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SG 43-96

Final Report to the Lake Erie Protection Fund
Grant No: SG 4380 "Plasmid Fingerprinting to Identify the Pollution
Source(s) at the Maumee Bay State Park".

PI: Dr. Lou Glatzer

Date: 10/25/98

A handwritten signature in black ink, appearing to read "Lou Glatzer", written over the printed name and date.

In order to solve a pollution problem which has closed the Lake Erie and "Inland Lake" beaches at the MBSP for a number of summers, it is necessary to identify the likely polluting sources of these waters. Locating this (these) sources has eluded previous tracing determinations because there is always substantial variation in the *Escherichia coli* titers from one day to the next during the swimming season. In fact, there have been numerous potential sources identified in the area of the park but no isolations of the organisms had been carried out until this project was conceived. A method was devised to identify **specific** bacterial strains causing the problem using unique biological parameters. A well-elaborated sampling protocol was developed for recovery of *E. coli* from these potential sources in the MBSP environs in co-operation with the ODNR, the Lucas County Department of Health, and the City of Toledo Department of Environmental Services and the Water Department.

Many enteric (intestinal) bacteria (including *E. coli*) contain plasmids which represent a partial identity of the inheritable content (DNA) of any given strain. Determining the bacterial plasmid patterns by DNA sizing in electrophoresis and enzyme digestion products (called restriction enzyme fragment length polymorphisms - RFLP), can provide the specificity needed to generate the genetic identities of the strains; strains that are also found in the Lake waters and which have caused beach closings. Identification of the source by identities of traceable plasmid DNA patterns in the *E. coli* isolates could be a major step to solving the problem.

The LEPF/Small grant permitted us to select 585 *Escherichia coli* isolates for this study. These were recovered from 5-8 locations in the Maumee Bay State Park area, which included three drainage ditch locations, the Lake Erie swimming beach, the Inland Lake beach, marsh drainage, ditch sediment release, and occasional sampling of goose and gull fecal matter. During the period of this grant, the strains were isolated and confirmed as *E. coli* from a collection of over 600 Idexx enumeration trays by four different criteria (Presumptive ID on Idexx enumeration plates, several *E. coli*-specific isolation petri plates, several key enzyme tests, and a battery of 30 biochemical identification criteria typically used in a clinical laboratory). These isolates were then placed in permanent, duplicate -70 degree freezer stocks for future studies. Confirmation of *E. coli* and establishment of the freezer stocks took the better part of the grant year. A number of student research assistants provided the labor for this monumental task which was enabled by the small grant fund. The early discovery that only 40-50% of the isolates contained plasmids led to a doubling in the number of isolates screened for recovery and substantially increased the time to complete the collection.

As a result of this effort, we now have available a rare collection of 585 regional isolates of *E. coli* which were contaminating the lake waters, the ditches, or the sediments in the MBSP area. These are now being characterized using several molecular technologies in several labs including mine.

Electrophoretic evaluation of the plasmids within these strains was initiated under this grant support. However, only a few were analyzed during the grant period as a result of the lengthy isolation/purification, confirmation, and storage protocols needed to guarantee purity and reliable access to these isolates. Currently several labs are involved in the analysis of DNA and membrane fatty acids, including my own.

The DNA electrophoresis gels showed only a few paired strains in spatial or temporal comparisons but instead showed great heterogeneity. Thus, it is clear that we are dealing with a rather large population of contaminating *E. coli* which could easily be due to multi-point sources including , in the Inland Lake, a regular population of gulls and (to a lesser extent), geese. Singular identities of isolates of any single sampling day from potential contaminating sources (such as the drainage ditches and

bird fecal matter) or in the Lake Erie Beach waters and the MBSP Inland Lake waters are rare. A few identities have been found on dates separated by weeks or over one month indicating a repeat source of contamination. Although limited in our observations, occasional detection of identical strains at different times and in different locations, is an indication that gull or goose populations are one potential contaminating source common to the MBSP area throughout the summer. We also believe that ditch and lake sediments may act as long term reservoirs for contamination deposits. These events could be due to repeated contamination of ditches and Wolf Creek (which enters Lake Erie at the park marina) by improperly serviced or nonfunctioning home or business septic systems.

Further work is now underway to expand our sampling to focus on bird populations as a contamination source and to trace the longevity of *E. coli* in the regional ditch sediments and lake sediments. An alternative technology called Pulsed-Field Electrophoresis which will use the chromosomal DNA of *E. coli* instead of plasmid DNA for tracing the bacterial strains is now under development. This work continues under additional and separate grants.

Thus, the primary outcome of these seed/small grant monies has been to provide us with a reliable population of pure, contamination-associated *E. coli*. These isolates are now available for study (including our current studies) of this MBSP pollution problem. They are also being used in the laboratory of Dr. Robert Sinsabaugh, UT-Lake Erie Center.

A first report of our findings is published in the Proceedings of the Eighth Meeting of the the International Society of Microbial Ecologists, August 1998.

The full citation is:

***ESCHERICHIA COLI* CONTAMINATION OF MAUMEE BAY STATE PARK WATERS.** Dr. Lou Glatzer and Chris Lauber, Biology Dept., The University of Toledo, 2801 W. Bancroft, Toledo, OH 43606. lglatze@uoft02.utoledo.edu

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