Monitoring the water quality through molecular techniques

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Abstract

Monitoring of the quality of water is mainly carried out through biochemical techniques with a consuming-time cost. The development of molecular techniques and specific probes to determine microorganisms has allowed a breakthrough in the validation of water quality not only for the technique sensitivity but also for its precision. In this work two attempts were carried out i) Identification of microorganisms through specific molecular probes and ii) Molecular characterization of structure of the bacterial communities.

The main bacterial groups which are included in the current legislation (*Escherichia coli*, *Enterococcus intestinalis*, *Salmonella spp.* and *Legionella spp.*) were analyzed in beaches and a wetland wastewater, through the design of specific probes from the 16S rRNA region. Potentially pathogenic filamentous fungi, belonging to genera *Cladosporium spp.*, *Aspergillus spp.* and *Penicillium spp.*, were also identified using primers from the Internal Transcribed Spacer region (ITS).

Specific probes for the identification of theses microorganisms were designed and tested, from cultures in microbiological media, for both groups and their corresponding genera. DNA isolation was performed with lithium acetate, and fragments amplified were sequenced and further analyzed in the BLAST database. Phylogenic analysis was confirmed with MEGA v 5.05 software in order to generate the corresponding phylogeny trees. Results revealed the specificity of the probes for each one of the microorganisms tested.

To continue, the molecular method of Amplified Ribosomal DNA Restriction Analysis (ARDRA) was performed with well–characterized molecularly strains. This would allow further the characterization of the structure of the bacterial community and the validation of the effectiveness of different wastewater treatments. This fingerprint method is based on the amplification of the 16S rRNA region for the family Enterobacteriaceae, the genus Enterococcus and specifically *E.coli*, digestion by restriction enzymes namely AluI, TaqI, MspI, HaeIII, HhaI and MseI, and followed by electrophoresis detection.

The different sized-band profiles from ARDRA revealed a characteristic pattern for different bacterial strains. Moreover an exhaustive molecular method, such as Terminal-Restriction Fragment Length Polymorphism (T-RFLP) will be used to qualify and quantify bacterial or fungal communities.

Results highlight the value of molecular techniques for the improvement and optimization of water quality monitoring for these microorganisms.

References

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