African Journal of Environmental Science and Technology Vol. 1 (1), pp. 007-013, August 2007
Available online at http://www.academicjournals.org/AJEST

Full Length Research Paper

Using nutrient utilization patterns to determine the source of *Escherichia coli* found in surface water

Jacinta C. Uzoigwe, Eric H. O'Brien and Edward J. Brown*

Environmental Programs, University of Northern Iowa, Cedar Falls, IA 50614-0421, USA.

Accepted 31 July, 2007

Identifying the sources of fecal contaminants in surface water bodies such as rivers, lakes and beaches is of importance for environmental safety, public health safety, food safety and regulatory purposes. Nutrient utilization patterns (NUPs) were used as a bacterial source tracking technique to identify the possible sources of fecal coliform bacteria, *Escherichia coli* in Silver Lake, Delaware County, Iowa. A total of three hundred (300) *E. coli* isolates collected from different sources (water, birds, geese, cattle, hogs and soil contaminated by feces) were analyzed. A database was built from these isolates by using discriminant analysis to identify the nutrient utilization patterns that best classify all 300 isolates by source. The average rate of correct classification by source was 89.5% when applying the nutrient utilization patterns database. After this verification, the NUP for *E. coli* isolates from Silver Lake water were measured. Based on the NUPs of the Silver Lake isolates, 73.1% were found to originate from cattle and hogs. Smaller percentages were predicted to be coming from birds and geese. None of the isolates were predicted to be originating from the human source. The results indicate that livestock are the primary contributors to fecal pollution in this hypereutrophic Iowa lake.

Key words: Nutrient utilization patterns, *Escherichia coli*, bacterial source tracking, Silver Lake, Delaware, Iowa.

INTRODUCTION

Surface water quality in many lakes and rivers suffers by the presence of high levels of fecal coliform bacteria, which is indicative of contamination with the feces of warm-blooded animals (Ackman et al., 1997; Jones and Roworth, 1996). Fecal pollution is a serious environmental problem that affects many coastal regions in the United States and worldwide. Fecal contamination resulting from humans, domestic animals, and wildlife poses a serious threat to human health and the environment. The presence of pathogens leads to human diseases and economic losses for industries that depend on water quality of lakes, rivers and streams (Ackman et al., 1997). Contamination of water with fecal coliform bacteria of human origin may signal the presence of other potential human pathogens, such as *Salmonella* spp., *Shigella* spp., hepatitis A virus, and Norwalk group viruses (Guzewich and Morse, 1986; Orskov and Orskov, 1981). In order to protect United States' surface waters, there are federal guidelines limiting quantity of fecal bacteria (USEPA, 1986). In 1986, the Federal Water Pollution Control Administration (FWPCA) instituted criteria for testing water samples, which set the limit at 200 fecal coliform organisms per 100 ml of water. Also in 1986, the EPA published the *Ambient* Water Quality Criteria for Bacteria-1986, which reports the results of studies investigating the link between swimming associated illnesses and microorganisms. According to these studies, the specific fecal coliform bacteria, *Escherichia coli* showed a stronger correlation to these illnesses than total fecal coliform organisms, which were previously recommended as indicators in 1968 by the FWPCA (USEPA, 1986). Guan et al. (2002) reported that, one of the most important and identifiable aspects of water quality are the presence of fecal coliform bacteria, especially *E. coli*. Increased levels of fecal bacteria are a concern for recreational waters; however, the source of contamination is often unknown.

Counts of *E. coli* cells in water indicate the potential presence of pathogenic microbes of intestinal origin but

*Corresponding author. E-mail: ed.brown@uni.edu. Tel: (319) 273-2645. Fax: (319) 273-7125

Abbreviations: NUP, nutrient utilization pattern; BST, bacterial source tracking; TMDL, total maximum daily load; BMP, best management practice; MAR, multiple antibiotic resistance; PFGE, pulse field gel electrophoresis, TMDL, total maximum daily load.

give no indication of the sources of the microbial pollution. Despite efforts to minimize fecal input into coastal waterways and beaches, the problem persists, partly due to an inability to reliably identify non point sources. These sources may include inefficient sewage treatment plants, leaking septic systems, agricultural runoff, or wildlife (Strittholt et al., 1998).

Identification of the source of the bacterial contamination is an essential first step in seeking to control fecal contamination of water. In particular, it is important to determine whether the source of fecal contamination is of human, livestock, or wildlife origin, as microorganisms of human origin are regarded as having greater potential to cause disease in humans (Puech et al., 2001). Bacterial source tracking (BST) (also called microbial source tracking, fecal source tracking or fecal typing) is new methodology that is being developed to determine the sources of fecal bacteria from environmental samples (e.g. from human, livestock, or wildlife origins). According to Parveen et al. (1997), bacterial source tracking is the determination of the animal origin of fecal bacteria in natural waters which result from point or non point pollution. There are two types of BST methods, phenotypic and genotypic (Scott, 2002; Simpson et al., 2002). Phenotypic methods are based on characteristics expressed by fecal bacteria and genotypic methods are based on DNA sequences. Three primary genetic techniques are available for BST. Ribotyping characterizes a small, specific portion of the bacteria's DNA sequence (Samadpour et al., 2005; Scott et al., 2003); pulse-field gel electrophoresis (PFGE) is similar to ribotyping but typically is performed on the entire genome of the bacteria (Lu et al., 2004). The polymerase chain reaction (PCR), amplifies selected (nonribosomal) DNA sequences in the bacteria's genome (Makino et al., 1999). Phenotypic techniques generally involve either a nutrient utilization technique where different nutrient sources are used to produce a metabolic profile of microorganisms (Garland and Mills, 1991) or antibiotic resistance analysis, where resistance patterns from a suite of different concentrations and types of antibiotics are measured (Hagedorn et al., 1999; Wiggins, 1996). According to some researchers (Hagedorn, 2004; Martellini et al., 2005; Meays et al., 2004), several methods are currently available for bacterial source tracking, however all of the BST methods are still being developed and/or evaluated to varying degrees. The ultimate goal of bacteria source tracking (BST) is to identify the source of indicator bacteria isolated from surface waters. Fecal coliforms, *E. coli* and the bacteria of the genus *Enterococcus* are used extensively in the US and throughout the world as indicator organisms to signal fecal contamination in water (Garland and Mills, 1991). The objectives of this studywere: (1) to design a methodology to determine nutrient utilization profiles for *E. coli* isolates as a phenoltypic fingerprinting methodology, and (2) to identify the possible sources of the fecal coliform bacteria *E. coli* in Silver Lake, Delaware County, Iowa using the nutrient utilization technique.

MATERIALS AND METHODS

Sample collection and Isolation of E. coli

The *E. coli* isolates used in this study were obtained from fecal samples collected from Silver Lake and its Watershed, Delaware, Iowa. Out of a total number of 300 *E. coli* isolates, 123 isolates came from geese, 97 isolates came from cattle, 41 isolates came from lake water, 22 isolates came from hogs, 11 isolates came from birds, and 6 isolates came from soil near the outdoor restroom facilities. The fecal samples were made into slurry by putting 1 g of fecal material into sterile phosphate buffer solution (PBS). The slurry was then streaked onto MacConkey agar and incubated at 35°C for 24 h. All isolates exhibiting the characteristics of fecal coliforms were then further analyzed to confirm them as *E. coli* by growing them in EC broth with 4-methylumbelliferyl-β-D-glucuronide (MUG) (Fisher Scientific, Chicago, IL). After 24 h of incubation at 37ºC, test tubes were removed from the incubator and were observed using a long-wave ultraviolet (UV) light (365 nm). Most strains of *E.coli* produce β-glucuronidase which hydrolyzes MUG to the fluorogenic compound, 4-methyl-umbelliferone. Therefore any isolates fluorescing under the UV light were confirmed as *E. coli*. These positive samples were transferred to Tryptic Soy Agar (TSA) (Fisher Scentific, Chicago, IL) slants, properly labeled, grown at 35°C, and stored at 4°C, until needed for analysis.

BIOLOG GN2 microplate™ preparation

The method used in the study is modified from that developed by Biolog, Inc., (Hayward, CA.). The BIOLOG GN2 Microplates were used and consist of 96 wells, with 95 containing pre-selected nutrient sources, tetrazolium violet and a blank well (A1) with no substrate. The tetrazolium violet is a redox dye that serves as an indicator of the utilization of the nutrient. Prior to analysis, pure cultures of *E. coli* isolates were grown on Trypticase Soy Agar (TSA) (DIFCO Laboratories) at appropriate temperature 37ºC for up to 24 h. The cells must be freshly grown, since many strains lose viability and metabolic vigor in stationary phase. The innoculum was prepared in 20 mm diameter test tubes with sterile saline. A colony was chosen from the TSA plate using a sterile wire loop, and suspended in a 0.40% saline (0.4 g of sodium chloride in 100 mls of water) and mixed to obtain a uniform solution using a squeeze bulb pipette. Three drops of a 5 mM concentration of sodium thioglycolate (Sigma, Chicago) (7.6 g of thioglycolate in 100 ml of saline) were added to keep the cells from using their own biofilm or cell walls as a carbon source, which would give a false positive reaction. The thioglycolate is an anticapsule agent, and partially or completely inhibits the purple color in the A-1 well and other negative wells that can form when bacteria metabolize their polysaccharide capsule as a carbon source. The concentration of bacteria was determined by placing the tube containing the bacteria in a spectronic 20 spectrophotometer and adjusting the transmittance to 59 - 61%. This solution was then used to inoculate 96-well BIOLOG Microplates™ containing water blank well and 95 different dried carbon sources. All the wells started out colorless when inoculated, and the microplates were incubated at 37ºC for 24 h. After incubation, the wells are examined for the formation of purple color similar to the BIOLOG procedure. The appearance of a purple color indicates the utilization of the carbon source in a particular well. Because the cell can utilize the carbon source, it respires and after oxygen is depleted, the cells reduce the alternate electron acceptor, redox dye tetrazolium, to form the purple color. The absorbance of each well in the plates were then read at 590 nm for color, using a SPECTRA

Table 1. BIOLOG GN2Microplate™, nutrient sources, number and percentage of bird, goose, cattle, hog and soil isolates that grew in each. Those wells that were either constantly positive or negative in all the samples were not used in the construction of a library.

Well no.	Carbon source in well	No. (%) Bird isolates	No. (%) Goose isolates	No. (%) Cattle isolates	No. (%) Hog isolates	No. (%) Soil isolates
A4	Glycogen	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(100.0)
A6	Tween 80	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(100.0)
A9	Adonitol	9(81.8)	111(90.2)	6(6.2)	1(4.5)	0(0.0)
A11	D-Arabitol	9(81.8)	42(34.1)	87(89.7)	21(95.5)	0(0.0)
B1	i-Erythritol	1(9.1)	5(4.1)	11(11.3)	3(13.6)	6(100)
B3	L-fucose	10(90.9)	115(93.5)	89(91.8)	19(86.4)	0(0.0)
B7	m-Inositol	1(9.1)	4(3.3)	0(0.0)	0(0.0)	6(100.0)
B9	Lactulose	11(100.0)	109(88.6)	90(92.8)	20(90.9)	6(100.0)
C1	D-Melibiose	11(100.0)	112(91.1)	91(93.8)	20(90.9)	6(100.0)
C2	β-Methyl-D-Glucoside	4(36.4)	36(29.3)	93(95.9)	21(95.5)	0(0.0)
C12	Succinic Acid Mono-Methyl Ester	9(81.8)	107(87.0)	11(11.3)	4(18.2)	0(0.0)
D1	Acetic acid	11(100.0)	110(89.4)	83(85.6)	20(90.9)	0(0.0)
D8	D-Glucosaminic Acid	0(0.0)	0(0.0)	2(2.1)	1(4.5)	6(100)
E1	p- Hydroxyphenylacetic acid	1(9.1)	4(3.3)	87(89.7)	97(100)	0(0.0)
E4	α-Ketoglutaric acid	10(90.9)	112(91.1)	89(91.8)	3(13.6)	0(0.0)
E8	Propionic Acid	10(90.9)	113(91.9)	90(92.8)	21(95.5)	0(0.0)
E10	D-Saccharic Acid	1(9.1)	5(4.1)	6(6.2)	1(4.5)	6(100.0)
E11	Sebacic Acid	0(0.0)	8(6.5)	0(0.0)	0(0.0)	0(0.0)
E12	Succinic Acid	11(100.0)	120(97.6)	96(99.0)	20(90.9)	0(0.0)
F1	Bromosuccinic Acid	2(18.2)	10(8.1)	7(7.2)	2(9.1)	6(100.0)
F4	L-Alaninamide	1(9.1)	9(7.3)	88(90.7)	20(90.9)	6(100.0)
F5	D-Alanine	3(27.3)	23(18.7)	91(93.8)	21(95.5)	0(0.0)
F10	L-Glutamic Acid	5(45.5)	19(15.4)	97(100)	20(90.9)	0(0.0)
G3	L-Leucine	2(18.2)	7(5.7)	1(1.0)	1(4.5)	0(0.0)
G6	L-Proline	0(0.0)	0(0.0)	4(4.1)	3(13.6)	0(0.0)
G11	D,L-Carnitine	1(9.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
H8	2,3-Butanediol	1(9.1)	5(4.1)	0(0.0)	1(4.5)	0(0.0)
H9	Glycerol	11(100.0)	120(97.6)	90(92.8)	20(90.9)	6(100.0)
H10	D,L, α-Glycerol Phosphate	10(90.9)	113(91.9)	89(91.8)	22(100.0)	6(100.0)
H12	D-Glucose-6-Phosphate	4(36.4)	36(29.3)	90(92.8)	21(95.5)	0(0.0)

maxPLUX^{384} microplate spectrophotometer (Molecular Devices), in place of the BIOLOG plate reader. A BIOLOG plate reader considers any well with more color than the reference well as positive. Similarly, wells with absorbance readings ≥ 0.4 units on the SPECTRAmaxPLUX^{384} microplate reader displayed a purple color and were considered as positive (+) while wells with reading < 0.4 units remain colorless as does the reference well (A-1), and were scored as negative (-). The more the cell respires the more color that is formed. This signature of plus (+) and minus (-) created a nutrient utilization profile (NUP) for that isolate. The test yielded a characteristic pattern of purple wells, which constitute a "Metabolic Fingerprint". *E. coli* isolates from birds, geese, cattle, hog and soil produced different metabolic patterns.

RESULTS

Nutrient utilization patterns

The NUP for each isolate was further refined by performing several discriminant analyses, which consist of removing those wells that contributed nothing to separation of isolates by source, and to determine the level of pooling of source types that produced the best combination of wells to use. For the known source isolates selected from birds, geese, cattle, hog and soil, the best correct classification was achieved with 30 of the 95 carbon wells in the BIOLOG GN2Microplate™ (Table 1).

Discriminant analysis was performed as described by Wiggins (1996). The pattern of each isolate was entered into a spreadsheet (Excel 2002, Microsoft, and Redmond, WA). Discriminant analysis using software such as SAS (version 8.02 for Windows; SAS Institute, Inc.) can be used to classify the isolates by source. The table generated by the discriminant analysis procedure displayed the number and percent of isolates from each known source that are classified in each source category. The number of isolates from a given source that are placed in the correct source category by discriminant analysis is known

Table 2. Number and percentage of *E. coli* isolates from the known sources that were correctly classified and their average rate of correct classification

Sources of *E. coli* isolates	Total number of isolates	Number of isolates that were correctly classified	Percentage of isolates that were correctly classified (RCC)
Birds	11	10	90.9
Geese	123	112	91.1
Cattle	97	81	83.5
Hogs	22	18	81.8
Soil	6	6	100.0
ARCC			89.5

as the rate of correct classification (RCC). The RCC was established for isolates in each of the five host sources ranging from 81.8% for hog isolates to 100% for soil isolates. The average rate of correct classification (ARCC) was 90%. ARCC is determined by averaging the percentages of correctly classified isolates from each source (Table 2).

The metabolic fingerprints (NUPs) obtained from the nutrient utilization test of forty-one (41) isolates from lake water were then compared to the NUPs from the known samples. The NUP database created (Table 1) was used to predict the sources of *E. coli* in the water. That is, the identification of possible sources of the *E .coli* in lake water was determined by matching the NUPs of microbes from lake water samples with those of isolates from known sources (bird, geese, cattle, hog and soil). When this was done, 73.1% of the isolates from water had NUPs representing cattle and hog. Smaller percentages had NUPs similar to birds and geese, and none of the isolates were predicted to be originating from soil (human sewage) (Figure 2).

DISCUSSION

This study has designed a phenotypic method for differentiating *E. coli* isolates from livestock, wildlife, or human origin that might be used to predict the sources of fecal pollution in the Silver Lake, Delaware, Iowa. Table 1 shows the pre-selected carbon sources, number and the percentage of bird, goose, cattle, hog and soil isolates that grew in each well. Some phenotypic patterns, or strains, of *E. coli* are theoretically endemic to cattle, hog, wildlife and others to humans due to varying diets. The use of BIOLOG microtiter plates relies on dehydrogenase activity as a measure of microbial activity with a single carbon source. Color development in some wells reflects the phenotypes of the strain being tested. The rate and extent of color formation indicate the rate and extent to which respiration occurs with the substrate present in that well (Knight et al., 1997). Data from growth was analyzed and used to build a database for comparison. This NUP database shows the similarities or differences among carbon utilization by the various isolates. *E. coli* isolates from birds, geese, cattle, hog and soil showed different metabolic patterns (Figure 1). The NUPs displayed by isolates from soil was observed to be different from the pattern of growth substrates utilization by isolates from birds, geese, cattle, and hog (Figure 1).

The rate of correctly classifying isolates by source (Table 2) was determined using discriminate analysis. Since more than 80% of the *E. coli* isolates from birds, geese, cattle, hogs and soil, were correctly classified, the NUP system using BIOLOG plates was shown to be useful phenotypic method of tracking the source of the fecal coliform, *E. coli* in Silver Lake, Delaware, Iowa. Figure 2 shows the pie chart of the number and relative percentages of *E. coli* isolates from birds, geese, cattle and hog that are found in isolates from Silver Lake water. Some isolates were indicated as “unidentifiable” since they were from sources not in the source library. As shown in Figure 2, of the 41 isolates from Silver Lake, 7 have NUPs representing birds (17.1%), 2 represent gee-se (4.9%), 16 represent cattle (39.0%), and 14 represent hogs (34.1%). None of the Silver Lake isolates have a NUP which represents soil obtained near the outdoor lavatory facility in the Silver Lake recreation area (human). The source of 2(4.9%) *E. coli* isolates from water remain unidentified. In another study O’Brien and Brown (2003) using multiple antibiotic resistance (MAR) analysis to characterize the same *E. coli* isolates, a source was only able to be predicted in 39.0% of the 41 “environmental isolates”. Of the 16 isolates that were able to be used for prediction, 10 (62.5%) were predicted to be originating from the “cattle” class, 4 (25%) were expected to be originating from hog, and 2 (12.5%) were predicted to be from human source (O’Brien and Brown, 2003). This result is supportive of the results obtained in this study. Both the NUP and MAR results indicate that multiple sources contributed to the fecal contamination of Silver Lake, with the highest contribution made by cattle. In addition, Vogel et al. (2007) reported that the most likely target for best management practice (BMPs) to reduce *E. coli* loadings in the watershed is cattle.

Meyer et al. (2005) reported that successful use of two phenotypic methods, nutrient utilization patterns and antibiotic resistance pattern to determine the source of fecal contamination in a small reacreational lake in Iowa. In another study, Hagedorn et al. (2003) demonstrated that

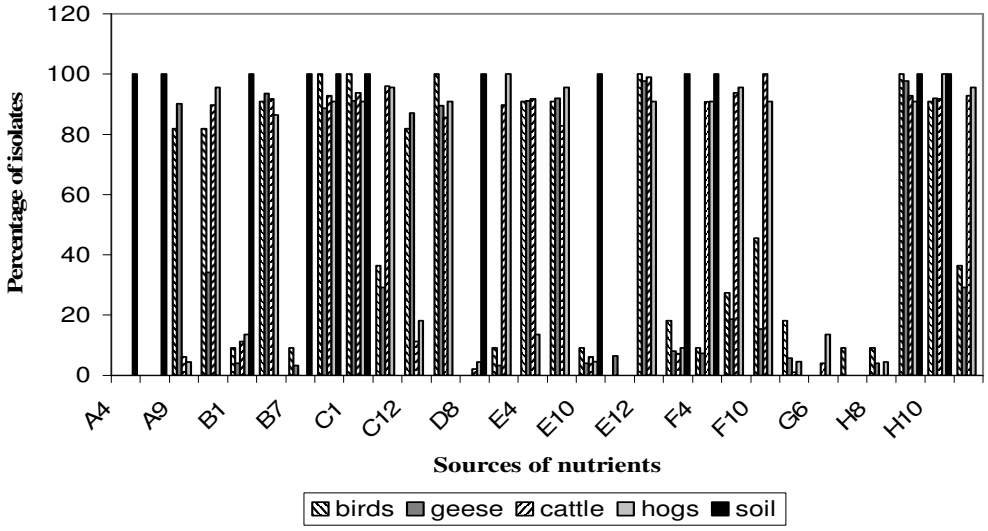

Figure 1. Histogram showing NUPs for birds, geese, cattle, hogs and soil (percentage of *E. coli* isolates from birds, geese, cattle, hogs and soil utilizing the substrates in the microtiter wells).

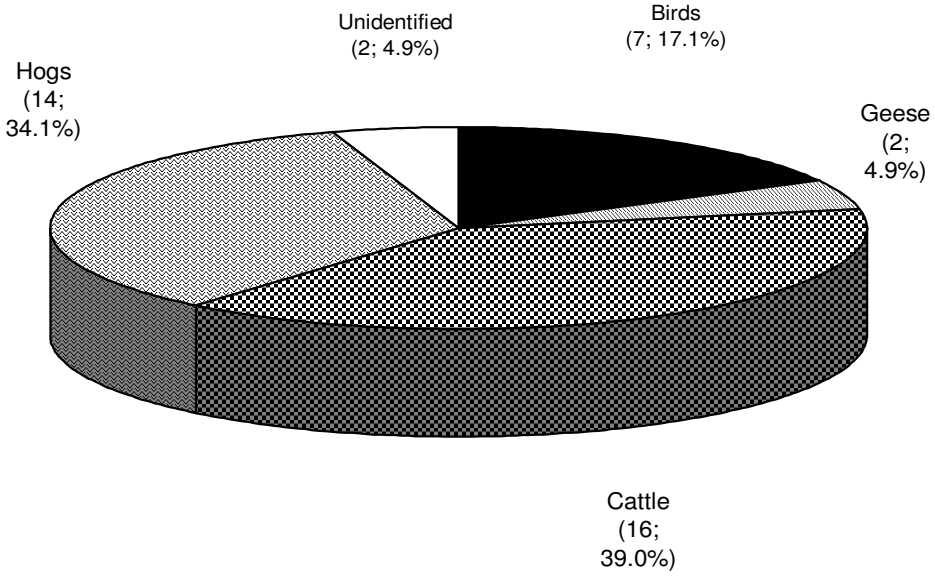

Figure 2. Pie chart showing the number and relative percentages of *E. coli* isolates from birds, geese, cattle and hog that are found in isolates from Silver Lake water. Isolates were indicated as "unidentifiable" if they were from sources not in the source library.

carbon source utilization profiles are a method to identify sources of fecal pollution in water. Hagedorn (1999) reported that NUP results appear to be compara-ble to MAR and PFGE in accuracy. According to Hage-dorn et al. (1999), the NUPs system is intermediate (bet-ween MAR and PFGE) in cost and time required to per-form the procedure. In the present study, cost was signi-ficantly reduced by using a standard plate reader and by not using commercial software to analyze and create NUPs. The NUP system is perhaps the most fool proof procedure because it uses an electronic plate reader. This removes judgment decisions by laboratory person-nel when evaluating plates (MAR and PFGE require such judgments). Bitton (2005) reported that an advantage of carbon utilization profile is its simplicity, necessitating only a microplate reader to determine carbon source uti-lization. This method requires less skill than antibiotic resistance analysis or genotypic methods (Bitton, 2005). Bennett and Odom (2002) conducted research on carbon utilization patterns as an indicator of host origins of *E. coli*. They sought to discover a method of differentiating between strains of *E. coli*, which might be endemic to cat-

tle or human, through the use of carbon source trac-king. Based on the results obtained, they concluded that the BIOLOG (Hayward, CA) method of nutrient utilization was not a useful method of *E. coli* strain differentiation. Their experiment was not successful probably due to the fact that all the 96 wells were used in the analysis and thus no patterns were obtained.

Conclusion

In the present study, the source tracking technique using carbon substrate utilization technique provided identification of *E. coli* contamination of Silver Lake from diverse sources that included (but were not limited to) birds, geese, cattles and hogs. Also, the results indicated that soil (humans) sources did not contribute to the fecal pollution of the Silver Lake. Being able to determine the source(s) of bacteria represent a significant advance in water quailty assessment and management. Also, understanding the origin of fecal pollution is paramount in assessing associated health risks as well as the actions necessary to remedy the problem while it still exists. For example, source tracking methodology using the nutrient utilization profiles has the potential to provide agencies responsible for water quality and public health with a resource to determine sources of fecal contamination. When and if fully implemented, source tracking methodologies could be widely used in the total maximum daily load (TMDL) program. A TMDL is a calculation of the maximum amount of a pollutant that a water body can receive (the sum of the allowable loads of a single pollutant from all contributing point and nonpoint sources) and still meet water quality standards. The calculation must include a margin of safety to ensure that the water body can be used for the purposes the state has designated (e.g., recreational uses, shellfish harvest, drinking water). The calculation must also account for seasonal variation in water quality. Allocations and allowable loads for pollutant sources implies that those sources can be accurately identified, and the inclusion of seasonal variation in the TMDL program will require longer-term fecal source identification studies than most of those reported to date (McClellan et al., 2000; McKenzie, 1998). Recommended strategies to reduce bacterial contamination include outreach to septic system and farm owners, as well as gardeners using manure fertilizers; careful monitoring of contaminated areas and conduction of further research on bacterial source tracking techniques. Finally and perhaps most important, findings from this bacterial source tracking project can be used to design prevention and remediation efforts for the Silver Lake watershed. However, it is not known if NUPs of isolates from one geographic location can be used to predict the source of isolates from different lakes in the same and in different regions. Therefore, further investigation is required to determine how useful the NUPs from one geographic area are in predicting the source of fecal contamination in a different area. Additionally, further studies to improve the NUPs and BIOLOG techniques is recommended, particularly a way to calibrate the changes in color intensities resulting from the utilization of redox dye should be investigated.

ACKNOWLEDGEMENTS

This research was supported, in part, by the Roy J. Carver Charitable Trust and Environmental Programs at the University of Northern Iowa.

REFERENCES

Ackman D, Marks S, Mack P, Caldwell M, Root T, Birkhead G (1997). Swimming-associated haemorrhagic colitis due to *Escherichia coli* O157:H7 infection: evidence of prolonged contamination of a fresh water lake. Epidemiol. Infect.119:1-8.

Bennet L, Odom K (2002). Carbon utilization patterns as an indicator of host origins of E. coli Oscar J. 10.http://www.selu.edu/Academics /ArtsSciences/oscar/journal10/bennett/bennett_odom.htm

Bitton G (2005). Microbial indicators of fecal contamination: applications to microbial source tracking. Report submitted to the Florida Stormwater Association, Tallahassee.

Garland JL, Mills AL (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57:2351-2359.

Guan S, Xu R, Chen S, Odumeru J, Gyles C, (2002). Development of a procedure for discriminating among *Escherichia coli* Isolates from animal and human Sources. Appl. Environ. Microbiol. 68:2690-2698.

Guzewich JJ, Morse DL (1986). Sources of shellfish in outbreaks of probable viral gastroenteritis; implications for control. J. Food Protec. 49: 389-394.

Hagedorn C, Crozier JB, Mentz KA, Booth AM, Graves AK, Nelson NJ, Reneau Jr RB (2003). Carbon source utilization profiles as a method to identify sources of fecal pollution in water. J. Appl. Microbiol. 94:792-799.

Hagedorn C, Robinson SL, Filtz JR, Grubbs SM, Angier TA, Reneau RB (1999). Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patters in fecal streptococci. Appl. Environ. Microbiol. 65:5522-5531.

Jones IG, Roworth M (1996). An outbreak of *Escherichia coli* O157 and campylobacteriosis associated with contamination of a drinking water supply. Pub. Hlth. (London). 110: 277-282.

Knight BP, McGrath SP, Chaudri AM (1997). Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc. Appl. Environ. Microbiol. 63:39-43.

Lu L, Hume ME, Sternes KL, Pillai, SD (2004). Genetic diversity of *Escherichia coli* isolates in irrigation water and associated sediments: Implications for source tracking. Water Res. 38:3899-3908.

Makinko S, Asakura H, Shirahata T, Ikeda T, Takeshi K, Arai K, Nagasawa MA, Sadamoto T (1999). Molecular epidemiological study of mass outbreak caused by enteropathogenic *Escherichia coli* O157-H45. Microbiol. Immunol. 43:381-384.

Martellini A, Payment P, Villemur R (2005). Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. Water Res. 39:541-548.

McClellan P, Shanholtz VO, Petrauskas B, Kern J (2000). Bacterial source tracking: A tool for total maximium daily load development. In T. Younos and J. Poff (ed.) Abstracts, Virginia Water Research Symp. 2000, VWRRC Spec. Rep. SR-19-2000. Virginia Water Resources Research Center, Blacksburg, VA. p. 17

McKenzie MC (1998). Total maximum daily load: A major step to restoring our waters. Small Flows 12:9–10.

Meays CL, Broersma K, Nordin R, Mazumder A (2004). Source tracking fecal bacteria in water: a critical review of current methods. J. Environ. Manage. 73:71-79.

Meyer KJ, Appletoft CM, Schwemm AK, Uzoigwe JC, Brown EJ (2005). Determining the source of fecal contamination in recreational waters. J. Environ. Health. 68: 25-30.

O'Brien EH, Brown EJ (2003). Non-point source fecal pollution in Silver Lake, Iowa. American society for microbiology. Annual meeting, Washington, D. C. Section N.

Orskov F, Orskov I (1981). Enterobacteriaceae. In Broude , A. I (ed), Medical microbiology and infectious diseases. The W.B. Saunders Co., Philadelphia, Pa. pp. 340-352.

Parveen S, Murphree RL, Edmiston L, Kaspar CW, Portier KM, Tamplin ML (1997). Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay. Appl. Environ. Microbiol. 63:2607–2612.

Puech MC, McAnulty JM, Lesjak M, Shaw N, Heron L, Watson JM (2001). A statewide outbreak of cryptosporidiosis in New South Wales associated with swimming at public pools. Epidemiol. Infect. 126: 389-396.

Samadpour M, Roberts MC, Kitts C, Mulugeta W, Alfi D (2005). The use of ribotyping and antibiotic resistance patterns for identification of host sources of *Escherichia coli* strains. Lett. Appl. Microbiol. 40: 63-68.

Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasik J (2002). Microbial source tracking: Current methodology and future directions. Appl. Environ. Microbiol. 68: 5796-5803.

Scott TM, Parveen S, Portier KM, Rose JB, Tamplin ML, Farrah SR, Koo A, Lukasik J (2003). Geographical variation in ribotype profiles of *Escherichia coli* isolates from humans, swine, poultry, beef, and dairy cattle in Florida. Appl. Environ. Microbiol. 69: 1089-1092.

Simpson JM, Santo-Domingo JW, Reasoner DJ (2002). Microbial source tracking: State of the science. Environ. Sci. Tech. 36: 5729-5289.

Strittholt JR, Garono RJ, Frost PA (1998). Spatial patterns in land use and water quality in the Tillamook Bay Watershed: A GIS Mapping Project. TBNEP technical report. Tillamook bay national estuary project. Garibaldi. p.62

US Environmental Protection Agency (1986). Ambient water quality criteria for bacteria. Washington, DC. USEPA Rep. 440/5-84-002. USEPA,

Vogel JR, Stoeckel DM, Lamendella R, Zelt RB, Santo Domingo JW, Walker SR, Oerther DB (2007). Identifying fecal sources in a selected catchment reach using multiple source-tracking tools. J Environ Qual 36: 718-729.

Wiggins BA (1996). Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and

animal sources of fecal pollution in natural waters. Appl. Environ. Microbiol. 62: 3997-4002