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Abstracts

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Development of an internal standard for quantitative PCR analyses of antibiotic resistance genes

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Introduction

Quantitative PCR (qPCR) is widely used to detect and quantify antibiotic resistance genes (ARGs) in the environment. Nevertheless, the ARGs quantification might be challenging since several factors influence qPCR experiments. Therefore, qPCR protocols normalization is needed.

Objectives

The main aim of this work was to develop an internal standard (IS) that would allow the comparability of DNA extraction and qPCR-based ARGs quantification in water samples. These efforts will contribute to establish guidelines for genes quantification using normalized methodologies, supporting inter-laboratory comparative analyses.

Methods

The gene *molA* that encodes a hydrolase to degrade the herbicide molinate (Duarte et al., 2011) was chosen as possible IS for genes quantification in environmental water samples. Conventional PCR was performed to selected a *molA* amplicon that was cloned in a commercial *Escherichia coli* strain (*). Several doses of fresh culture suspensions of *E. coli* harboring the recombinant *molA*-plasmid, which contains a fragment of that gene, were used to spike water and wastewater environmental samples. These samples were processed as usual and total DNA was extracted. The chromosomal genes 16S rRNA and *marA* and the IS *molA* gene were quantified using qPCR.

Results

The IS used enabled to assess the DNA losses that might occur in the DNA extraction and in the genes quantification. For DNA extraction, losses around <17% were observed, while for quantitative PCR losses were below 11%. The amount of IS to use and the effect of matrix were relevant variables in this analysis.

Conclusion

The qPCR protocols normalization using an IS is an adequate approach to understand the losses that occur during samples processing and genes quantification. The application of this procedure to quantify ARGs in environmental samples will improve the quality of comparative studies with data obtained from different environments or geographic locations.

Duarte M, Ferreira-da-Silva F, Lünsdorf H, Junca H, Gales L, Pieper DH, et al. (2011) *Gulosibacter molinativorax* ON4 T molinate hydrolase, a novel cobalt-dependent amidohydrolase. Journal of Bacteriology 193: 5810–5816. doi: 10.1128/JB.05054-11

*Courtesy of Prof. Olga Nunes (LEPABE, FEUP, Portugal)