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The Response of Three Bacterial Populations to Pollution in a Stream

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Abstract

Practical methods for biomonitoring of natural systems are still under development. Bacteria are potentially useful indicators of water quality because of their species diversity and ability to rapidly respond to changing environmental conditions. In this study, bacterial populations from unpolluted and polluted stream sites in two watersheds were compared to determine their suitability for use as environmental indicators. Upper Three Runs Creek and Four Mile Creek headwaters have had little anthropogenic disturbance, as opposed to lower Four Mile Creek which received thermal, radioactive, and chemical perturbations. Chemical and physical measurements provided evidence that seepage from holding ponds polluted Four Mile Creek. Polluted sites did not have altered total bacterial numbers but had decreased numbers of colony-forming units. Abundances of three bacterial species, *Acinetobacter calcoaceticus, Burkholderia cepacia,* and *Pseudomonas putida,* were determined by colony hybridization with species-specific rDNA probes. Contribution of *A. calcoaceticus* to the assemblage was higher at polluted sites, which indicated either tolerance of polluted conditions or the ability to utilize compounds existing at these sites to reach larger populations. No differences in *B. cepacia* populations were detected, and differences in *P. putida* populations could not be attributed solely to disturbance. The pollution of Four Mile Creek induced differences in bacterial populations that could be monitored using the described approach.

Introduction

Pollution often alters aquatic biota, and the response of some species makes them useful biological indicators. The development of biological indicators of water quality is appealing for two reasons. First, relative abundances of pollution-tolerant or -intolerant organisms indicates the response to stress conveyed to the system by disturbance. Second, evaluation of conditions using biological indicators can be more cost and time effective than complex chemical and physical analysis, and they can be monitored to assess recovery.

State and federal monitoring agencies currently use biological indicators to assess stream population [e.g., 25, 27, 41]. Typically, a community-level approach is taken, in

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which a representative sample of fish, macroinvertebrates, algae, or bacteria are sampled and identified. Results are compared to established taxa-based indices [1, 16, 28, 30, 42]. This process is often labor intensive, costly, and inconclusive due to regional variability, making subsequent monitoring prohibitive.

The use of bacterial indicators is usually limited to the measurement of the abundance of the fecal coliform group, and rarely is species tolerance (tolerant or intolerant) of chemical pollution monitored. If changes in bacterial assemblages associated with stream pollution could be assessed, then discovery of sensitive biological indicators, as well as evaluation of remediation progress, would be expedited. Methodological constraints have impeded our ability to examine the response of natural populations of bacteria to pollution. However, a combination of assessment of the disturbance (environmental monitoring), determination of effect of disturbance on the biota (ecology), sampling of bacteria (microbiology), and identification to the species level (molecular biology) makes this approach possible at this time.

In this study, responses of bacterial populations to anthropogenic disturbance were evaluated by comparing abundances in polluted and unpolluted streams. Abundances of three species of common bacteria, *Acinetobacter calcoaceticus, Burkholderia cepacia,* and *Pseudomonas putida,* were determined. A culture-based approach for sampling bacteria was coupled with nucleic acid–based identification. Although culturing may be a liability when attempting to describe total bacterial assemblages, it offers advantages for monitoring species-specific responses, if proper criteria are met.

Traditional methods for culturing and identifying bacterial communities limit the portion of the total assemblage that can be monitored [21]. However, certain species have been well-studied; monitoring the response of these populations of bacteria, rather than the entire assemblage, is potentially a productive approach to this problem. The amenability of the study species to cultivation becomes a principal concern. Although our understanding of stream bacterial communities is in its infancy [19], we do know that some species are difficult, perhaps impossible, to culture. In addition, some species such as *Vibrio cholerae* enter a viable, nonculturable state [34]. The organisms employed in this investigation do not appear to enter such a state [14] (Lemke, unpubl. data), and it can be assumed that all metabolically active individuals were enumerated through culturing. Results from different sites and over time can be compared because cultivation conditions were constant.

Species selection was based on genetic and physiological descriptions, and the potential response (i.e., sensitivity) of the bacteria to perturbations. Only species with species-specific oligonucleotide probes available at the time of study were considered. Species with a range of characteristics associated with bioremediation were selected, and a response based on abundance, as opposed to presence or absence, was measured.

A. calcoaceticus was chosen because it is ubiquitous in soil and water, and was expected to thrive in polluted environments. Unlike most aerobic bacteria, relatively few strains use glucose as a carbon source [6]. Instead, they derive energy from aliphatic alcohols, decarboxylic and fatty acids, unbranched hydrocarbons, and recalcitrant aromatic compounds [15]. These physiological characteristics have lead to its use in biodegradation of industrial pollutants and hydrophobic substrates (e.g., crude oil) [5, 32, 33, 38], and are predicted to enhance its success in polluted waters.

Like *A. calcoaceticus, Burkholderia* (*Pseudomonas*) *cepacia* is a common soil and freshwater bacterium that utilizes a wide variety of substrates [9, 10, 29]. It is one of the most nutritionally versatile of the pseudomonads [4]. In addition, it exhibits the highest level of genetic diversity of any species studied [22, 23]. Based on the described physiologic diversity, *B. cepacia* populations should be successful in both polluted and unpolluted environments.

P. putida is also a potentially useful bacterium for degradation of common chemical pollutants. It degrades a different class of carbon substrates, such as toluene [36], styrene [26], and naphthalene [12]. Differences in carbon compound utilization among the three species suggests that each will respond differently to pollution.

This research was conducted at the U.S. Department of Energy's Savannah River Site (SRS) which provided a unique opportunity to evaluate anthropogenic disturbance. Weapons-grade nuclear material was produced at the site beginning in the early 1950s. Many of the watersheds were, and are, impacted by chemical and radioactive contamination. The lower reaches of Four Mile Creek (FOU) were heavily polluted in comparison to Upper Three Runs Creek (UTR) and the FOU headwaters. The downstream FOU community has been affected by thermal pollution from water used to cool a nuclear reactor, and holding ponds that seep chemical and radioactive contaminants.

Table 1. Baseflow stream site characteristics for at Savannah River Site, Aiken, S.C. Values represent means

	Unpolluted			Polluted	
	UTR1	UTR2	FOU1	FOU2	FOU3
Stream characteristics					
Order	Third	Fourth	First	Second	Third
% Stream cover	0	90	100	0	50
Monthly vol. (liters × 10^9)[a]	8.4	17.8	ND[b]	9.3	14.0
Flow rate (m s^{-1})	1.8	3.3	2.2	1.4	4.0
Physical measurements					
Temperature (°C)	24.0	23.6	24.5	26.5	26.1
pH	6.2	5.9	6.0	5.9	6.2

[a] See [3]
[b] ND, not determined

Materials and Methods

Study Site Description

Two watersheds, Four Mile Creek (FOU) and Upper Three Runs Creek (UTR), in the 768-km^2 SRS, were sampled in July 1995. Average July air temperature was 27.1°C, and total precipitation was 11.7 cm [39]. UTR originates outside the SRS facilities in Aiken County, S.C. and represents an undisturbed blackwater stream, typical of the Upper Coastal Plain [44]. It is spring fed and has a 492-km^2 catchment [18]. FOU is completely contained within the boundaries of SRS and drains a 91-km^2 area. It is impacted by point and nonpoint pollution sources immediately downstream from its headwaters.

Five stream sites were chosen so that meaningful comparisons could be made. Stream characteristics (i.e., volume, order, water temperature, pH, and flow rate; Table 1) were similar between the upper unpolluted (UTR1) and polluted (FOU2) sites, and the lower unpolluted (UTR2) and polluted (FOU3) sites. An unpolluted site at the Four Mile Creek headwaters (FOU1) was included for within-stream comparisons.

Release of radioactive and chemical compounds during SRS operations has impacted some freshwater environments. The highest radioactive release to streams occurred circa 1964 (i.e., 111,000 Ci tritium; [3, 40]) with about 8,800 Ci tritium being released to streams in 1994. About 6,980 Ci tritium, as well as strontium-90, iodine-129, cesium-137, technetium-99, and radium-223, -224, and -226, also entered FOU below the headwaters from nearby seepage basins in 1994. Relative to lower FOU, the UTR sites were minimally impacted and designated as unpolluted. The upstream site (UTR1) has not been disturbed, and lower reaches received only about 389 Ci tritium in 1994 [2]. The average tritium concentration (μCi × 10^{-6} ml^{-1}) in FOU stream water was high near FOU2 (2,600) and FOU3 (485), but very low at the upper reaches of UTR (0.46) [2].

Curently, many chemical pollutants enter FOU through groundwater about 1,500 m upstream from FOU2 from open-air holding ponds and from other ponds just below FOU2. FOU3 was located 10.5 km downstream from FOU2, and discharge from the seepage basins, coal-pile runoff, burning pits, and other disposal facilities near FOU2 affected FOU3 via ground and surface water [2].

Groundwater entering FOU often had a large amount of suspended solids (<60 ppm), and high turbidity (about 18 NTU) and alkalinity [2, 3]. Groundwater pollutants included inorganic compounds (sodium hydroxide, sodium chloride, arsenic, cyanide, nitric acid, nitrate-nitrite salts, sulfates) and metals (mercury, cadmium, chromium, aluminum, lead, zinc). Organic pollutants included oil, grease, heptachlor epoxide, and organic halogens (carbon tetrachloride, trichloroethylene, tetrachloroethylene, 1,1-dichloroethane, PCBs, dichloromethane, trichlorofluormethane) at concentrations to 275 μg $liter^{-1}$ [8]. Levels of contaminants were frequently above drinking water standards.

Soils, which may effect water chemistry and serve as a source of bacteria to streams, were similar at all sites. Soils at FOU1, FOU2, and UTR1 were poorly drained, floodplain Pickney sand containing dark, loamy acidic (pH 3.6–6.5) surface soils with 2–10% clay and 3–15% organic matter. Although soils at FOU3 and UTR2 were classified as Fluvaquents, they resembled soils at other sites with pH 4.5–5.5, 2–12% clay, and 1–5% organic matter [39].

Riparian vegetation at the undisturbed stream areas was about 40% trees and 15% shrub/vine, and classified as bottomland hardwoods. Overstory vegetation was mesic, mixed hardwood forest [44]. Thermal effluent from nuclear plant operations left few remnants of the bottomland hardwoods at FOU2. Alluvial-filled sloughs supporting marsh sedges (*Carex* spp. and *Cyperus* spp.) and shrub-scrub associations dominated by willow and button bush have replaced the original vegetation at these sites.

Collection and Processing of Samples

Physical measurements ($n = 3$) of stream conditions taken at time of sampling were: pH, temperature (Oakton WD-35615 pH/mV/Temperature Meter, Singapore), turbidity (2100P Hach Turbidimeter, Loveland, Colo.), total dissolved solids (TDS; Hach 44600 Conducitivity/TDS meter), and flow rate (FP 101/201 Global Flow

Probe, Global Water Instruments, Fair Oaks, Calif.). Five surface water samples (about 60 ml) were collected from midstream at each site on July 24–25, 1995, and kept at 4°C until processed later the same day. Chemical analyses on three of the five replicates included dissolved organic carbon (DOC) (samples filtered through GF/F glass fiber filters; analyzed on OI Model 700 total carbon analyzer, College Station, Tx), nitrate (NO_3) (Autoanalyzer II Continuous Flow System, Technicon/Bran-Luebbe, Buffalo Grove, Ill.), and a 20-element plasma emission spectrum (Jarrell-Ash 965ICP, Franklin, Mass.), performed at the University of Georgia Chemical Analysis Laboratory. Five milliliters of water per replicate, per site, (from each replicate) ($n = 5$) was preserved with 0.2 ml formalin (for each site) for total bacterial enumeration using the DAPI (4′,6-diamidino-2-phenylindole) microscopy method [31].

Three modified nutrient agar [20] plates were inoculated with 10 or 100 µl of water from each of the 25 samples. Plates were incubated for 3 days at 23°C, and colonies were enumerated. About 50 cfu per sample was transferred to the same medium for colony hybridization, as described in Leff et al. [21]. Colonies were blotted onto MSI MagnaLift nylon filters (Westboro, Mass), lysed with 0.5 M NaOH, washed with 0.5 M Tris buffer (pH 8.0), 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2), and 95% ethanol [37], followed by heating (80°C, 2 h) to anneal DNA to the filter.

Colony Hybridization

Hybridization of filters was done in 5 × SSC, 0.5% Boerhinger-Mannheim blocking reagent, 0.1% sodium *N*-lauryl sarcosine, 0.02% sodium dodoecyl sulfate (SDS), at 47°C (*B. cepacia*) or 50°C (*A. calcoaceticus* and *P. putida*) for 12–18 h, followed by three washes with 1 × SSC, 0.1% SDS for 20 min, at the respective hybridization temperature. Bacterial species-specific probes were: 5′-AGC ATC CTA TCG CTA GGT A-3′ for 16S rDNA of *A. calcoaceticus* [7], 5′-CCC ATC GCA TCT AAC AAT-3′ for 23S rDNA of *B. cepacia* [35], and 5′-GCT GGC CTA ACC TTC-3′ for 16S rDNA of *P. putida* [35]. Probes were labeled with digoxigenin, following the Boerhinger-Mannheim Genius protocol (Indianapolis, Ind.) and excess label was removed using NucTrap probe purification columns (#400701, Stratagene Cloning Systems, LaJolla, Calif.). Probe specificity was determined by inclusion of ATCC type strain controls, tested with samples to verify hybridization with the target species and to demonstrate a lack of hybridization with other, closely related species. Species used as control were: *B. cepacia* (25416), *P. putida* (12633), *A. calcoaceticus* (23055). *B. solanacearum* (11696), *P. fluorescens* (13525), *P. aeruginosa* (10145), *P. chlororaphis* (9446), *Aeromonas hydrophila* (7966), *Comamonas testosteroni* (11996), *C. acidovorans* (15668), and *Hydrogenophaga pseudoflava* (33668). Following hybridization, filters were processed by the recommended Boerhinger-Mannheim procedure, and autoradiographs were produced by exposing filters saturated with Lumiphos (Boerhinger-Mannheim) to X-ray film for 1–2 h. The number of cells of a given species per milliliter was determined by multiplying the percent of colony-forming units positive for a particular probe by the number of colony-forming units per milliliter.

Results were compared statistically between polluted (FOU2, FOU3) and unpolluted (FOU1, UTR1, UTR2) sites, using an unpaired-sample *t*-test. Intra- (FOU2, FOU3 vs. UTR1, UTR2) and interstream (FOU1 vs. FOU2, FOU3) differences were detected with the same test using StatView (ver. 4.02, Abacus Concepts, Berkley, Calif.). Percent culturable was arcsine-transformed before analysis.

Results

Water chemistry and physical data illustrated the differences between polluted and unpolluted sampling sites. Polluted sites had at least twice the concentration of magnesium (Mg) (0.7–0.8 ppm polluted, 0.3–0.4 ppm unpolluted, $n = 3$, $P < 0.001$), and four times higher concentrations of calcium (Ca), and sodium (Na) (Fig. 1A) ($P < 0.001$). Although iron (Fe) concentrations were much lower at FOU3 than FOU2, no statistically significant differences were found. Manganese (Mn) was undetectable at all sites except the most polluted, FOU2 (1.44 ppm), and NO_3 concentration at FOU3 was the greatest measured at any site (0.53 ppm; other sites 0.001–0.071 ppm; data not shown). DOC concentrations were not significantly different among sites, however, dissolved inorganic carbon (DIC) and Ca followed similar trends ($P = 0.001$) (Fig. 1B).

Of the physical measurements made, TDS was three times higher at the polluted sites (Fig. 1C; $P < 0.0001$). Even though turbidity at FOU2 was twice that measured at unpolluted sites, no consistent correlation existed between pollution and this measurement. Precipitation during sampling was 0.0–0.41 cm for all sites except FOU3 (0.86 cm), which had only 0.10–0.18 cm the four days prior to sampling for all sites (except FOU3, 0.76 cm) (Savannah River Technical Center, unpublished data).

Assemblage-level responses to pollution were seen among the culturable bacteria. A significant reduction in colony-forming units per milliliter occurred at polluted sites ($P = 0.002$) (Table 2). This was especially apparent at the area of highest impact (FOU2), at which the cfu ml^{-1} results were 50% lower than at the unpolluted sites (Fig. 2). Although there was a threefold decrease in total bacterial numbers from FOU2 to FOU3, no significant differences between unpolluted and polluted sites were found. The ratio between total bacterial numbers and colony-forming units per milliliter gives percent culturable, or a measure of bacteria that responded to culturing conditions out of the total assemblage. The percent culturable was lower at polluted sites in

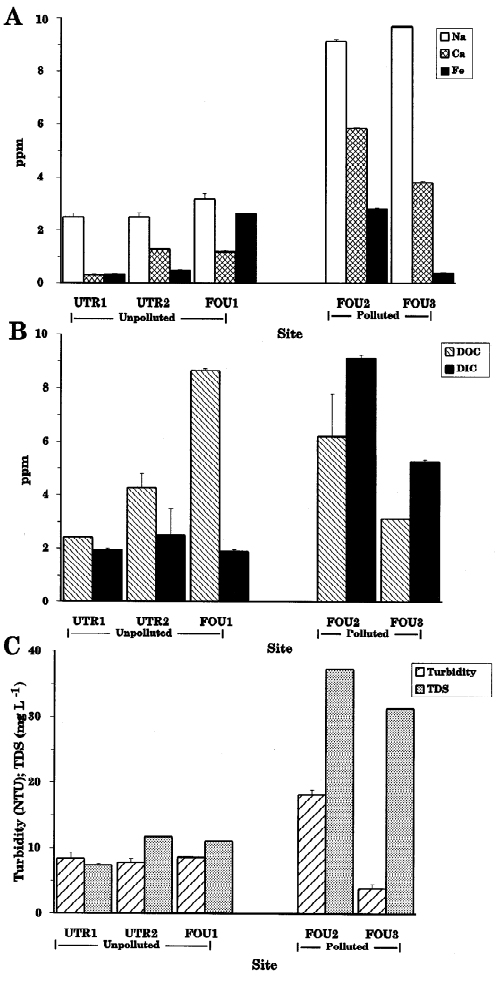

Fig. 1. Chemical and physical measurements for stream sampling sites. (**A**) Concentrations of sodium (Na), calcium (Ca), and iron (Fe) from water chemical analyses; (**B**) physical measurements of turbidity and total dissolved solids (TDS); and (**C**) carbon: dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) (average + standard error; $n = 3$).

general (Table 2; $P = 0.014$), and at FOU2 in particular (0.04%; Fig. 2).

The number of individuals of a given species per unit volume of stream water represents a quantifiable population response representative of the metabolically active bacteria in the assemblage. This measure of relative contribution allows us to compare the success of different species under particular conditions. Of the three bacterial species examined, only *A. calcoaceticus* showed a consistent response to the pollution at SRS (Fig. 3). The contribution of *A. calcoaceticus* to the total bacterial assemblage (number per milliliter) was nearly nine times higher at the polluted sites than at unpolluted sites (Fig. 3A; avg. 381, SEM 166, $n = 10$ polluted; avg. 43, SEM 13, $n = 15$ unpolluted; $P = 0.020$). No

Table 2. Assemblage-level bacterial response at polluted and unpolluted stream sites[a]

	Unpolluted	Polluted
Number of bacteria ml^{-1} ($\times 10^6$)	1.41(0.18)	2.06(0.36)
cfu ml^{-1} ($\times 10^3$)	3.15(0.21)[b]	1.96(0.28)
% Culturable	0.26(0.03)[b]	0.15(0.04)

[a] Mean, standard error of the mean in parentheses; $n = 10$ polluted (FOU2 and FOU3), $n = 15$ unpolluted (FOU1, UTR1, UTR2)
[b] Significant difference at alpha ≤ 0.05

differences were seen in the relative contribution of either *B. cepacia* (Fig. 3B; avg. 113, SEM 26, $n = 10$ polluted; avg. 109, SEM 22, $n = 15$ unpolluted) or *P. putida* (avg. 75, SEM 21, $n = 9$ polluted; avg. 202, SEM 70, $n = 15$ unpolluted). The number of *P. putida* at FOU1 was significantly higher than at downstream sites (intrastream comparison; $P = 0.013$) (Fig. 3C).

Discussion

In contrast to UTR, the waste seepage area at FOU affected the aquatic chemical composition and associated bacterial assemblages. Abnormally higher concentrations of Na at FOU2 and FOU3 were consistent with reported seepage of sodium hydroxide and sodium salts from holding ponds into FOU [2, 3, 40]. Other waste components (oxides and hydroxides of Mn, Fe, and aluminum, sodium nitrate, nitrite, and aluminate salts) were also reflected in water chemistry data. Although nitrates, nitrites, and nitric acid were reported in groundwater up- and downstream from FOU2 [3, 11], the origin of elevated NO_3 detected at FOU3 was apparently holding ponds downstream from FOU2. Simultaneously high concentrations of Ca and DIC resulted from pollutants locally elevating pH that, in turn, increased solubility of calcium carbonate [40]. Abnormally high turbidity

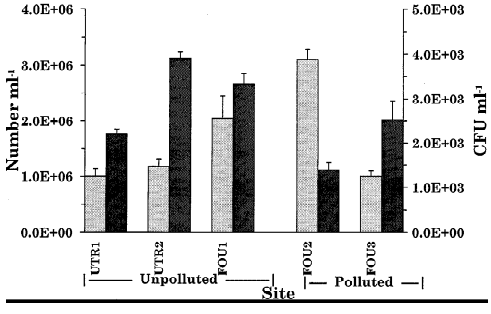

Fig. 2. Total bacterial number (*gray bars; left axis*) and 3-day viable populations grown on modified nutrient agar (*dark bars; right axis*) (average + standard error; $n = 5$) for stream water collected at the Savannah River Site.

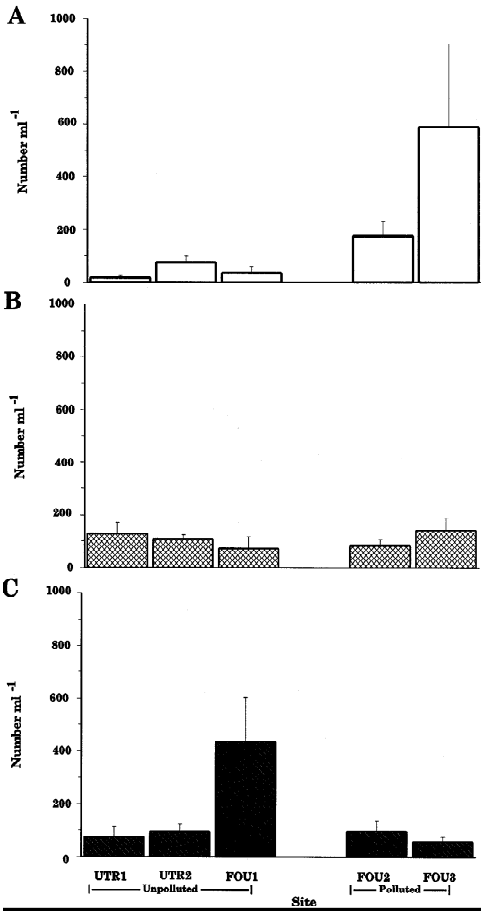

Fig. 3. The number of bacteria identified as either *A. calcoaceticus* **(A),** *B. cepacia* **(B),** or *P. putida* **(C)** in stream water, polluted and unpolluted stream sites in two SRS drainages (average + standard error of the mean; $n = 5$).

at FOU2 probably resulted from a high level of suspended solids in groundwater [3]. Precipitation, which can cause an influx of particles and bacteria to streams due to overland runoff, may induce erosion to a greater extent along thermally altered streams [13]. However, rainfall was too low at the time of sampling to account for the high turbidity.

Bacterial assemblage composition in polluted reaches of FOU differed from upstream areas. Although bacteria were first exposed to pollutants near FOU2, the true impact of the exposure may not be evident until FOU3, at which point cells would have been in prolonged contact with contaminants. The effect of pollution may be best realized, on an assemblage level, by the relatively low number of culturable bacteria at FOU2. Pollutants present at FOU2 may have killed planktonic bacteria, or made them metabolically inactive.

A. calcoaceticus numbers were highest at FOU3 (Fig. 3A), accounting for nearly 20% of the culturable assemblage. The ability of this organism to degrade chemical pollutants and aromatic compounds [33, 38] may contribute to its tolerance of conditions in lower FOU, as well as allow it to utilize substrates unavailable to other bacteria. Along with organic contaminants in the groundwater, other xenobiotic compounds suitable as carbon substrates for use by *A. calcoaceticus* possibly came from coal-pile run-off at FOU2 [40]. The ability to utilize alternate carbon sources may give this species a competitive advantage in the polluted stream, as compared to conditions in the reference stream. Increased abundance of *A. calcoaceticus* at polluted sites makes it a good biological indicator of pollution in these systems.

Similar abundances of *B. cepacia* at all sites indicates that it is adapted to a wide variety of conditions, because of its metabolic and genetic diversity [23, 29, 43]. *B. cepacia* has been shown to degrade trichloroethylene [17], as well as many other xenobiotics present in the SRS waters. The fact that it did not thrive in these polluted sites implies that pollution-associated factors (i.e., alternative substrates, toxic properties) do not affect *B. cepacia* populations, whereas ubiquitous factors that control bacterial populations may be more important (i.e., nutrient availability, predation, or competition).

While *P. putida* is able to degrade specific recalcitrant carbon compounds in the laboratory [12, 26, 36], comparatively little is known about its metabolic limitations in nature. *P. putida* abundance was highest at FOU1 but decreased at FOU2 and FOU3; the opposite trend was seen for *A. calcoaceticus* at these sites. It did not show similarly high abundances at other unpolluted sites. The fact that this species accounted for over 25% of the culturable assemblage only at the FOU headwaters, and not at other undisturbed sites, suggests that its abundance is site-related; factors other than pollution affect its contribution to the bacterial assemblage.

Pollution in Four Mile Creek, apparent from longitudinal changes in water chemistry and physical characteristics, impacted the percent culturable bacteria and contributions of specific species to the culturable assemblage. Of the three species examined through colony hybridization, *A. calcoaceticus* and *P. putida* exhibited the greatest response; the decline in *P. putida* abundance was probably not attributable to pollution. The fact that *A. calcoaceticus* thrived at the polluted sites suggests that it will serve as a useful biological indicator of chemical pollution.

Previous studies using bioindicators to examine effects of SRS operations on lotic ecosystems have yielded mixed re-

sults. Streams impacted by SRS operations drain to the Savannah River, at which surveys of the algal, protozoan, macroinvertebrate, insect, and fish communities have been conducted. Of these studies, only decreased diatom diversity could be attributed to poor water quality [3], suggesting that either adequate water conditions exist or there is a need for a more sensitive indicator.

This study represents an initial effort to use select bacterial species responses as a method of biomonitoring, as well as identification of species useful for bioremediation. Colony hybridization allows species identification and maintenance of strains, so subsequent research utilizing isolates may be performed. Costs for labeling probes and hybridization may limit the widespread use of this monitoring method. However, as with monitoring of coliforms, it may be possible to use selective culture media to enumerate known bacterial indicators or, alternatively, develop immunological tests using labeled antibodies for routine identification during monitoring.

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References

1. American Public Health Association (1992) Standard methods for the examination of water and wastewater, 18th edn. American Public Health Association, Washington D.C., pp 9.1–10.137
2. Arnett MW (ed) (1994) Savannah River Site environmental data for 1994. Publication WSRC-TR-95-077. USDOE Westinghouse Savannah River Co., (Savannah River Site,) Aiken S.C.
3. Arnett MW, Mamatey AR, Spitzer D (eds) (1994) Savannah River site environmental report for 1994. Publication WSRC-TR-95-077. USDOE Westinghouse Savannah River Co., Savannah River Site, Aiken S.C.
4. Ballard RW, Palleroni NJ, Doudoroff M, Stainer RY, Mandel M (1970) Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia, P. marginata, P. alicola, and P. caryophyll.* J Gen Microbiol 60:199–214
5. Bar-Or Y, Shilo M (1988) Cynobacterial flocculants. Methods Enzymol, Acad Press, San Diego, CA, 167:616–622
6. Bauman P (1968) Isolation of *Acinetobacter* from soil and water. J Bact 96:39–42
7. Braun-Howland EB, Vescio PA, Nierzwicki-Bauer SA (1993) Use of simplified cell blot technique and 16S rRNA–directed probes for identification of common environmental isolates. Appl Environ Microbiol 59:3219–3224
8. Chase J (1995) Mixed waste management facility FSS well data groundwater monitoring report. Fourth quarter 1994 and 1994. Publication WSRC-TR-94-0610 (NTIS No. DE 95060118). USDOE Westinghouse Savannah River Co., Aiken, S.C.
9. Daubaras DL, Hershberger CD, Kitano K, Chakrabarty AM (1995) Sequence analysis of a gene cluster involved in metabolism of 2,4,5-trichlorophenoxyacetic acid by *Burkholderia cepacia* AC1100. Appl Environ Microbiol 61:1279–1289
10. Daugherty DD, Karel SF (1994) Degradations of 2,4-dichlorophenoxyacetic acid by *Pseudomonas cepacia* DBO1 (pRO101) in a dual-substrate chemostat. Appl Environ Microbiol 60:3261–3267
11. Dixon KL, Rogers VA (1992) Semi-annual sampling of Fourmile Branch and its seeplines F and H areas of SRS: July 1992 (U). Publication WSRC-TR-93-289. USDOE Westinghouse Savannah River Co., Aiken, S.C.
12. Guerin WF, Boyd SA (1995) Maintenance and induction of naphthalene degradation activity in *Pseudomonas putida* and an *Alcaligenes* sp. under different culture conditions. Appl Environ Microbiol 61:4061–4068
13. Hauer FR (1985) Aspects of organic matter transport and processing within Savannah River plant streams and the Savannah River flood plain swamp. Savannah River Ecology Lab, Aiken, S.C.
14. Janakiraman A (1996) Survival of introduced bacteria in steam microcosm. MS thesis, Kent State University
15. Juni E (1978) Genetics and physiology of *Acinetobacter.* Annu Rev Microbiol 32:349–371
16. Kentucky Department of Environmental Protection (1993) Methods for assessing biological integrity of surface waters. Kentucky Department of Environmental Protection, Frankfort, Ky.
17. Landa AS, Sipkema Em, Weijma J, Beenackers AACM, Dolfing J, Janssen DB (1994) Cometabolic of trichlorethylene by *Pseudomonas cepacia* G4 in a chemostat with toluene as the primary substrate. Appl Environ Microbiol 60:3368–3374
18. Langley TM, Marter WL (1973) The SRP plant site. Publication DP-1323. Savannah River Laboratory. E.I. DuPont de Nemours and Co., Aiken, S.C.
19. Leff LG (1994) Stream bacterial ecology: a neglected field? ASM News 60:135–138
20. Leff LG, Meyer JL (1991) Biological availability of dissolved organic carbon to bacteria along the Ogeechee River continuum. Limnol Oceanogr 36:315–323
21. Leff LG, Kernan RM, McArthur JV, Shimkets LJ (1995) Identification of aquatic *Burkholderia* (*Pseudomonas*) *cepacia* by

hybridization with species-specific rRNA gene probes. Appl Environ Microbiol 61:1634–1636
22. McArthur JV, Kovacic DA, Smith MH (1988) Genetic diversity in natural populations of a soil bacterium across a landscape gradient. Proc Natl Acad Sci USA 85:9621–9624
23. McArthur JV, Leff LG, Smith MH (1992) Genetic diversity of bacteria along a stream continuum. J N Am Benthol Soc 11: 269–277
24. Meador MR, Cuffney TF, Gurtz ME (1993) Methods for sampling fish communities as part of the National Water-Quality Assessment Program. U.S. Geological Survey, open-file report 93-104. Raleigh, N.C.
25. Metcalfe JL (1989) Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. Environ Pollut 60:101–139
26. O'Connor K, Buckley CM, Hartmans S, Dobson ADW (1995) Possible regulatory role for nonaromatic carbon sources in styrene degradation by *Pseudomonas putida* CA-3. Appl Environ Microbiol 61:544–548
27. Ohio Environmental Protection Agency (1987) Biological criteria for the protection of aquatic life, vol 1. The role of biological data in water quality assessment. Division of Water Quality Monitoring and Assessment, Columbus, Ohio
28. Ohio Environmental Protection Agency (1987) Biological criteria for the protection of aquatic life, vol 3. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Division of Water Quality Monitoring and Assessment, Columbus, Ohio
29. Palleroni NJ (1975) General properties and taxonomy of the genus *Pseudomonas.* In: Clarke PH, Richmond MH (eds) Genetics and biochemistry of *Pseudomonas.* Wiley Press, London, pp 1–36
30. Plafkin JL, Barbour MT, Porter KD, Gross SK, Hughes RM (1989) Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. U.S. Environmental Protection Agency, Washington D.C.
31. Porter KG, Fieg YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–947
32. Rosenberg E, Parry A, Gibson D, Gutnick DL (1979) Emulsifier of *Arthrobacter* RAG-1: specificity of hydrocarbon substrate. Appl Environ Microbiol 37:409–413
33. Rosenberg E, Rubinovitz C, Gottlieb A, Rosenhak S, Ron EZ (1988) Production of biodispersan by *Acinetobacter calcoaceticus* A2. Appl Eviron Microbiol 54:317–322
34. Roszak DB, Colwell RR (1987) Survival strategies of bacteria in the natural environment. Microbiol Rev 51:365–379
35. Schleifer KH, Amman R, Ludwig W, Rothemund C, Springer N, Dorn S (1992) Nucleic acid probes for the identification and *in situ* detection of pseudomonas. In: Galli E, Silver S, Witholt B (eds) *Pseudomonas:* molecular biology and biotechnology. American Society for Microbiology, Washington D.C., pp 127–134
36. Schwartz A, Bar R (1995) Cyclodextrin-enhanced degradation of toluene and *p*-toluic acid by *Pseudomonas putida.* Appl Environ Microbiol 61:2727–2731
37. Shimkets LJ, Asher SJ (1988) Use of recombination techniques to examine the structure of the *csg* locus of *Myxococcus xanthus.* Mol Gen Genet 211:63–71
38. Towner KJ (1992) The genus *Acinetobacter.* In: Balows A, Trüper HG, Dworkin M, Hardner W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer-Verlag, New York, pp 3137–3143
39. U.S. Department of Agriculture Soil Conservation Service (1990) Soil survey of the Savannah River Plant area, parts of Aiken, Barnwell, and Allendale counties, South Carolina. USDOE, USDA, USFS, S.C. Agricultural Experiment Station, S.C. Land Resources Conservation Commission, Aiken, S.C.
40. U.S. Department Energy (1991) Savannah River site environmental report for 1991. Westinghouse Savannah River Co., Aiken, S.C., pp 199–442
41. Voshell JR, Hiner SW (1988) Shenandoah National Park long-term ecological monitoring system, Section III. Aquatic component user manual. MAR-38-III, Shenandoah National Park, Luray, Va.
42. Whitehurst IT (1991) The *Gammaurus:Asellus* ratio as an index of organic pollution. Water Res 23:333–339
43. Wise MG, Shimkets LJ, McArthur JV (1995) Genetic structure of a lotic population of *Burkholderia* (*Pseudomonas*) *cepacia.* Appl Environ Microbiol 61:1791–1798
44. Workman SW, McLeod KW (1990) Vegetation of the Savannah River Site: major community types. Savannah River Site National Environmental Research Park Program, Aiken, S.C.