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A real-time qPCR assay for the detection of the nifH gene of *Methanobrevibacter smithii*, a potential indicator of sewage pollution

C. Johnston[1,2], J.A. Ufnar[3], J.F. Griffith[4], J.A. Gooch[2] and J.R. Stewart[2,5]

[1]*Jardon and Howard Technologies Incorporated, Orlando, FL*

[2]*Center for Coastal Environmental Health and Biomolecular Research and Hollings Marine Laboratory, U.S. National Oceanic and Atmospheric Administration, Charleston, SC*

[3]*Center for Science Outreach, Department of Biological Sciences, Vanderbilt University, Nashville, TN*

[4]*Southern California Coastal Water Research Project (SCCWRP), Costa Mesa, CA*

[5]*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC*

ABSTRACT

Methanobrevibacter smithii is a methanogenic archaea prevalent in the intestinal tract of humans. Due to its abundance in the human gut and low likelihood of regrowth in the environment, the *nifH* gene of *M. smithii* may be a useful marker of sewage pollution in water. This paper describes the development of a real-time quantitative polymerase chain reaction (RT-qPCR) assay to detect the *nifH* gene of *M. smithii*. Quantification standards were prepared from *M. smithii* genomic DNA dilutions and a standard curve used to quantify the target gene and calculate estimated genome equivalency units (GEUs). A competitive internal positive control was designed and incorporated into the assay to assess inhibition in environmental extracts. Testing the assay against a panel of 23 closely related methanogen species demonstrated specificity of the assay for *M. smithii*. A set of 36 blind water samples was then used as a field test of the assay. The internal control identified varying levels of inhibition in 29 of 36 (81%) samples, and the *M. smithii* target was detected in all water samples with known sewage input. Our results suggest that the quantitative polymerase chain reaction (qPCR) assay targeting the *M. smithii nifH* gene developed in this study is both sensitive and rapid, and shows promise as a reliable indicator of sewage in environmental waters.