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Probiotics for amphibians: Advances in the selection of lactic acid bacteria for chytridiomycosis control

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Statement of the Problem: Chytridiomycosis is an amphibian skin disease caused by *Batrachochytrium dendrobatidis* (Bd) that produces extinction around the world. Some amphibian-skin bacteria have been proposed as probiotic for chytridiomycosis control but they excluded the lactic acid bacteria (LAB) group. Here in, we advanced in the selection of indigenous LAB from bullfrog (considered as a Bd carrier) skin to design probiotic for application during the *ex situ* breeding of endangered amphibians.

Methodology: To determine the anti-Bd activity, co-culture assays between Bd strains (CFLT 159 from Brazil; AVS4 and AVS7 from Chile) and potentially LAB isolates were performed. Isolates previously shown exopolysaccharide (EPS) synthesis and/or auto aggregation (AA) ability were evaluated for biofilm formation by using polystyrene plates. Compatibility assays were performed to evaluate the possibility to formulate a mixed probiotic.

Findings: From 62 potentially LAB, 48 isolates had any anti-Bd activity. The 16s RNA sequence analysis allowed obtaining 97-99% of identity that matches with *Enterococcus* and *Lactobacillus*. Thus, *Enterococcus* sp. 90, 564, 747, 762; *Lactobacillus* sp. 10, 529, and *Enterococcus gallinarum* CRL 1826 (previously characterized) inhibited all the Bd strains. Three LAB isolates exhibited low biofilm formation, while *E. gallinarum* showed moderate production. This ability was not always associated with AA or EPS synthesis. The compatibility assays indicated that the LAB isolates could be included in mixed probiotic with the exception of *Enterococcus* sp. 742 that was inhibited by *E. gallinarum*.

Conclusion & Significance: *E. gallinarum* CRL 1826 resulted the best strain for a probiotic since it has many beneficial properties: anti-Bd activity, AA, EPS synthesis, biofilm formation, medium hydrophobicity and GRAS properties according to in vitro and in vivo tests (3-7). However, *Enterococcus* sp. 747 would maximize some probiotic properties of the CRL strain; therefore, a mixed probiotic can be proposed.

Biography

Sergio E Pasteris has his expertise in the study of lactic acid bacteria (LAB) metabolism as well as in the isolation and evaluation of its beneficial properties to design probiotics for amphibian culture. Taken into account the development of resistant bacteria, some LAB strains represent an alternative instead chemotherapeutics to prevent epizootics in bullfrog systems breeding. Since bullfrog is a carrier of the etiological agent of chytridiomycosis, probiotics by using native LAB from bullfrog skin are being developed to be applied during the *ex situ* breeding of endangered amphibian species.

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