

Identification and diversity of selected model bacteria from different aquatic ecosystems

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INTRODUCTION

Bacteria from the genera *Pseudomonas*, *Aeromonas* and *Vibrio* are ubiquitous members of water column and aquatic organisms. Due to their ability to develop antimicrobial resistance under altered conditions, they could be used as suitable indicators of potential risk of water pollution. The aim of the present study was to identify and molecularly characterize selected model bacteria (SMB) isolates of *Pseudomonas*, *Aeromonas* and *Vibrio* from different aquaculture, feral and wild aquatic ecosystems, including water column, sediment and aquatic organisms. Specific tasks of the study were: 1) comparison of MALDI-TOF and genotyping methods for SMB identification, 2) characterization of virulence properties of selected bacteria, 3) comparison of SMB communities from several aquatic ecosystems.

MATERIALS AND METHODS

Samples were collected from marine and freshwater ecosystems in autumn 2016: 1) Ilova River and nearby common carp (*Cyprinus carpio*) farm; 2) Krka River and rainbow trout (*Oncorhynchus mykiss*) farm on that river; 3) Bay of Mali Ston and sea bass (*Dicentrarchus labrax*) farm in that marine ecosystem. Water samples were collected using Niskin sampler at four sea depths and one river depth. Sediment samples were collected using gravity corer. The fish sampling was performed by nets and electrofishing according to Croatian standard HRN EN 14011 (2005) in freshwater catching of Prussian carp (*Carassius gibelio*) from Ilova River and brown trout (*Salmo trutta*) from the Krka River. As an indicator of *Vibrio* species, mussels were collected from the feral sea water manually. Samples from marine ecosystem were plated on Marine agar and TCBS, while samples from the freshwater ecosystem were submitted to TSA and selective plates. Bacterial isolates with different morphologies were further processed to MALDI TOF MS. Molecular analyses included DNA extraction of selected bacteria isolates, PCR amplification of *rpoD* and *gyrB* genes (Mulet et al., 2010; Soler et al., 2004) accompanied by commercial sequencing (Macrogen Inc.) and bioinformatics analyses. The presence of 16 genes important for the virulence and pathogenicity of *Aeromonas* was investigated (Sun et al., 2016).

RESULTS

Comparison of MALDI-TOF and genotyping methods for SMB identification

From 63 morphologically different bacteria isolated from the marine ecosystem, 19 were members of the genus *Pseudomonas*, 2 of *Aeromonas* and 21 of *Vibrio*. MALDI-TOF identified 7 different *Pseudomonas*, 1 *Aeromonas* and 9 *Vibrio* species. Molecular analyses based on *rpoD* and *gyrB* genes confirmed presence of 5 *Pseudomonas*, 1 *Aeromonas* and 4 *Vibrio* species, with determination of one MALDI-TOF-unidentified *Vibrio* species. From 121 bacteria isolated from freshwater ecosystems, 49 were members of the genus *Pseudomonas* and 15 of *Aeromonas*. MALDI-TOF identified 17 different *Pseudomonas* and 10 *Aeromonas* species. Molecular analyses based on *rpoD* and *gyrB* genes confirmed presence of 9 *Pseudomonas* and 7 *Aeromonas* species. Phylogenetic analyses allowed further characterisation of bacteria isolates by grouping them with isolates available from the GenBank.

Characterization of virulence properties of selected bacteria

From 16 investigated virulence-related genes, 2 namely *ahp* and *exu* were not detected in the analyzed isolates; the gen *ompAII* was detected in only 1 isolate, the gene *eprCAI* in 2 isolates, gen *hly* in 4 isolates, while the other genes were present in 5 or more isolates.

Comparison of SMB communities from several aquatic ecosystems

Investigated sea ecosystem samples were characterized by the presence of *Vibrio* species in mussels and lower number of *Pseudomonas* and *Aeromonas* community members, which were also less diverse than freshwater ecosystem samples. However, some species from marine and freshwater ecosystem samples were grouped together in phylogenetic trees showing ubiquitous presence of investigated bacteria.

DISCUSSION AND CONCLUSIONS

MALDI TOF and molecular analyses gave the same results for most of the samples. Due to different species indexed in the base of MALDI TOF platform and GenBank, in cases of some bacteria isolates, different results were obtained. For molecular identification of SMB, the best results were obtained while using both molecular markers, *rpoD* and *gyrB*, which is confirmed previously (Mulet et al., 2010; Soler et al., 2004). Virulence properties of *Aeromonas* species based on the determination of 16 virulence-related genes from the water environment/aquatic organisms samples showed profiles different from described in the literature (Sun et al., 2016), probably due to the different sample source and different species. Marine and freshwater ecosystem samples were characterized by different *Pseudomonas*, *Aeromonas* and *Vibrio* communities.

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