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Comparison of DNA extraction methods for the direct quantification of bacteria from water using quantitative real-time PCR

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Polymerase chain reaction (PCR) has traditionally been performed on single isolates and from sample enrichments. Enrichments cannot estimate the bacterial counts; you only get an idea of presence and absence. Since viable but non-culturable bacteria cannot be isolated by standard culture based methods, the simplest way to overcome this would be to isolate DNA from bacterial cells concentrated directly from the water samples, thus circumventing the need for culturability. The aim was to develop a method to concentrate the bacterial cells directly from water samples followed by DNA extraction from the cells. This method was compared to commercially available water testing DNA extraction kits. The modified in house DNA extraction method was compared to the Water Master™ DNA purification kit (Separations), Ultra Clean Water DNA isolation kit (Optima Scientific), Aquadien™ kit (Biorad) and Metagenomic DNA isolation kit for water (Separations) using an optimized gas quantitative real-time PCR (qPCR) protocol. DNA was extracted from serial diluted bacterial cells using the various kits and used for the construction of the standard curves using the qPCR results. The in house DNA extraction method R2 (0.99 and 0.99) and slope (-3.48 and -3.65) showed similar results for the 2 repeats done in triplicate. The R2 and slope for the Water Master™ DNA purification kit (R20.34 and 0.73; slope -5.73 and -4.45); Ultra Clean Water DNA isolation kit (R2 0.97 and 0.28; slope -3.89 and -8.84); Aquadien™ kit (R20.98 and 0.77; slope -3.59 and -5.94) and Metagenomic DNA isolation kit (R20.65 and 0.77; slope -3.83 and -4.89) showed higher variability than the in house method. The results showed that the in-house DNA extraction protocol has the potential for good DNA recovery, repeatability and reproducibility and quality for PCR analysis. It is a suitable and most importantly cost effective alternative to the available commercial DNA extraction water testing kits.

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Evaluation of anti-biofilm activity of synthetic peptides analogous to human cathelicidin LL-37 in clinical isolates of *Staphylococcus* species

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Biofilms, multicellular communities formed by bacteria causes the majority of infections and exhibit increased resistant to antibiotics. Staphylococcus spp. is a clinical pathogen that forms biofilm infections on nearly all types of medical devices and no antimicrobials have been developed specifically to treat biofilms yet. Synthetic peptides with anti-biofilm activity represent a novel approach to treat biofilm infections. In this context, it is necessary to implement strategies to neutralize the ability of biofilm formation. Therefore, short analogs of LL-37, a pleiotropic peptide with antibiofilm activity were designed and synthesized to try to maintain/improve the activity of the native molecule against biofilm, their helical structure and the possible interaction with the bacterial membrane. A bioinformatics tool was used to predict important antimicrobial residues and generate fragments of the complete sequence. Four different peptides were manually synthesized using F-MOC technique and characterized by RP-HPLC and MALDI-TOF. The biofilm forming capability of Staphylococcus was linked to the presence of the ica operon and the amount of biofilm formation measured by the crystal violet (CV) adherence assay. The antibiofilm activity of the peptides was tested; the results showed an inhibition concentration of 5 uM using a flow cell system by confocal microscopy and in between 25-50 uM by using CV assay. Staphylococcus spp. was characterized for its ica status using PCR and its biofilm forming ability over 3 days. According to the results, these peptides represent a promising alternative for the treatment of biofilm-associated infections or could be combined with conventional antibiotics.

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