Quantifying dominant bacterial genera detected in metagenomic data from fish eggs and larvae using genus-specific primers

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Abstract

The goal of this study was to design genus-specific primers for rapid evaluation of the most abundant bacterial genera identified using amplicon-based sequencing of the 16S rRNA gene in fish-related samples and surrounding water. Efficient genus-specific primers were designed for eleven bacterial genera including Alkalimarinus, Colwellia, Enterovibrio, Marinomonas, Massilia, Oleispira, Phaeobacter, Photobacterium, Polarbacerium, Pseudomonas, and Psychrobium. The specificity of the primers was confirmed by the phylogeny of the sequenced polymerase chain reaction (PCR) amplicons that indicated primers were genus-specific except in the case of Colwellia and Phaeobacter. Copy number of the 16S rRNA gene obtained by quantitative PCR using genus-specific primers and the relative abundance obtained by 16S rRNA gene sequencing using universal primers were well correlated for the five analyzed abundant bacterial genera. Low correlations between quantitative PCR and 16S rRNA gene sequencing for Pseudomonas were explained by the higher coverage of known Pseudomonas species by the designed genus-specific primers than the universal primers used in 16S rRNA gene sequencing. The designed genus-specific primers are proposed as rapid and cost-effective tools to evaluate the most abundant bacterial genera in fish-related or potentially other metagenomics samples.

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