J. Great Lakes Res. 32:401–405
Internat. Assoc. Great Lakes Res., 2006

NOTE

Persistence and Potential Growth of the Fecal Indicator Bacteria, *Escherichia coli*, in Shoreline Sand at Lake Huron

Elizabeth Wheeler Alm•, Janice Burke†, and Erin Hagan‡

Department of Biology
Central Michigan University
157 Brooks Hall
Mount Pleasant, Michigan 48859

ABSTRACT. *Recent investigations found high abundances of the fecal indicator* Escherichia coli *in shoreline sand at freshwater beaches, but it is not known whether these high numbers are due to passive filtration/trapping of the bacteria, or to colonization and growth. This study was initiated to test the hypothesis that high abundance can be explained, at least in part, by the ability of* E. coli *to persist and grow in beach sand. A combination of laboratory and field studies was used to monitor the densities of environmental isolates of* E. coli *in beach sand. In controlled laboratory microcosm studies using autoclaved beach sand inoculated with* E. coli *strains previously isolated from ambient beach sand,* E. coli *densities increased from 2 CFU/g to more than* 2×10^5 *CFU/g sand after 2 days of incubation at 19°C, and remained above* 2×10^5 *CFU/g for at least 35 days. In field studies utilizing similarly inoculated beach sand in diffusion chambers incubated at a Lake Huron beach,* E. coli *also grew rapidly, reaching high densities (approximately* 7.5×10^5 *CFU/g), and persisting in a cultivable state at high density for at least 48 days. In comparison,* E. coli *levels in ambient beach sand adjacent to the chambers always had densities <100 CFU/g. Lake Huron beach sand clearly provides nutrients, temperatures, and other conditions needed to support growth of* E. coli. *The growth of* E. coli *in sterile sand diffusion chambers to higher levels than occurs in ambient beach sand may indicate the presence in ambient sand of biological controls on bacterial growth, such as predation or competition.*

INDEX WORDS: *Water quality, beach monitoring, sand, fecal indicator,* E. coli*, persistence, growth.*

INTRODUCTION

Increased monitoring of fecal indicator bacteria in water at recreational beaches in North America has resulted in increased numbers of beach advisories and closings. Fecal indicator bacteria are abundant in human and other animal feces and are recovered frequently from natural environments. Epidemiological studies have shown that in freshwater environments, high densities of *Escherichia coli* are correlated with gastrointestinal symptoms among swimmers (Cabelli *et al*. 1982) and *E. coli* are recommended as bioindicators for monitoring fecal contamination at freshwater beaches (U.S. EPA 1986). However, if *E. coli* can persist in freshwater environments throughout the warm season (transient persistence) or even persist from one year to another (indigenous persistence) as some have suggested (Whitman and Nevers 2003), then the usefulness of *E. coli* as an indicator of recent contamination at beaches is compromised.

Beach sand may be one component of the freshwater environment where *E. coli* persist and perhaps grow in the Great Lakes region. Recent studies demonstrate high densities of *E. coli* in exposed sands around the Great Lakes, even when their densities in the water are low (Alm *et al*. 2003, Haack *et al*. 2003, Whitman and Nevers 2003) and sand populations of *E. coli* have been suggested as a source of *E. coli* to recreational water (Byappanahalli *et al*. 2003, Whitman and Nevers 2003, Whit-

•Corresponding author. E-mail: alm1ew@cmich.edu
†Current address: Faculty of Forestry and the Forest Environment, Lakehead University, Thunder Bay, Ontario.
‡Current address: Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109.

man *et al*. 2003). Therefore, we tested the hypothesis that *E. coli* isolated from the freshwater sand environment can grow in sand under summer beach conditions.

METHODS AND RESULTS

Conger Lighthouse Beach (42.9708 N by 82.4250 W) is located on Lake Huron just north of Port Huron, Michigan. Water samples were collected just offshore at a 1-m depth into acid washed Nalgene® bottles (Nalge Nunc International) attached to the end of a 2-m pole. Sand samples were collected from the wave-washed (swash) zone of the shoreline into sterile Whirlpak® bags. All samples were kept on ice during transport and until analysis.

Controlled laboratory microcosm experiments were performed to determine whether *E. coli* could grow in sand. Two bacterial isolates were recovered from the top 10 cm of swash-zone sand at Conger Beach on 9 July 2002 by sand slurry filtration (Alm *et al*. 2003) and overnight incubation on mTEC plates according to U.S. EPA guidelines (U.S. EPA 2000) and were then immediately frozen in glycerol and stored at –80°C. Testing of these isolates in API20E test strips (bioMerieux) determined a > 92.5% probability of the isolates being *E. coli*. Further evidence in support of an identification of the beach isolates as *E. coli* was provided by positive polymerase chain reaction assays for the *E. coli* malate dehydrogenase gene (S.T. Walk, L.M. Calhoun, L.J. DeRose-Wilson, E.W. Alm, and T.S. Whittam, Abstr. 105th Amer. Soc. Microbiol., abstr. Z009, 2005). Prior to sand amendment, the frozen isolates were rejuvenated by overnight incubation in Tryptic Soy Broth. Following overnight incubation, the culture was washed four times (microcentrifugation at 6,000 rpm for 10 min followed by resuspension in 1 mL 0.8% [w/v] NaCl) to remove media and then resuspended to an approximate density of 1.5×10^3 colony forming units (CFU)/mL as determined by comparison to a 0.5 MacFarland turbidity standard; initial densities were confirmed by plate count. One mL of *E. coli* suspension was added to 200 g of autoclaved sand (121ºC, 30 min) collected from Conger Beach, and 10 g of inoculated sand were transferred to 50-mL centrifuge tubes. Microcosms containing only sterile sand served as uninoculated controls. Immediately after inoculation (time 0), duplicate inoculated microcosms and one control microcosm were sampled. Sand slurries were prepared and filtered and

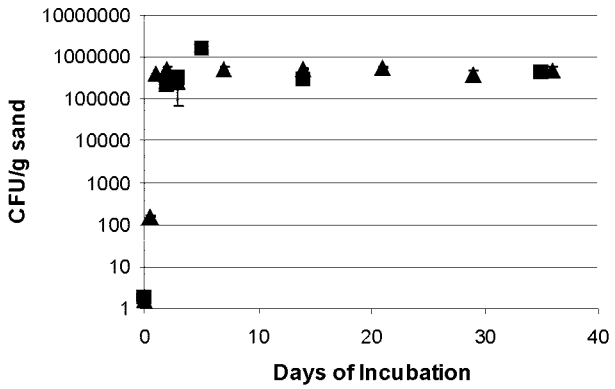

FIG. 1. Mean (± standard deviation) abundance of beach* E. coli *in duplicate laboratory sand microcosms incubated at 19°C. Experiment 1 (■) and Experiment 2 (▲).

colonies were enumerated on mTEC plates as described above. Remaining sand microcosms were incubated in the dark at a constant temperature of 19ºC (average summer sand temperature, 2002) and colonies were enumerated from duplicate inoculated microcosms and from an uninoculated control microcosm at 2, 3, 5, 14, and 35 days (Experiment 1). Data from Experiment 1 demonstrated that most *E. coli* growth occurred between time 0 and day 2, therefore the experiment was repeated with finer sampling intervals during this period (8 hours and 1, 2, 3, 7, 14, 21, 29, and 36 days; Experiment 2).

In Experiment 1, *E. coli* densities at time 0 were 2 CFU/g sand (wet weight; mean of duplicate columns) and increased to 2×10^5 CFU/g by day 2, and then persisted above 2×10^5 CFU/g sand to day 35 (Fig. 1). In Experiment 2, *E. coli* densities in sand microcosms at time 0 were 1 CFU/g sand, and increased to 2×10^2 CFU/g sand by 8 hours. *E. coli* densities reached 4×10^5 CFU/g sand by day 1 and remained above 4×10^5 CFU/g sand to day 36 (Fig. 1). The growth rate (determined in Exp. 2 according to Madigan *et al.* 2003) appeared logarithmic at 8 hours with approximately 72 minutes/generation and slowing to 471 minutes/generation by day 7.

To determine whether *E. coli* have the potential to grow in sand at a beach under ambient conditions, in situ studies were performed at Conger Beach from 2 July to 19 August 2003. Diffusion chambers (13 × 5 cm, length by diameter) were constructed from opaque PVC pipe. Each chamber was packed with 400 g autoclaved sand from Conger Beach that was inoculated with the same *E. coli* isolates described above. Membrane filters (Milli-