TITLE: CHARACTERIZATION OF VIRULENCE FACTORS OF LATIC ACID BACTERIA ISOLATES AND SIMULATION OF GASTROINTESTINAL TRANSIT TOLERANCE OF AUTOCHTHONOUS LACTIC ACID BACTERIA FROM SILAGE

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ABSTRACT

Quality control of probiotics in aquaculture is very important. The virulence profile of a probiotic strain has a critical importance in the selection process since the final product will be administered in the diet of healthy animals and must not offer risks to the health. The objective of the present study was to evaluate virulence and resistance factors of the isolates to passage through the gastrointestinal tract (GIT). The six strains selected were isolated from Elephant grass cv. Cameroon silage supplemented with grape residues and the species were confirmed by 16S rRNA gene sequence analysis. DNase was determined by the addition of one stripe of the isolate in the DNase agar surface with toluidine blue at 0.1%. Bacteria were then incubated at 37ºC for 48 h. The formation of rose-pink zones around the colonies indicated a positive result for the presence of DNase. For the coagulase test, 1µL of each culture through a calibrated loop was transferred to sterile tubes containing 0.3 mL of horse plasma, incubated at 37ºC ± 1 for 6 h. For the hemolysis test, the LAB isolates were cultured in agar dishes supplemented with 5% defibrinated sheep blood. After 48-72h, the hemolytic reaction was evaluated by observing hemolysis pattern. Tests to simulate survival in GIT (survival rate higher pH 2, bile tolerance test, pancreatic fluid tolerance test and tolerance test to intestinal juice) were performed, in which LAB isolates were tested in an *in vitro* model that chemically replicates the physiological conditions. The strains for each test were initially cultured for 24 h in De Man, Rogosa and Sharpe (MRS) broth at 37ºC. The isolates did not show activity of the enzymes DNase and coagulase in phenotypic tests, such as in the hemolysis test, they did not show β-hemolytic activity. The absence of hemolytic activity is a selection criteria for candidate strains with potential for application in animal feeds. All LAB isolates demonstrated a survival rate of 75.0% for the pH 2.0 and 100% of LAB isolates showed a survival rate (90.0%) in intestinal and pancreatic juice. All LAB isolates demonstrated a survival rate exceeding 95.0%. A high survival rate on TGI passage indicates that LAB strains can be used as probiotic feeds additives as they demonstrate safety and resistance.

Keywords: probiotic, hemolysis, DNAse, resistance

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