Title

Comparison between classical and molecular (FISH and PCR) methods for *Lactobacillus spp.* detection in Clinical Samples.

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Abstract

Lactobacillus species constitute the main beneficial bacteria in our body by inhibiting the growth from pathogenic microorganisms. Fluorescence In Situ Hybridization (FISH) is an ideal method for cultivation-independent detection of microorganisms in microbial communities or clinical samples.

Therefore, the current aims of this research are to identify and discriminate Lactobacillus spp. contained in clinical samples by the use of PNA-FISH methodology. In spite this method is proved to be useful to visualize target cells in natural habitats, it wasn't possible to find a Lactobacillus spp. 16S conservative region that allowed an unique and efficient identification in clinical samples. To overcome this problem, we used morphological visualization to differentiate Lactobacillus genus from another relative genera of the same Lactobacillaceae order. In addition, we also needed to overcome some methodological limitations, such as minimizing probe penetration problems and increasing hybridization efficiencies. As a result, we investigated the effect of different pre-treatment procedures of the exopolymer cell walls prior to the hybridization step, such as, several types of fixation compounds (paraformaldehyde and ethanol percentages), buffer steps and enzymatic (lysozyme and protease) pre-treatment. Furthermore, we modified PNA FISH protocol in several steps, for instance, hybridization and washing steps.

In resume, the use of PNA probe specific for Lactobacillus spp. in situ hybridization by fluorescence microscopy could be perfectly used to study the complex and spatial organization of vaginal microbial samples.

To conclude, we validate Lactobacillus spp. PNA probe by FISH to quantify and characterize in mixed microbiologic populations present in clinical samples.