Biosolids are generated from the treatment of human waste and upon treatment specified by regulatory agencies they can be land applied on fields for waste disposal and as a soil amendment. Current methods for assessing water quality around land application sites cannot distinguish biosolid runoff from other sources of pollution. The goal of this research was to identify, develop, and validate novel biosolid microbial source tracking (MST) markers that can be used for tracking biosolid materials following land application. Biosolid samples were collected from two different wastewater treatment plants (WWTPs) in the Southeastern region of the US. Total community DNA was extracted and high-throughput 454 pyrosequencing was performed to examine the microbial communities (Archaea and Bacteria) present in the samples. Microbial markers were designed and validated using polymerase chain reaction (PCR) for presence/absence in additional biosolids and animal manure samples. Using MST techniques in a field study, surface waters near biosolid land application fields were tested to demonstrate environmental detection of fecal indicators, human-specific HF183, and candidate microbial biosolid markers. The combined pyrosequencing and PCR analysis identified several candidate sequences within the Archaea and Bacteria kingdoms as potential microbial markers, and upon further in silico analysis, we selected sequences belonging to the following genera to target: unclassified Betaproteobacteria, Leptotrichiaceae, Methanosarcina, and an unclassified uncultured Archaea. The validation study confirmed these microbial biosolid markers are sensitive to biosolid materials. However, initial tests with treated animal wastes suggest that these markers are not specific for biosolid materials but can be found in other digested wastes. The field study resulted in the environmental detection of candidate biosolid markers in surface water samples, along with fecal indicators and other microbes of public health concern. This research provides an approach for understanding the potential transport of biosolid materials following land application, and potential impacts on environmental quality.

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