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FLUCTUATIONS OF SOME PHYSIOLOGICAL CHARACTERISTICS OF AN AQUATIC BACTERIAL COMMUNITY

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ABSTRACT

The percentages of the surface water, heterotrophic bacterial community of Reelfoot Lake which were oxidase positive, could hydrolyze starch, could hydrolyze gelatin, could reduce nitrate, could ferment glucose and could ferment sucrose were determined periodically during a year period at two sampling stations located approximately 6.5 km apart. The percentages of the community characterized by each of these physiological capabilities fluctuated widely during the study, suggesting that the species composition also fluctuated dynamically. Oxidase positivity, glucose hydrolysis, and starch hydrolysis fluctuated similarly at the two stations with a seasonal pattern. It was concluded that these physiological traits were selected by general environmental factors exerting influences on both stations. In contrast, nitrate reduction, sucrose fermentation, and glucose fermentation fluctuated sporadically and independently at the two sampling stations.

INTRODUCTION

Studies of the population dynamics of aquatic bacterial communities are limited because of the difficulty of routinely identifying the diversity of species which are present under natural conditions. Consequently, most aquatic investigations have dealt with either the total numbers or the biomass of bacteria as a function of time (Potaenki, 1968; Abdirov, et al., 1968). In spite of the limitation on taxonomically-oriented bacterial ecology, functionally-oriented investigations are feasible because physiological characterization of bacterial strains is a fundamental technique in microbiology. Although functionally-oriented studies can not be a substitute for taxonomically-oriented investigations, these two approaches can be adjuncts to each other. The data gathered from functionally-oriented studies should provide a better understanding of the composite physiological capabilities of a bacterial community, or segments of it; of the temporal fluctuation of the bacterial populations defined by physiological capabilities; and of the environmental factors which select for or against specific physiological components of the bacterial community.

The objective of the investigation reported in this paper was to determine the seasonal fluctuations of five bacterial physiological traits: nitrate reduction, oxidase positivity, starch hydrolysis, gelatin hydrolysis, and anaerobic fermentation of glucose and of sucrose. The seasonal fluctuations of bacteria with these characteristics were approximated by determining monthly the percentages of the isolates from a defined segment of the bacterial community which were oxidase positive, could hydrolyze starch, could hydrolyze gelatin, could ferment glucose, could ferment sucrose, and could reduce nitrate.

This study was limited to the heterotrophic, aerobic bacteria isolated from the surface water of Reelfoot Lake, Tennessee on a complex medium incubated at 30 C. Reelfoot Lake is a shallow, non-thermally stratified lake located in Northwest Tennessee. The lake is approximately 29 km long and 6.5 km wide and is divided into two major open water basins by encroaching peninsulas of riparian swamp forest.

MATERIAL AND METHODS

Two widely separated sampling stations were selected, one in each of the major basins of the lake. Station I was located on the southern basin of the lake at the end of a 6m pier at Samburg, Tennessee, while collection Station II was located on the northern basin approximately 6.5 km from Station I at the end of a 9m pier at Gray's Camp Resort.

Water samples were collected aseptically from both stations at intervals of approximately one month between June 28, 1968 and April 25, 1969. Samples were kept packed in ice until they were diluted and placed on agar medium. All inoculations were completed within two hours of collection.

Colonies were cultivated by spreading diluted water samples on the surface of sterile Trypticase Soy Agar in Petri dishes and by incubating this medium for 72 h at 30 C. Dilutions of 10^{-1} to 10^{-2} were made in sterile saline solution, and ten plates were prepared from each dilution. Both total and differential colony counts were made from those plates containing between 75 and 150 colonies. Differential counts comprised the number of each different type of colony on a plate of medium. Differential counts were made using a binocular microscope to distinguish colony characteristics such as texture, pigmentation, and type of edge. A representative of each colony type was isolated and maintained on Trypticase Soy Agar slants. Each isolate then was tested to determine if it was oxidase positive, could hydrolyze gelatin, could hydrolyze starch, could ferment glucose anaerobically, could ferment sucrose anaerobically, and could reduce nitrate. The percentages of the community which was positive for each characteristic was estimated from the differential counts and the data from the physiological tests.

Gelatin hydrolysis was detected by a modification of the Frazier method (Frazier, 1926). The surface of Trypticase Soy Agar to which gelatin was added at 5 g per liter was inoculated in Petri dishes and subsequently incubated at 30 C for 72 h. The unhydrolyzed gelatin was precipitated with a saturated aqueous solution of ammonium sulfate containing 1.5 percent (wt/vol) mercuric chloride. A clear zone of 1.0 mm or more surrounding the growth was recorded as positive for gelatin digestion.

Starch hydrolysis was detected by inoculating sterile Difco starch agar in Petri dishes with the test organism, incubating the inoculated medium at 30 C for 72 h, and subsequently covering the surface of the medium with Gram's iodine solution for three minutes. Positive results were detected as a clear zone of 1.0 mm or more surrounding the growth.

The oxidase test was performed by the method described by Stanier et al. (1966) using a one percent aqueous solution (wt/vol) of N, N′-dimethyl-p-phenylenediamine. An isolate was recorded as positive if it turned dark red within 30 s.

Anaerobic fermentation of carbohydrates was determined by inoculating each isolate into a Durham fermentation tube containing a Difco phenol red carbohydrate broth and by incubating the inoculated medium at 30 C for 24 h. Only tubes which were distinctly yellow were recorded as positive.

The test for nitrate reduction was performed by inoculating each organism into Trypticase Soy Broth which was supplemented with KNO_3 at a rate of one percent (wt/vol), incubating the inoculated medium at 30 C for 48 h, and then testing for nitrite by the method recommended by the Society of American Bacteriologist Committee on Bacteriological Technic (1957). All tubes which were negative for nitrite during the initial test subsequently were tested for the presence of nitrate by adding a small amount of zinc. Those tubes which at the end of incubation contained nitrite or which contained no nitrate, as determined by the zinc test, were recorded as positive.

Results

From June 1968 through April 1969, twelve collections were analyzed from each of the two stations. Collection dates, surface water temperatures, the number of isolates obtained from each sample, and the precipitation during the 72 h preceding the collection are presented in table 1. The water temperature fluctuated widely during the study from a high of 34 C to a low of 1 C. The number of isolates among the several collections and between samples from each station during any one collection varied considerably. The amount of precipitation prior to each collection had no predictable effect on the number of isolates obtained. There was a total of 129 strains isolated from Station I and 121 strains isolated from Station II.

TABLE 1: Sampling dates, water temperature, precipitation during the 72 hour prior to sampling, and the number of bacterial isolates obtained from each sample at two stations on Reelfoot Lake, Tennessee.

			Station I		Station II	
Sample Number	Sample Date	Precipitation prior 72 hours	Temp, C	No. Isolates	Temp, C	No. Isolates
1	6/28/68	1.87	29	8	28	17
2	7/16/68	0.00	31	12	32	11
3	8/08/68	0.00	33	9	34	7
4	8/27/68	0.00	30	9	31	11
5	9/22/68	0.00	29	6	26	9
6	10/15/68	0.22	21	8	22	11
7	11/17/68	0.21	13	8	12	12
8	12/11/68	0.00	4	15	4	10
9	1/10/69	0.19	1	15	2	14
10	2/28/69	0.28	4	17	4	9
11	3/29/69	0.00	8	19	11	7
12	4/26/69	0.00	16	12	16	9

The seasonal fluctuations of the physiological characteristics are presented in figures 1 and 2 as the percentages of the organisms with each physiological trait in the monthly samples. These traits are divisible into two distinctive groups based on their patterns of fluctuation. Gelatin digesters, starch digesters, and oxidase positive organisms comprise Group I. The patterns of fluctuation of organisms with these characteristics were similar at the two sampling stations although they were sometimes out of phase by approximately one month (Figure 1). There was a definite pattern of fluctuation of the Group I traits. The percentages of organisms capable of gelatin digestion remained relatively constant throughout the period of the study. The percentages of the organisms capable of starch digestion and exhibiting oxidase positivity remained relatively constant for sustained periods; they were more prominent between July and October and were less prevalent between December and February. Disregarding sporadic variation which may, in part, result from the sampling technique, organisms with Group I traits demonstrated parallel seasonal fluctuations at the two sampling stations.

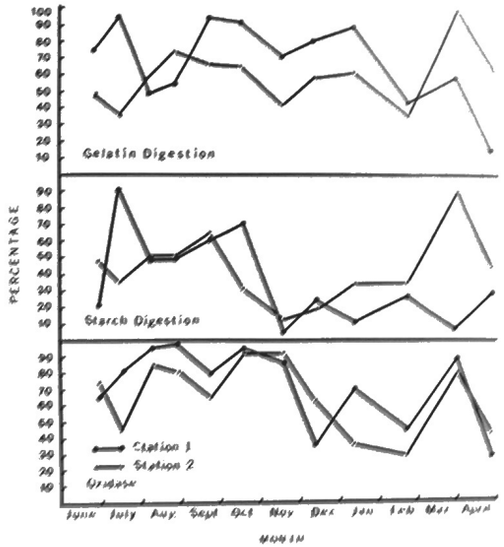

FIG. 1: *Monthly changes in the percentages of the isolates which could digest gelatin, could digest starch, and were oxidase positive. The fluctuations of these traits followed definite seasonal patterns which were roughly parallel at the two sampling stations.*

Organisms capable of reducing nitrate, fermenting glucose, and fermenting sucrose comprise Group II. Data relating the monthly percentages of the isolates with these characteristics are presented in figure 2. These three traits fluctuated independently at the two stations and sporadically during the course of the study at each sampling station. Although there were short periods when one or more of these characteristics fluctuated with similar patterns at the two sampling stations, it is difficult to discern a definite pattern in these data or to relate the fluctuations to seasonal changes.

Discussion

The percentages of the bacterial community which were positive for each of the physiological characteristics included in this investigation fluctuated widely during the period of this study (figures 1 and 2). The most reasonable inference from these data is that the fluctuations in the physiological characteristics were caused by corresponding changes in the species composition of the bacterial community. It is difficult to explain the physiological fluctuations without including species fluctuations. The data from this investigation provide apparent indirect evidence for a bacterial community with a dynamically fluctuating species composition.

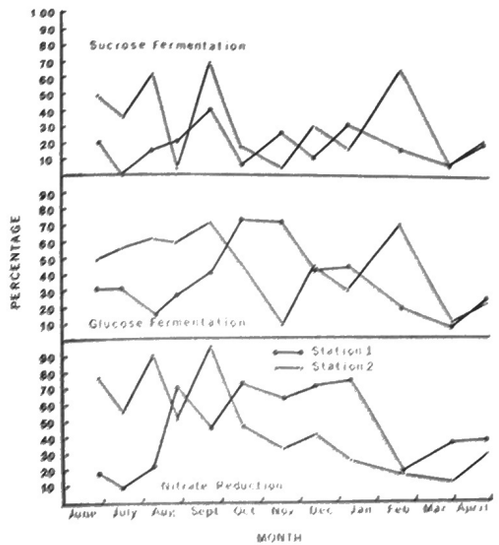

FIG. 2: *Monthly changes in the percentages of the isolates which could ferment sucrose, ferment glucose, and reduce nitrate. These traits fluctuated independently at the two stations and sporadically at each station.*

Many environmental factors can be expected to determine the species and the functional composition of bacterial communities. Some factors, such as water chemistry and run-off, may be primarily local, influencing only a small geographical area. Other factors, such as temperature, may be more general, exerting a relatively uniform effect on habitats within a large region. The composition of a bacterial community will likely result from complex interactions between the selective influences of both types of factors with the dominant influence alternating between these two types of factors.

General environmental influences associated with seasonal changes appear to have been dominant in determining the percentages of the community which were oxidase positive, could hydrolyze starch, and could hydrolyze gelatin (Figure 1). As a result, the percentages of each of these Group I characteristics fluctuated approximately together at the two sampling stations, both of which are under the influence of the general selective factors, and remained at constant levels for relatively long time intervals, adjusting gradually to higher or lower magnitudes as the factors change. However, some of the sporadic fluctuations may be due to local environmental influences affecting each sampling station independently.

Nitrate reduction, sucrose fermentation, and glucose fermentation each fluctuated sporadically with time and independently at the two sampling stations. It is hypothesized that these three Group II physiological characteristics were not of adaptive advantage to the organisms under the environmental conditions prevalent during this study and that they fluctuated randomly as the species composition changed due to selection for other physiological capabilities which were adaptive. Since nitrate reduction and the ability to ferment are adaptions for anaerobic environments and since all organisms included in this investigation were isolated from aerobic surface water, it is unlikely that these traits would be adaptive for members of this community.

An aquatic habitat usually contains many niches which are apparently occupied by organisms selected for their unique functional traits rather than for their taxonomic identities. A bacterial community which is functionally stable can probably be maintained even if the species composition fluctuates rapidly. During this process, the inhabitant of a niche may be displaced by an invading propagule because the latter has an adaptive advantage and has the essential functional traits required by the niche. The data from this study, although not conclusive, suggest that the species composition of the bacterial community is fluctuating rapidly. The magnitude of the fluctuations of the Group II traits (Figure 2) would be difficult to attribute to anything other than changes in species composition. Although the species composition is fluctuating continually, the proportion of the isolates with the apparent adaptive characteristics respond to general environmental changes, exemplified by the seasonal fluctuations noted in this study (Figure 1). Under this model, non-adaptive traits would fluctuate sporadically while the adaptive traits would remain as constant as the environment.

The speculations presented in this discussion are indicative of a need for further functionally-oriented investigations designed to discriminate between those adaptive physiological characteristics upon which the environmental selective factors act and those non-adaptive characteristics which drift sporadically. It is important that we identify ecologically significant physiological characteristics of bacterial communities and that we determine which environmental factors are selective for each physiological trait.

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