A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE RAPID DETECTION OF *ENTEROCOCCUS* SPP. IN WATER

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INTRODUCTION

Microbial pollution of water resources through faecal input is a threat to public health in developing as well as in industrialised countries. According to the WHO, 9% of the world's population are without access to safe drinking water [1], which is why the improvement of drinking water quality and sanitation is one of the United Nations Sustainable Development Goals [2]. The microbiological quality of water used for drinking, bathing, or irrigation can be determined by the detection of standard faecal indicator bacteria (SFIB) such as *Escherichia coli* or certain *Enterococcus* species. The major shortcomings of the conventional detection methods include the requirement for a microbiological laboratory and that SFIB can only be identified and quantified with certainty after up to 48 hours of growth. Therefore, molecular detection methods based on quantitative polymerase chain reaction (qPCR) are nowadays being used extensively for routine water quality monitoring. However, expensive equipment and computer software are required for their application and the interpretation of the results. To overcome these drawbacks, isothermal amplification methods have recently become a useful alternative to the molecular detection method of qPCR, allowing molecular diagnostics directly at the point-of-care with simple or no instrumentation and without the need for highly trained personnel [3].

AIM OF THE WORK

The aim of this study was the development of a novel method for the rapid molecular detection of *Enterococcus* spp. in water by loop-mediated isothermal amplification (LAMP) and its comparison to the qPCR method 1611 recommended by the US Environmental Protection Agency [4].

RESULTS AND DISCUSSION

The developed LAMP assay can be completely performed at 65 °C and does not require any temperature changes throughout the 45 minute reaction. Therefore, a simple heating block is

sufficient to fulfil the requirements for the implementation of the *Enterococcus* LAMP. The sensitivity and specificity tests were performed using a set of 30 *Enterococcus* and non-target bacterial reference strains, which showed that the developed LAMP assay is equally sensitive and specific as the reference qPCR assay. The LAMP method had a limit of detection (95% probability of detection) of 130 DNA target molecules per reaction. Additionally, enterococci isolated from Austrian surface water bodies as well as a set of DNA extracts from environmental waters were tested. Contingency analysis demonstrated a highly significant correlation between the results of the developed LAMP assay and the reference qPCR method. The simple naked-eye identification of the LAMP products was achieved within one minute by the addition of a DNA-intercalating fluorescence dye.

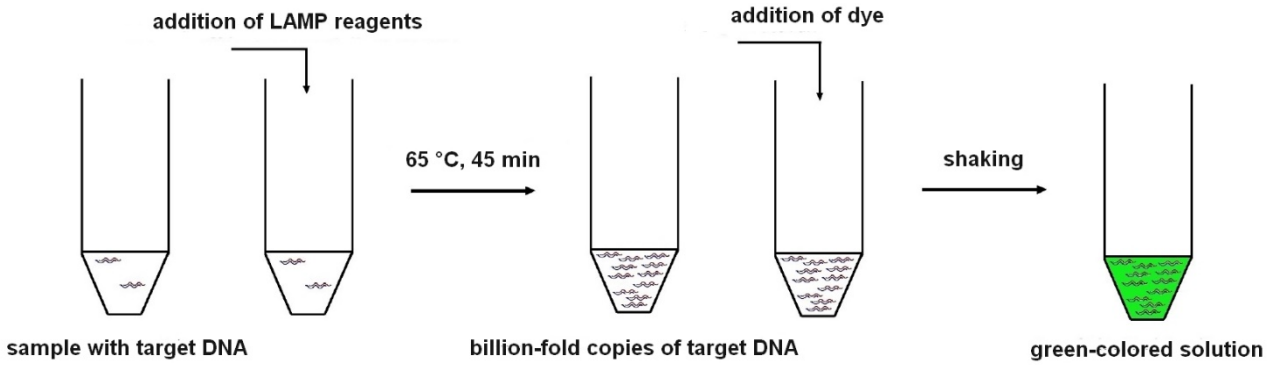

Figure: LAMP detection scheme.

CONCLUSION

In our study, a novel loop-mediated isothermal amplification (LAMP) assay for the molecular detection of enterococci in water samples was developed. The LAMP assay does not require expensive instrumentation and can be performed in less than one hour. In conclusion, this method represents an essential component for the efficient screening and testing of water samples in low-resource settings without sophisticated laboratory equipment and highly specialised personnel, e.g. in developing countries. In order to provide an entire on-site applicable workflow for the assessment of water quality, our future work will focus on simple filtration methods as well as on the development of a user-friendly and time-saving DNA extraction protocol for bacterial DNA from water.

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