachates and stream water were measured with a Beckman 915-B Total Organic Carbon Analyzer (Beckman Instruments, Inc., Irvine, Calif.). Humic materials were estimated by passing 50 ml of leachate, which had been diluted to stream concentrations of DOC (ca. 25 mg/liter), through an XAD resin (Rohm and Haas, Philadelphia, Pa.) column to remove the humic material. The adsorbed material was removed from the column and analyzed in the Beckman 915-B. The results are expressed as percent humics (13).

Bacterial isolates and inocula were collected from four sites along the watercourse. Initial observations were made by spreading each plate with 10 μl of ambient stream water. All counts of bacteria were viable cell counts.

Gradient plates (12) were made from minimal salts medium poured over a wedge of leachate medium from bur oak or big bluestem grass. The DOC concentration on the richest

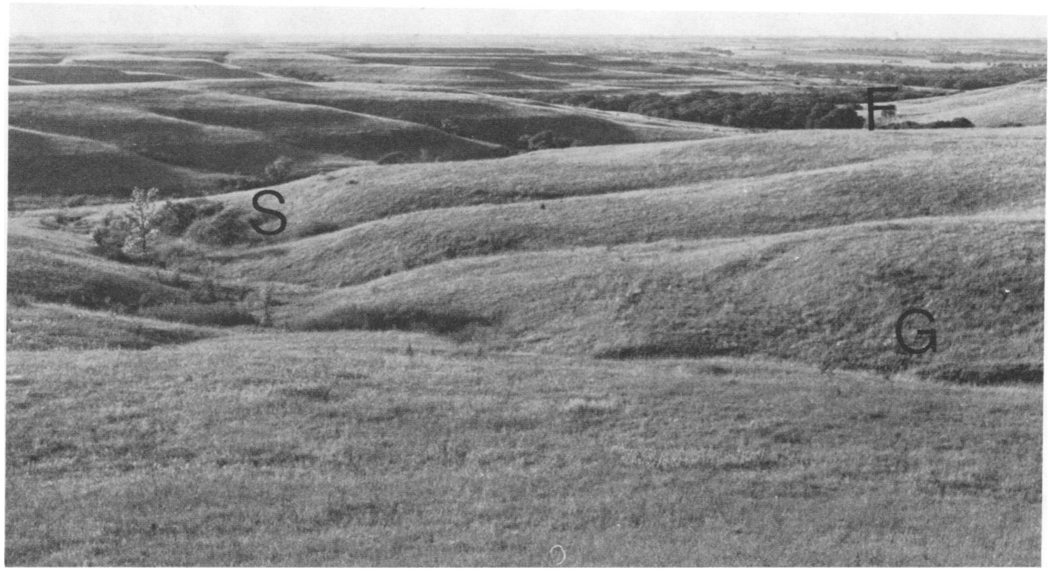

FIG. 1. Watershed in the tallgrass prairie. Note the dominance of grasses in the headwaters and the abrupt transition to gallery forest bordering the lower channels. Relative positions of sample sites are indicated by G (grassland zone), S (prairie-shrub zone), and F (gallery forest).

side of the plates (400 mg of C per liter) was higher by more than 16-fold than the highest stream concentration. The concentration from watershed sources was near 0 on the side of the plate opposite the leachate wedge. These plates were streaked with bacteria isolated from sediments and stream water collected at prairie, prairie-shrub, or gallery forest sites. All incubations were carried out at 20°C.

Initial observations on the differences in the growth of natural assemblages of bacteria on substrates from different sources were made on plates containing leachate from bur oak leaves and on plates made from ambient stream water.

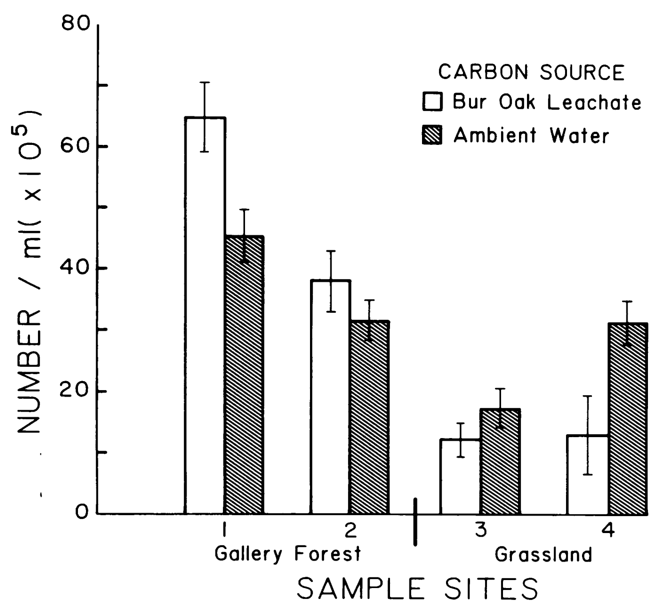

FIG. 2. Differential response of natural assemblages of bacteria from raw water samples to media prepared from ambient water and from soluble organic substrate leached from the leaves of bur oak (*Quercus macrocarpa*), a common gallery forest tree. The bars represent standard errors of the mean.

The highest number of viable cells was present in the inoculum from the gallery forest site (Fig. 2). The numbers of colonies were lower in inocula from sites higher in the watershed where grasses dominate the vegetation. The highest total number of viable cells in the inoculum from gallery forest stream was observed when cells were grown on the bur oak leachate. The lowest total number of viable cells in the inoculum from the grassland reach of the stream was observed when cells were grown on the bur oak leachate. Thus, at least some bacteria in the gallery forest section of stream grew better on carbon derived from gallery forest vegetation (bur oak leachate) than they did on ambient carbon sources in the stream water. Fewer bacterial cells

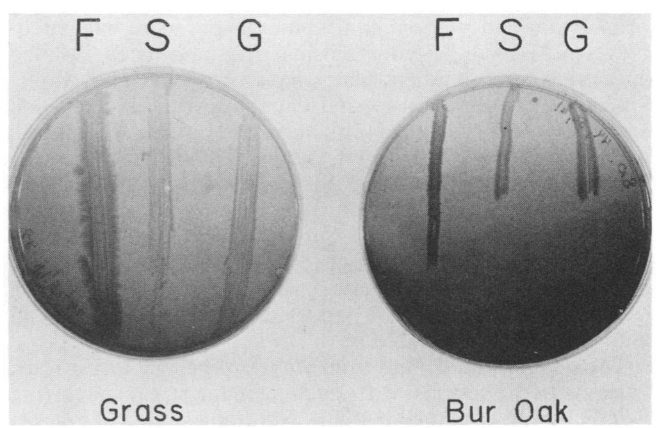

FIG. 3. Gradient plates made from minimal salts medium poured over a wedge of leachate medium from bur oak leaves (right plate) or big bluestem, a C4 grass (left plate). The DOC concentration on the richest side of the plates (bottom of photo) was higher by more than 16-fold (400 mg of C per liter) than the highest stream concentration. The concentration from watershed sources was near 0 on the side of the plate opposite the leachate wedge. The bacterial streaks are from the same isolates on both plates and were collected from (left to right) the gallery forest (F), the prairie-shrub zone (S), and the grassland zone (G).

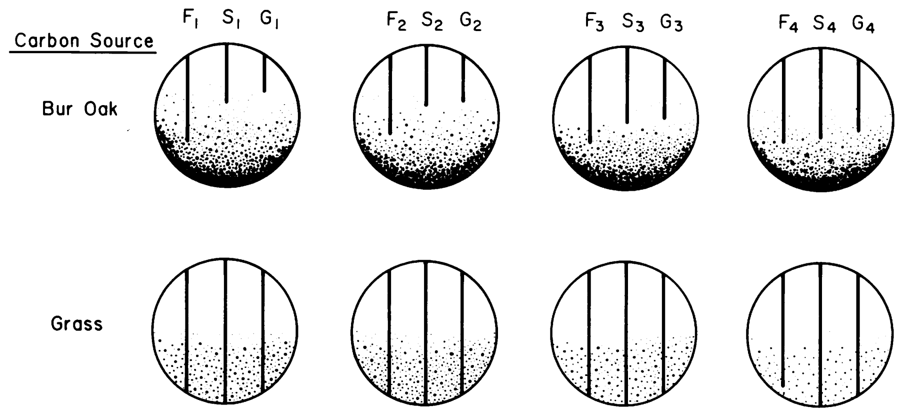

FIG. 4. Comparison of growth response of different bacterial isolates from the three vegetational zones to bur oak and grass leachate wedges. From each site, gallery forest stream (F), prairie-shrub stream (S), and grassland stream (G), four bacterial types, determined to be different by colony morphology, texture, and color, were isolated, purified, and streaked to gradient plates. Each isolate is designated by the site of isolation and isolate number (e.g., F1, F2, F3, and F4). The substrate from which the plates were made is the same as shown in Fig. 3.

from grassland stream inocula grew on the bur oak leachate than grew on the ambient stream water.

The inhibition of growth of the bacteria from the grassland stream suggested an inadequate substrate, toxic substances, or both. The results of the toxicity experiments showed that diffusion from the wells in which bur oak leachate medium had been placed inhibited some cells around the wells, and therefore the bur oak leachate was toxic to some isolates and not toxic to others.

The responses of streaks of pure cultures of bacteria, isolated from stream water from grassland, prairie shrub, and gallery forest vegetation zones, to various substrates on gradient plates were clear and dramatic (Fig. 3 and 4). Only bacteria isolated from the gallery forest stream sites grew into the region of the plate containing high levels of leachate from bur oak leaves. The DOC leached from grasses supports both grassland and gallery forest isolates, and growth of the streaks on grass leachate was often luxurious. Not all kinds of bacteria from these sites have been evaluated; only a few of the more common ones, as determined by frequency of isolation and predominant colony type, were evaluated. Nevertheless, all microorganisms that have been isolated and streaked on gradient plates have responded this way.

Leachates from grasses and oaks differ in concentration and quality of dissolved organic matter. Oak leachate is higher in dissolved humics (35 versus 27%) (J. V. McArthur, Ph.D. dissertation, Kansas State University, Manhattan, 1984). Bur oak leachate also has a lower acidity than leachates from grasses, and bur oak leachate differs in the concentration of inorganic nutrients (J. V. McArthur, Ph.D. dissertation). These leachates were the only two carbon sources used for the work reported here, whereas DOC in nature is much more diverse.

The quality of leachate from leaves of gallery forest trees varies with the species, with leaching in the stream occurring mainly during autumnal leaf fall. Natural leaching of grasses in the headwaters does not occur with the same autumnal magnitude of deciduous trees because most of the grass biomass stays in place throughout winter. Even so, the quality of leachates from standing dead grasses during spring snow melt will likely be different from actively growing grasses leached during a summer thunderstorm. Floodplain litter, in various stages of decomposition, offers further potential for DOC diversity. Often imports typically occur in pulses, some of which are predictable. The rapid unidirectional transport of the mobile carbon resources (10) in streams, especially as it occurs in pulses, is a strong selective agent for strains of bacteria most efficient in taking up DOC at high rates. Figures 3 and 4 are evidence in support of selection but are far from sufficient. Natural selection should favor microbes that can use high concentrations of DOC at maximal rates; otherwise, DOC is exported downstream, especially at high rates of discharge when DOC concentrations are highest (3).

Since microbial species are characterized, in part, by their specific use of various organic compounds (11), change in types of compounds required for growth is evidence of enzyme induction or mutants; that is, the change in response may be physiological, genetic, or both.

The differential use of DOC compounds by natural aquatic bacterial species is related to their exposure to these compounds in nature. The growth of streaks into the portions of the plates with high concentrations of leachate confirmed that these strains could use or tolerate high concentrations. The most fastidious types, and fewer kinds of them, should occur in the upstream reaches where the diversity of substrates is expected to be lowest, despite its apparent high quality (Fig. 2 gives evidence for lower population density but no indication of diversity). Since the distribution of DOC compounds changes downstream (14) and since a shift in bacterial metabolic capabilities seems to be driven by that change, metabolically flexible strains, and more kinds of them, should be found in the gallery forest where the diversity of organic compounds is expected to be highest. Our results generally support these theoretical considerations.

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