MSc in Environmental Contamination and Toxicology EMJMD
UPV/EHU – UBx – ULiège
UPPA – NTNU – UPO
an Erasmus+ Consortium
www.ectplus.eu

Co-funded by the Erasmus+ Programme of the European Union

MASTER THESIS PROJECT

Evaluations of strengths and weaknesses of 16S and 23S rRNA genes in differentiation of closely related *Vibrio* species

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PLENTZIA (UPV/EHU), JULY 2022

Abstract

The members of *Vibrio* genus play a crucial role in nutrient cycling with several species acting as symbionts or pathogens of marine organisms and humans. *Vibrio* spp. abundance is greatly affected by human activities and intensive aquaculture practices, thus making it necessary to monitor the presence of these aquatic bacteria in various environmental samples. Identification of Vibrio species is associated with a set of problems due to high sequence similarity of phylogenetic markers, high plasticity of their genomes, and intra- and interspecific horizontal gene transfer. The present study evaluated the potential of ribosomal operon genes 16S and 23S rRNA for *Vibrio* species identification. All full-length gene copies of 16S and 23S rRNA from representative *Vibrio* genomes were downloaded from several online sources. A maximum likelihood 16S rRNA-based phylogenetic tree revealed cases of distinct topological placements of several gene copies coming from single genomes confounding the taxonomic assignment of *Vibrio* species. Assignment was improved in a 23S rRNA-based tree using sequences from the same set of *Vibrio* genomes. We identified the unique nucleotide positions in outlier 16S rRNA copies and demonstrated their role in determining tree topology by introducing additional sequences containing the same nucleotides at these positions. The main reason why one monophyletic clade clustered within the other in the 16S rRNA-based tree was the lack of unique informative nucleotides shared inside gene copies from the same species. This hypothesis was supported by analysis of sequence variability of 16S and 23S rRNA genes in *Vibrio* and phylogenetic tree construction based on their concatenation. Finally, we evaluated primers to amplify full-length 16S rRNA bacterial loci and suggested terminal conserved regions of the 23S rRNA gene as targets to amplify multiple copies of a nearly full-length 16S-23S operon. Our results lay the foundation for the application of sequenced *Vibrio* operons from metagenomic DNA samples and the development of monitoring strategies of *Vibrio* in the environment.