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Lactose Variability of *Escherichia coli* in Thermally Stressed Reactor Effluent Waters

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Lactose-utilizing and nalidixic acid-resistant populations of *Escherichia coli*, having an optimum growth temperature of 37°C, were placed in modified diffusion chambers. The chambers were submerged in the epilimnion and hypolimnion of a 1,100-hectare lake (Par Pond) which receives cooling water from a nuclear production reactor. Control chambers were placed in a deep-water reservoir and a Flowing-Streams Laboratory, both of which had comparable temperatures to Par Pond. The populations of *E. coli* were sampled regularly for up to 3 weeks. Viability of the bacteria was determined by dilution plating to nutrient agar followed by replicate plating onto selective medium to determine lactose utilization and nalidixic acid sensitivity. Initial populations of *E. coli* were lactose positive but changed to lactose negative in Par Pond when the reactor was operating (i.e., cooling water from the heat exchangers was being discharged to the lake). This alteration occurred most rapidly in the chambers closest to the cooling-water discharge point. Such changes did not occur in a deep-water reservoir, in Par Pond when the reactor was not operating, or in the Flowing-Streams Laboratory. The nalidixic acid-resistant characteristic remained stable regardless of the chambers' placement or reactor operations. Although the reasons for such alterations are unclear, it appears that lactose-negative populations of *E. coli* are selected for in these reactor effluent waters. The loss of the lactose characteristic prevents the recognition and identification of *E. coli* in this cooling lake (when the reactor is operating) and may prevent the assessment of water quality based on coliform recognition.

Escherichia coli is recognized as a member of the coliform group of organisms which are used as indicators of fecal pollution (4). No other single bacterium is as well characterized from a biochemical, physiological, and genetic standpoint as *E. coli*, and its occurrence and survival have been studied in different aquatic systems (3, 5, 13). Extensive sampling regimes and water quality assessments have been based on the visualization of a diagnostic characteristic of the coliform group, i.e., lactose utilization through the use of selective media and temperature (1). Although extensive monitoring information is based on measuring lactose utilization, practically nothing is known about the stability of the lactose expression of the coliforms in situ and specifically for *E. coli*. Recent observations by Gorden and Fliermans (9) on the survival of *E. coli* in a thermally altered reservoir showed the spontaneous occurrence of non-lactose-fermenting *E. coli*.

This paper describes the effect of reactor cooling waters on the survival of *E. coli* under in situ conditions (in the lake) and the fermentative utilization of lactose. The data contribute to the knowledge of *E. coli* as an indicator organism in stressed habitats.

MATERIALS AND METHODS

Study sites. Studies were conducted at the Savannah River Plant near Aiken, S.C., which is a National Environmental Research Park operated for the Department of Energy by E. I. du Pont de Nemours & Co. Experiments were conducted on Par Pond, a 1,100-hectare lake; (7) in the thermally altered streams at the Flowing Streams Laboratory (10); and at Clark Hill, a 10,000-hectare, man-made reservoir on the Savannah River. Ambient temperature water from Par Pond, along with 10% makeup water from the Savannah River, is used to cool a nuclear production reactor. Cooling water is discharged from the reactor's heat exhangers through 11 km of canals and lakes before discharging back into Par Pond at the hot dam (Fig. 1). During reactor operations, the warm arm of Par Pond is generally 7 to 12°C warmer than ambient temperature surface waters common for other southeastern lakes.

The Flowing Streams Laboratory at the Savannah River Plant is a greenhouse laboratory housing six

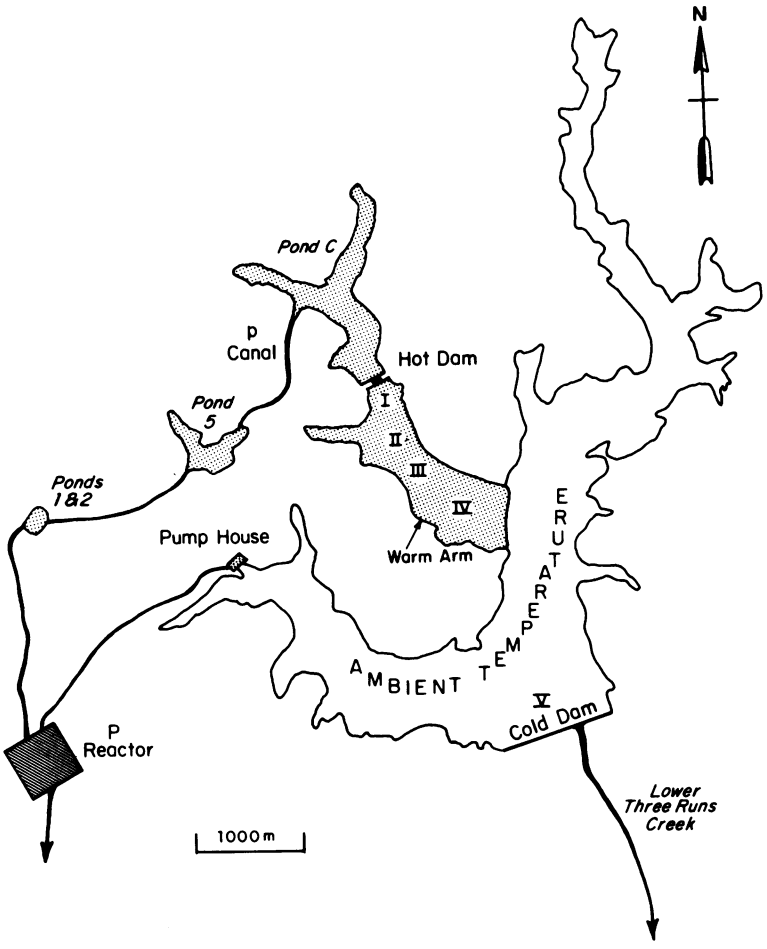

FIG. 1. *Par Pond system showing the ambient and thermal (shaded) temperature regions. Sampling stations are numbered.*

artificial streams (10). Duplicate streams receive water directly from the parent stream and reflect its temperature fluctuations. Additional streams are maintained at temperatures of 2.5, 5.0, 7.5, and 12.5°C above the parent stream. The six streams are reflective of once-through cooling systems and have in situ temperatures similar to the temperatures observed at different locations of Par Pond.

Clark Hill is approximately 130 river km upstream from the Savannah River Plant on the Savannah River. The reservoir, which serves as a source for hydroelectric power, receives no thermal effluent from any power plants. For these studies, Clark Hill served as a control system where the lactose characteristic could be observed in an additional deep-water system.

Permanent stations were established at each of the sampling sites. Stations at Par Pond were located at different distances from the input, whereas stations at the Flowing Streams Laboratory were at ambient temperature and 12.5°C above ambient. A single station was located in the deepest portion of Clark Hill. Temperature, dissolved oxygen, pH, conductivity, and oxidation/reduction potentials were measured for each depth at each selected station (Table 1) with a Hydrolab Surveyor Multiprobe Analyzer (Hydrolab Corp., Austin, Tex.). Study sites and sampling locations were selected to compare results of experiments conducted during various thermal alterations.

Lactose variation studies. A clinical isolate of *E. coli* F^- (1533-68) with an optimal growth temperature of 37 ± 1°C was obtained from the Center for Disease Control (Atlanta, Ga.). This strain had previously and repeatedly been shown by the Center for Disease Control and our laboratory to be lactose positive (Lac^+) and genetically stable for the measured diagnostic character. Over 4×10^4 colony-forming units (CFU) were tested for reversion and mutation frequency of the lactose expression without observing a single revertant or mutant colony. For several of the studies, this strain, which was nalidixic acid sensitive (Nal^s), was modified to produce a nalidixic acid-resistant (Nal^r) substrain by direct selection for Nal^r mutants on nutrient agar containing 200 μg of nalidixic acid per ml. Cultures were grown from a single colony

in nutrient broth (Difco) at 37°C for 24 h. Cells were aseptically washed three times in 0.01 M phosphate-buffered saline (pH 7.2) and concentrated by centrifugation. To determine initial population densities and genetic stability of Lac^+ and Nal^r characters, washed cells were diluted in filter-sterilized buffer (0.22 μm), plated on nutrient agar, incubated for 12 to 18 h at 37°C, and then replicated (11) to eosin-methylene blue agar (Difco) with and without 100 μg of nalidixic acid per ml.

E. coli populations of a known density (ca. 4×10^{10} CFU/ml) were aseptically placed in sterile diffusion chambers (6). The chambers were then attached to stainless steel chains and lowered into the water column at each of the stations. At each station, duplicate chambers were located in the epilimnion (1 m below the surface) and in the hypolimnion (1 m above the sediments), except at Clark Hill where equal sampling depths were maintained. Stations within Par Pond were chosen to represent the extreme conditions where genetic variation had been detected previously (9). Thermal control chambers were maintained in the artificial streams of the Flowing Streams Laboratory at ambient temperatures and at 12.5°C above ambient.

Samples were taken from duplicate chambers twice daily (approximately every 12 h) over the study periods. During sampling, chambers were removed from the lake, agitated for mixing, and sampled aseptically with a sterile syringe. The samples were then transferred into sterile plastic tubes, and the chambers were returned to the lake. Samples were immediately returned to the laboratory, diluted in sterile phosphate buffer (pH 7.2), and plated as previously described. Colonies from replicated plates were differentiated into one of the four following classes: (i) Lac^+ and Nal^r, (ii) Lac^+ and Nal^s, (iii) Lac^- and Nal^r, (iv) Lac^- and Nal^s. Throughout the study, colonies were picked at random, restreaked, and tested in duplicate with the API 20 E System (Analytab Products, Inc., Plainview, N.Y.) to confirm identification as to species of the wild-type *E. coli*.

RESULTS

Physical and chemical parameters measured at the sampling stations during both reactor conditions indicate differences between locations, particularly when the reactor was operating (Table 1). Chambers placed in the epilimnion when the reactor was operating exposed the *E. coli* populations to warm aerobic waters with a neutral pH and low conductivity. Populations in the hypolimnion were exposed to anaerobic, cooler waters having generally low oxidation/reduction potential and relatively higher conductivity. When the reactor was not operating, the chambers were exposed to waters with neutral pH and low conductivity both at Par Pond and Clark Hill. The $\Delta T = 12.5$°C stream at the Flowing Streams Laboratory had temperatures comparable to station I at Par Pond when the reactor was operating.

The data in Table 2 demonstrate the changes in population density of *E. coli* during the time that the bacteria were suspended in the various habitats. The data demonstrate that comparable levels of survival occurred in all the habitats over a 96-h period regardless of reactor influences. The survival of *E. coli* was generally better in the hypolimnion than in the epilimnion. The population densities reflect measurements on duplicate chambers and have been shown in previous studies to have standard deviations within 10% of the arithmetic mean (6).

At each of the designated time intervals, the percentage of lactose-utilizing *E. coli* was measured (Table 3). The data indicate that epilimnion populations all demonstrated a more rapid appearance of Lac^- cells than did hypolimnion populations at the same site. When the reactor was operating, the emergence of Lac^- cells occurred most rapidly near the discharge point of the cooling water (station I), and dominated the population by 72 h (Fig. 2a). When the reactor was operating, the Lac^- character appeared within 72 h in all the chambers suspended in Par Pond regardless of their position (Fig. 2a, b, and c). By 96 h, epilimnion populations at stations I and II were totally Lac^- as well as the hypolimnion populations at station I (Fig. 2a and b). Lac^- populations were observed neither in chambers placed in Par Pond when the reactor was not operating nor in the streams of the Flowing Streams Laboratory nor in Clark Hill.

Lac^+ and Lac^- colonies picked at random from enumeration plates were consistently identified as *E. coli* by using the API 20E System. The Nal^r character remained stable for all the populations tested regardless of the chamber depth, station, reactor operating condition, or sampling site.

DISCUSSION

The experimental design and use of diffusion chambers provide a means for continually sampling a given microbial population subjected to in situ conditions over an extended period of time and the subsequent measuring of the response of those microorganisms. The simple design of the chambers affords extensive flexibility to study the responses of both axenic and mixed populations of microorganisms under in situ conditions.

The data indicate that an emergence of lactose-negative *E. coli* occurs from a lactose-positive population when the latter is placed in sterile diffusion chambers and subjected to in situ aquatic conditions peculiar to the effluent discharged from this nuclear production reactor. Although only 4×10^4 CFU were tested for reversion and mutation frequency, the sponta-

TABLE 1. *Physical and chemical parameters measured at the stations used for lactose variability studies in diffusion chambers*

Station	Depth (m)	Temp (°C)	Eh (mv)	D.O. (ppm)	pH	Cond. (μmho/cm²)
Habitat:		*Par Pond, Reactor Operating*				
I	1	33.0	380	7.4	6.9	70
	7	25.0	450	0.4	6.6	80
II	1	30.0	330	7.2	6.9	70
	9	23.0	160	0.3	6.5	100
V	1	28.5	310	7.9	7.9	80
	15	19.0	N.D.[a]	0.2	6.7	120
Habitat:		*Par Pond, Reactor Not Operating*				
I	1	30.5	290	8.4	8.0	80
	7	25.0	390	0.3	6.1	90
II	1	30.5	300	8.2	7.9	80
	9	23.5	400	0.3	6.2	110
V	1	29.0	280	8.5	8.3	70
	15	17.0	400	0.3	6.7	110
Habitat:		*Flowing Streams Laboratory*				
ΔT = 0°C	Surface	21.9	N.D.	6.0	5.9	22
ΔT = 12.5°C	Surface	34.5	N.D.	7.5	5.9	21
Habitat:		*Clark Hill—No Reactor*				
	1	30.5	325	5.8	8.8	370
	5	28.5	315	5.9	8.8	370
	10	21.0	340	3.8	8.8	330
	15	17.5	350	3.4	8.8	310
	20	17.0	330	2.1	8.8	325
	25	16.0	340	2.0	8.8	345
	30	15.5	350	1.6	8.8	370
	35	14.5	340	1.1	8.8	350
	40	13.5	335	0.8	8.8	350
	45	13.0	360	0.8	8.8	390
	50	13.0	350	0.8	8.8	430

a. N.D. = Not Determined.

neous reversion and mutation rate for stable genes is generally 10^{-7} to 10^{-8} CFU (12). Similar frequencies are assumed for the lactose expression in this strain of *E. coli*. The observed effect is not merely a direct temperature phenomenon because *E. coli* placed in chambers subjected to similar temperatures in other natural systems did not vary in their lactose utilization characteristic. Additionally, *E. coli* in chambers placed in deeper, cooler waters during reactor operating conditions varied in their lactose characteristic but to a slower degree than those in the overlying warmer epilimnion waters. It is of interest that the data in Fig. 2 suggest that changes in the lactose expression of the *E. coli* population occur in a sequential manner with distance from

TABLE 2. *Population densities (CFU/ml) of E. coli from diffusion chambers placed in selected habitats for different periods of time*

Station	Depth (m)	Time (hr) 0	12	24	36	48	72	96	120
Habitat: Par Pond — Reactor Operating									
I	1	4.1×10^{10}[a]	ND[b]	5.5×10^{9}	ND	7.7×10^{8}	3.7×10^{8}	5.5×10^{7}	ND
	7	4.1×10^{10}	ND	3.1×10^{10}	ND	1.6×10^{10}	6.9×10^{9}	1.1×10^{9}	ND
II	1	4.1×10^{10}	ND	2.1×10^{10}	ND	1.7×10^{10}	1.5×10^{9}	1.6×10^{8}	ND
	11	4.1×10^{10}	ND	1.5×10^{10}	ND	2.1×10^{10}	7.4×10^{9}	1.6×10^{9}	ND
V	1	4.1×10^{10}	ND	1.6×10^{10}	ND	1.7×10^{10}	5.5×10^{9}	4.9×10^{9}	ND
	15	4.1×10^{10}	ND	1.4×10^{10}	ND	1.6×10^{10}	4.5×10^{9}	9.7×10^{8}	ND
Habitat: Par Pond — Reactor Not Operating									
I	1	2.1×10^{10}	1.1×10^{10}	5.5×10^{9}	2.2×10^{9}	4.3×10^{8}	3.1×10^{8}	9.1×10^{7}	4.7×10^{7}
	7	2.1×10^{10}	8.4×10^{10}	7.1×10^{10}	4.4×10^{10}	9.1×10^{9}	8.1×10^{9}	9.3×10^{8}	2.6×10^{8}
II	1	2.1×10^{10}	1.5×10^{10}	7.2×10^{9}	4.1×10^{9}	6.5×10^{8}	3.7×10^{8}	5.7×10^{7}	1.9×10^{7}
	11	2.1×10^{10}	7.7×10^{10}	5.1×10^{10}	3.6×10^{10}	1.1×10^{10}	2.4×10^{9}	6.6×10^{8}	3.8×10^{8}
V	1	2.1×10^{10}	2.2×10^{10}	ND	1.9×10^{9}	5.9×10^{8}	7.6×10^{7}	3.1×10^{7}	9.4×10^{6}
	15	2.1×10^{10}	6.3×10^{9}	5.1×10^{9}	2.8×10^{9}	9.3×10^{8}	7.3×10^{8}	4.1×10^{8}	ND
Habitat: Flowing Streams Laboratory									
$\Delta T = 0°C$		4.1×10^{10}	ND	5.0×10^{10}	ND	1.6×10^{10}	5.7×10^{9}	4.1×10^{8}	ND
$\Delta T = 12.5°C$		4.1×10^{10}	ND	2.1×10^{10}	ND	2.1×10^{9}	2.9×10^{8}	6.7×10^{7}	ND
Habitat: Clark Hill — No Reactor									
I	1	7.2×10^{10}	1.1×10^{10}	1.2×10^{9}	7.1×10^{8}	4.7×10^{8}	7.7×10^{7}	4.8×10^{7}	ND
	15	7.2×10^{10}	5.3×10^{10}	4.5×10^{10}	3.1×10^{10}	1.7×10^{10}	1.6×10^{9}	2.6×10^{8}	ND

a. All population estimates are means of duplicate chambers.
b. ND = Not determined.

TABLE 3. *Percentage of E. coli that remained Lac⁺ when placed in selected habitats for different periods of time*

Station	Depth (m)	Time (hr) 0	12	24	48	72	96	120
Habitat: Par Pond – Reactor Operating								
I	1	100 (123)[a]	100 (269)	100 (180)	82 (116)	26 (156)	0 (211)	ND[b]
	7	100 (123)	100 (296)	100 (121)	100 (317)	46 (242)	0 (188)	ND
II	1	100 (123)	100 (318)	100 (77)	96 (171)	72 (256)	0 (322)	ND
	11	100 (123)	100 (315)	100 (126)	100 (140)	82 (202)	52 (188)	ND
V	1	100 (123)	100 (297)	100 (318)	100 (172)	89 (157)	62 (227)	ND
	15	100 (123)	100 (145)	100 (271)	100 (184)	95 (250)	89 (100)	ND
Habitat: Par Pond – Reactor Not Operating								
I	1	100 (160)	100 (242)	100 (319)	100 (411)	100 (302)	100 (187)	100 (112)
	7	100 (160)	100 (117)	100 (159)	100 (214)	100 (181)	100 (231)	100 (269)
II	1	100 (160)	100 (221)	100 (171)	100 (191)	100 (91)	100 (114)	100 (281)
	11	100 (160)	100 (159)	100 (110)	100 (212)	100 (56)	100 (120)	100 (240)
V	1	100 (160)	100 (211)	ND	100 (118)	100 (191)	100 (300)	100 (171)
	15	100 (160)	100 (141)	100 (110)	100 (212)	100 (138)	100 (219)	ND
Habitat: Flowing Streams Laboratory								
ΔT = 0°C		100 (140)	ND	ND	ND	ND	100 (124)	ND
ΔT = 12.5°C		100 (140)	ND	ND	ND	ND	100 (54)	ND
Habitat: Clark Hill – No Reactor								
I	1	100 (144)	100 (333)	100 (247)	100 (161)	100 (176)	100 (166)	ND
	15	100 (144)	100 (253)	100 (317)	100 (258)	100 (153)	100 (218)	ND

a. Numbers in parentheses indicate the number of colonies tested for lactose utilization.

b. ND = Not determined.

the cooling-water discharge location. The epilimnion populations closest to the discharge location are affected first.

Although "a cooling-water factor" is suggested by the data, the studies in Par Pond with the reactor operating and not operating were not conducted concurrently. Experiments were initially conducted with the reactor not operating. When the reactor had been operating for 30 days and the physical and chemical conditions appeared to stabilize in the lake, the "reactor operating" experiments were begun. Thus, it is unlikely that a pulse of a cooling-water factor moved down the lake in a stepwise fashion. Furthermore, subsequent dye releases into Par Pond at the cooling-water discharge location indicate that the dye remains in the top 2 m of the water column, during the 6-h study period. Additionally, the dye studies demonstrated that the travel time to station I was less than 5 min, and travel time to station II was 90 min. No data are available for station V. Thus, the cooling-water input may be primarily a surface phenomenon and of sufficient magnitude to have affected stations I and II by the time the chamber studies began.

Several possible reasons for the lactose variability may be put forward. Plating on nutrient agar, once the *E. coli* was removed from the chambers, might have selected for a Lac⁻ population because no substrate was present to induce the enzyme system for lactose utilization. However, such plating was consistently done for all sets of samples, so controls and test samples would have the same selectivity. The high initial cell densities might have masked the presence of a Lac⁻ substrain present in the initial population. The exposure to conditions in the cooling water may select against Lac⁺ cells with Lac⁻ cells present initially in the population, either increasing, maintaining, or decreasing less slowly than Lac⁺ cells. Such a differential in growth would cause the Lac⁻ cells to become dominant.

Additionally, some factor(s) in the cooling-water effluent may have forced the conversion of Lac⁺ cells to Lac⁻ cells. However, the dilution of the cells in phosphate-buffered saline before plating should have diluted such factors; thus, a greater number of Lac⁺ cells would have been present in the greater dilutions. Such was not the case because colonies from all dilutions were consistent in their lactose-utilizing ability. Although levels of lactose in Par Pond have not been measured, no a priori data suggest that the levels of lactose changed during reactor operations and affected the ecosystem.

A phage or transmissible plasmid may explain the observed phenomenon, because phage φX174 has been reported to reduce β-galactosidase synthesis in *E. coli* (8). A loss of β-galactosidase would prevent the hydrolization of lactose and subsequent utilization. If φX174 were present in the aquatic system, the pore sizes of the diffusion chambers, 0.4 μm, would not exclude the phage. The differences observed in lactose variability between reactor operating and not operating conditions may be explained by the presence of phage, because the high temperatures that occur in the reactor subject the cooling water to high temperatures (>70°C) and appear to lyse entrained microorganisms (Fliermans, unpublished data). If these microorga-

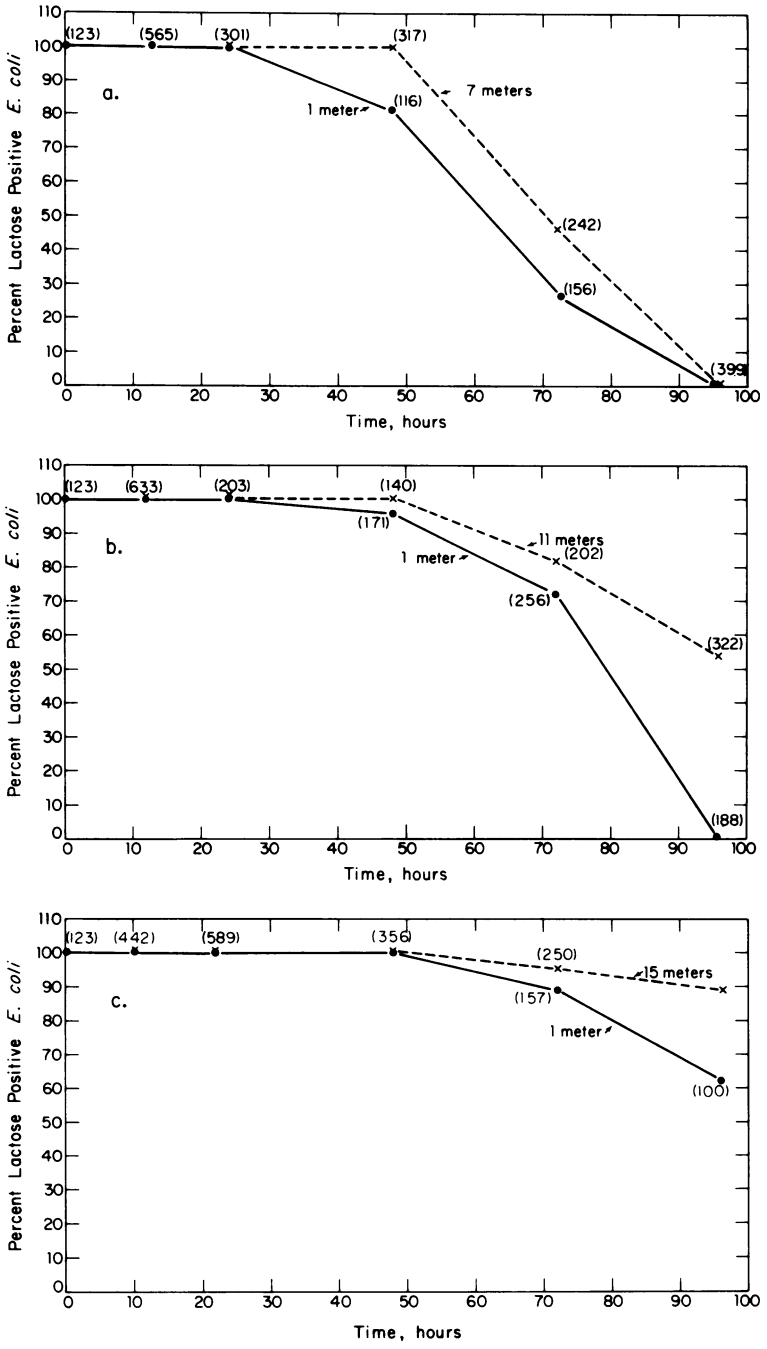

FIG. 2. *Lactose changes of E. coli in Par Pond at station I (a) station II (b), and station V (c) when the reactor was operating. The numbers in parentheses represent the number of colonies replicated to eosin-methylene blue plates.*

nisms harbor the appropriate phage particles, then their lysis may release these particles into the aquatic ecosystem. Such an hypothesis does not appear to explain the differences among chambers and stations. Additionally, no evidence of phage or prophage induction has been observed. The *E. coli* strain used is resistant to infection by MS II phage. This resistance indicates that it does not harbor the F factor either chromosomally or as a plasmid (11). With the

plasmid mobilization techniques of Skerman et al. (14), this strain of *E. coli* was shown to be free of detectable transmissible plasmids (S. Richardson, personal communication).

In these experiments, the appearance of Lac^- cells was always accompanied by Nal^r cells. Because nalidixic acid resistance maps at 30 min and the lactose operon is located at approximately 8 min on the *E. coli* genome (2), it appears that the observed phenomenon is localized. The absence of Lac^- cells in Par Pond when the reactor is not operating, in the Flowing Streams Laboratory, and in Clark Hill where no reactor input occurs indicates a cooling water effect of some kind.

It is not clear whether the observations are due to genotypic or phenotypic changes because Lac^- cells could repress the lactose operon at the transcriptional or translational level. Lac^- cells may have lost all or part of their lactose operon during replication of DNA. Regardless of the type of change(s) occurring in the population or the cause, the result is the same. That is, it is not possible to easily detect and quantify *E. coli* by standard methods (1) in Par Pond when the reactor is operating. The applicability of this research is broad if the other coliform bacteria in these systems respond like *E. coli*. The assessment of water quality based on coliform measurements in lakes such as Par Pond would result in data which would indicate that the water quality is better than it really is. The data further suggest that the use of standard coliform measurements in natural waters, without establishing the stability of the diagnostic characteristic in situ, may lead to erroneous results.

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LITERATURE CITED

1. **American Public Health Association.** 1976. Standard methods for the examination of water and wastewater, 14th ed., p. 982-986. American Public Health Association, Washington, D.C.
2. **Bachmann, J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116-167.
3. **Baross, J. A., F. J. Hanus, and R. Y. Morita.** 1975. Survival of human enteric and other sewage microorganisms under simulated deep-sea conditions. Appl. Microbiol. **30**:309-318.
4. **Faust, M. A.** 1976. Coliform bacteria from diffuse sources as a factor in estuarine pollution. Water Res. **10**:619-627.
5. **Faust, M. A., A. E. Aotaky, and M. T. Hargadon.** 1975. Effect of physical parameters on the in situ survival of *Escherichia coli* MC-6 in an estuarine environment. Appl. Microbiol. **30**:800-806.
6. **Fliermans, C. B., and R. W. Gorden.** 1977. Modification of membrane diffusion chambers for deep-water studies. Appl. Microbiol. **33**:207-210.
7. **Fliermans, C. B., R. W. Gorden, T. C. Hazen, and G. W. Esch.** 1977. *Aeromonas* distribution and survival in a thermally altered lake. Appl. Environ. Microbiol. **33**:114-122.
8. **Ghosh, A., and R. K. Poddar.** 1977. Reduced synthesis of β-galactosidase in *Escherichia coli* infected with phage ϕX174. Can. J. Microbiol. **23**:1069-1077.
9. **Gorden, R. W., and C. B. Fliermans.** 1978. Survival and viability of *Escherichia coli* in a thermally altered reservoir. Water Res. **12**:343-352.
10. **Harvey, R. S.** 1973. A flowing stream laboratory for studying the effects of water temperature on the ecology of stream organisms. Assoc. Southeast Biol. Bull **20**:3-7.
11. **Hayes, W.** 1968. Genetics of bacteria and their viruses, 2nd ed. John Wiley & Sons, Inc., New York.
12. **Lederberg, J., and E. M. Lederberg.** 1952. Replica plating and indirect selection of bacterial mutants. J. Bacteriol. **63**:399-406.
13. **McFeters, A., and D. G. Stuart.** 1972. Survival of coliform bacteria in natural waters: field and laboratory studies with membrane-filter chambers. Appl. Microbiol. **24**:805-811.
14. **Skerman, F. J., S. B. Formal, and S. Falkow.** 1972. Plasmid-associated enterotoxin production in a strain of *Escherichia coli* isolated from humans. Infect. Immun. **5**:622-624.