A COMPLEMENTARY ISOTHERMAL AMPLIFICATION METHOD TO THE U.S. EPA qPCR APPROACH FOR THE DETECTION OF ENTEROCOCCI IN ENVIRONMENTAL WATERS

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INTRODUCTION

Faecal indicator bacteria such as intestinal enterococci are used around the globe to assess the microbiological quality of environmental waters. In this respect, quantitative polymerase chain reaction (qPCR) has become powerful detection tool within the last decade to monitor the levels of enterococci more rapidly. Despite high sensitivity and specificity, however, qPCR is limited to specialized laboratories having access to high-end instruments and extensively trained personnel to perform the method, as well as to analyze and interpret the obtained data. Isothermal amplification methods – such as helicase-dependent amplification (HDA) – represent a novel group of DNA-based detection techniques that mainly differ from PCR-technology in the temperature conditions needed to amplify a specific DNA target region. Unlike qPCR, HDA can be performed at a constant temperature (~ 65 °C) and thus offers considerable advantages especially for resource-limited settings. Furthermore, HDA is a promising technique for incorporation into portable, battery-operated devices and microfluidic systems (lab-on-a-chip).

AIM OF THE WORK

The aim of this work was to design and develop an HDA assay that is complementary to existing qPCR assays of the United States Environmental Protection Agency (US EPA) for the detection of enterococci. To that end, the qPCR US EPA Method 1611 [1] – targeting the 23S rRNA gene as a marker for fecal pollution – was translated into the HDA reaction format. The performance of the developed *Enterococcus* HDA assay was evaluated and compared to reference qPCR with respect to specificity, sensitivity, limit of detection, and analysis of environmental isolates, as well as its applicability to environmental water samples.

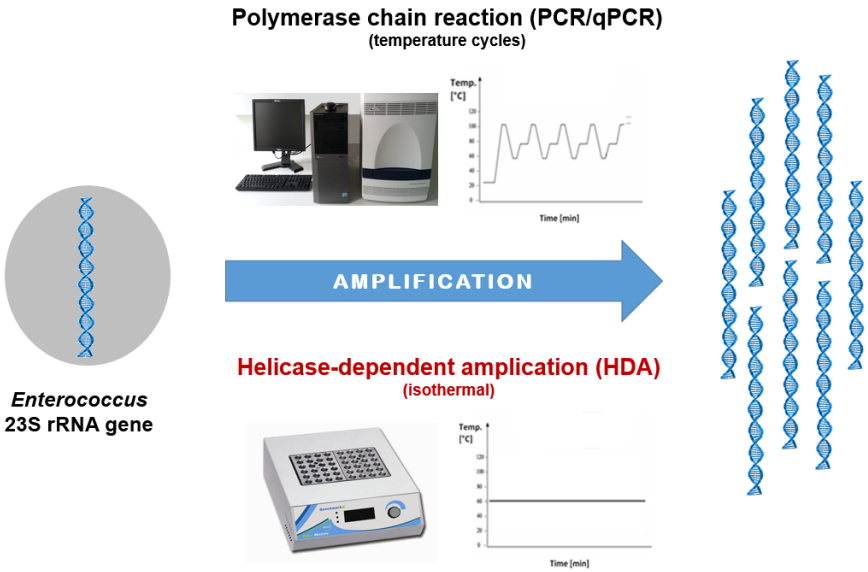

Figure 1: Schematic representation of instrument complexity and temperature requirements for polymerase chain reaction (PCR) and helicase-dependent amplification (HDA) technology

RESULTS AND DISCUSSION

The developed *Enterococcus* HDA assay successfully discriminated 15 enterococcal from 15 non-enterococcal reference strains and reliably detected 48 environmental isolates of enterococci. The limit of detection determined by analyzing a dilution series of *Enterococcus faecalis* genomic DNA was 25 target copies per reaction, only three times higher than that of qPCR. The applicability of the assay was tested on 30 environmental water samples, simulating a range of fecal pollution. Despite the isothermal nature of the reaction and the use of simple instruments, the HDA results were consistent with those of the qPCR reference.

CONCLUSION

This novel HDA assay targets the same *Enterococcus* 23S rRNA gene region as the existing qPCR assays of the US EPA but can be entirely performed on a simple heating block. Given the performance, we conclude that the developed *Enterococcus* HDA assay has great potential as a complementary screening method to the qPCR. This amplification platform can broaden the applicability of molecular methods (e.g., for high-throughput analysis) and greatly increase their overall accessibility (e.g., in resource-limited settings or developing regions).

ACKNOWLEDGEMENTS

This study was part of the Life Science Call 2013 project LSC13-020 funded by the Niederösterreichische Forschungs- und Bildungsgesellschaft (NFB) and supported by the Austrian Science Fund (FWF) project P23900. This study was a joint collaboration of the Interuniversity Cooperation Centre Water & Health (www.waterandhealth.at).

REFERENCES

[1.] USEPA *Method 1611: Enterococci in Water by TaqMan quantitative polymerase chain reaction (qPCR) assay*; EPA-821-R-12-008; U.S. Environmental Protection Agency, Office of Water, Washington, D.C.: 2012