



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

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BACKGROUND

Introduction

The last decades has seen the emergence of several virulent wildlife pathogens. These emerging infectious diseases have decimated wildlife populations globally and contributes to the global loss of biodiversity (1). Amphibians are considered one of the most threatened group of vertebrates on earth, and the recently emerged disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) has decimated species of amphibians in many parts of the world (2). The microbiota associated to the host can play an important role in health and diseases development (3). Some amphibian-skin bacteria have been proposed as probiotic for chytridiomycosis control (4) 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 probiotics for application during the *ex situ* breeding of endangered amphibians species.

METHODOLOGY

To determine the anti-Bd activity, co-culture assays (5) between Bd strains (CFLT 159 from Brazil; AVS4 and AVS7 from Chile) and potentially LAB isolates were performed (Fig. 1). Isolates that previously shown exopolysaccharide (EPS) synthesis (Fig. 3) and/or autoaggregation (AA) ability (6) were evaluated for biofilm formation by using polystyrene plates (7). Compatibility assays (6) were performed to evaluate the possibility to formulate a mixed probiotic.

RESULTS

- 62 presumptive LAB (pLAB) from *L. catesbeianus* skin and 2 LAB strains from raniculture were evaluated using protocols to analyse antimicrobial activity and surfaces properties to select potentially probiotics to prevent Chytridiomycosis.
- 48 presumptive LAB (77.42%) showed any anti-Bd activity against the tested Bd strains (Fig. 2)
- 8 presumptive LAB (12.9%) showed anti-Bd activity against all indicator Bd strains. Two of them showed strong inhibition against AVS7 and CLFT 159 strains.
- A low number of microorganisms were EPS-producers.

DISCUSSION

All the LAB isolates showed a different profile of inhibition on Bd strains that it was not related with the Bd lineage since all the strains belong to the *Bd GPL* genotype. Thus, this behaviour could be due to the amphibian species and environmental factors related to the geographical area of isolation of Bd strains (cold and warm regions). It could be supposed that the environment would modified the expression of some surface proteins that could be target sites of the antifungal compound produced by the LAB isolates.

By considering that *Enterococcus* sp 747 and *E. gallinarum* CRL 1826 showed the best probiotic properties, complete genotypic identification of *Enterococcus* sp 747 must be performed as well as compatibility assays with *E. gallinarum* CRL 1826 to know if they can be included in a mixed probiotic. Moreover, safe and technological studies must be carried out to advance in the design of the probiotic.

Since species specificity is considered a desirable property in the search for probiotic bacteria, *in vivo* tests should be carried out on endangered amphibian species to evaluate the protective effect of the LAB against a Bd amphibian infection model.

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Fig. 1 Co-culture assay

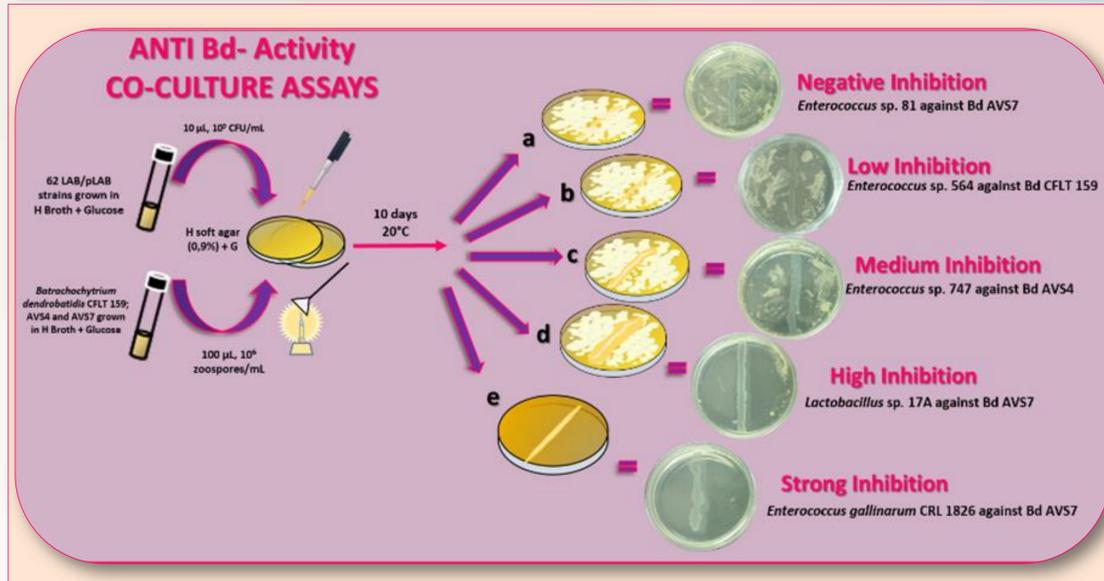


Fig. 2 Anti-Bd activity

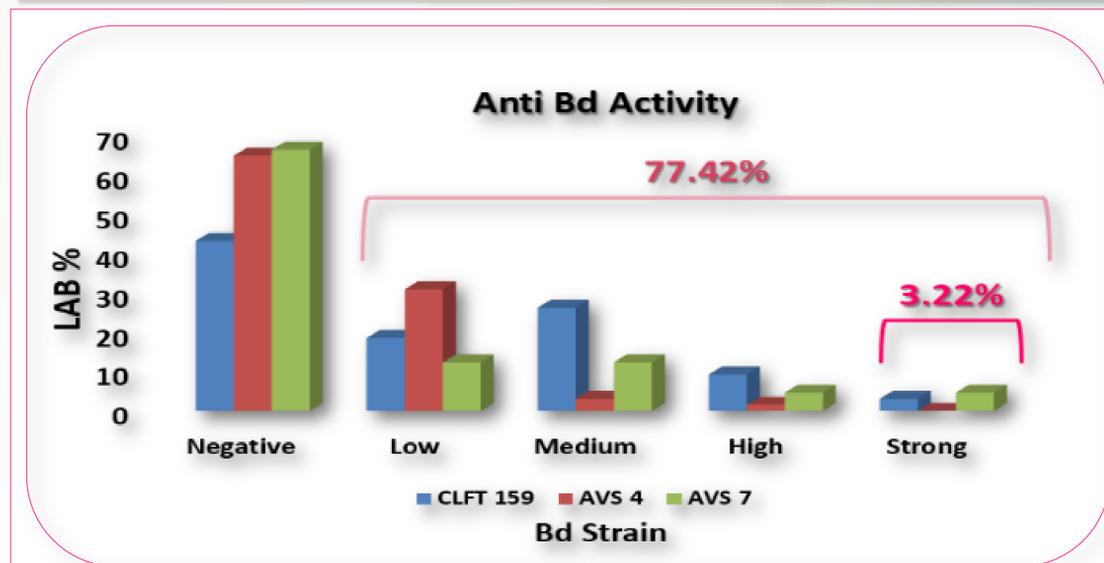
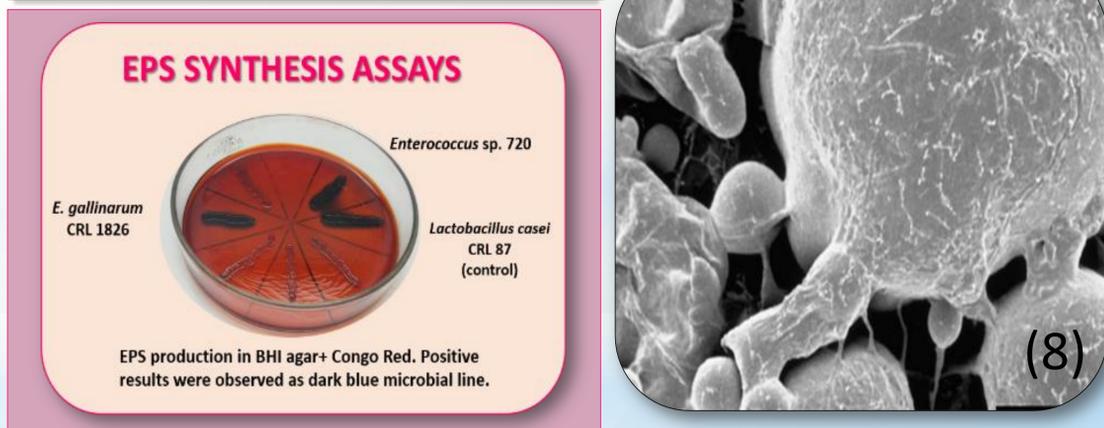


Fig. 3 Exopolysaccharide synthesis



CONCLUSIONS

• *E. gallinarum* CRL 1826 resulted the best strain for a probiotic since it has many beneficial properties (anti-Bd activity against all Bd strains, autoaggregation, EPS synthesis, biofilm formation, medium hydrophobicity and GRAS properties according to *in vitro* and *in vivo* tests).

• *Enterococcus* sp. 747 would maximize some probiotic properties of the CRL 1826 strain; therefore, a mixed probiotic can be proposed.