

EVALUATION OF THE PROBIOTIC POTENTIAL OF WEISSELLA CONFUSA AND LACTOBACILLUS PARACASEI STRAINS ISOLATED FROM NIGERIAN TRADITIONAL DAIRY FERMENTED FOODS AND COW'S INTESTINES

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INTRODUCTION

Infectious diseases are still major causes of death in developing countries especially Africa. Current chemotherapy rely almost exclusively on the use of antibiotics, however this is becoming ineffective due to global problem of antibiotic resistance. Hence the need for alternative chemotherapy through the use of viable Lactic Acid Bacteria (LAB) as probiotics.

Probiotics have been defined by the WHO/FAO as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO, 2006)

According to the guidelines proposed by these Organisations, one of the criteria for the selection of probiotic strains is their ability to transiently colonise the human mucosa (WHO/FAO, 2006).

This property could help to maintain or improve the health of the intestinal and vaginal environments and thereby the wellbeing of the consumer

In developed countries, the search for new strains with functional properties is of great interest from both health and industrial points of view. In this way, the traditional fermented foods from non-industrialised countries constitute a reservoir to search for new strains with novel functional properties, the study of the properties of the isolated strains could contribute to improve the safety, quality and industrial value of traditionally fermented foods and could also open the possibility of their use for different applications.

In a previous work, we have reported the antimicrobial ability of isolates from Nigerian fermented foods and animal intestines against clinical pathogens strains obtained from patients with urinary tract infections (Ayeni et al., 2009).

This study was carried out to characterize the probiotic potential of selected LAB strains isolated in Nigeria from traditional fermented dairy foods and cow's intestines samples in order to select strains for probiotic use in the gastrointestinal and urogenital tracts that will lessen possible infections in the tracts through the study of their ability to survive simulated gastrointestinal digestion, and to adhere to epithelial cell lines

RESULTS

After gastric juice simulation, all the tested LAB survived better than *Lactobacillus rhamnosus* GG LMG18243 which has a survival of 40%.

W. confusa UI7 resisted better the high bile salt concentration of DJ with 65% survival. (showing lower viability decreases than the other isolates and the positive control *L. rhamnosus* GG (0.1%).

The final challenge of the IJ (containing 0.3% bovine bile and pancreatin) did not affect the viability of isolates.

Overall, viability decrease (respect to the initial values) was improved for all isolates if the initial pH of GJ was adjusted to 3.0 or the GJ pH 2.0 was supplemented with 10% skim milk whereas, survival percentages of isolates under pH 2.0 challenge were lower than 0.003%.

The highest survival after passage through gastrointestinal conditions was observed with *W. confusa* UI021 (45.0%). while all the LAB survived better than *L. rhamnosus* GG LMG18243 which has a survival of 0.01%.

Adhesion of the four selected isolates to HT-29-MTX monolayer was better than the adhesion of the positive control *L. rhamnosus* GG (0.18%). *Weissella confusa* FAA006 adhered best to the cell line with 0.81% adherence.

L. paracasei UI14 isolate showed better adhesion (4%) to HeLa cell monolayers than strain *L. rhamnosus* GG (2%)

METHOD

The survival of 3 *Weissella confusa* and 2 *L. paracasei* strains in chemically simulated gastrointestinal transit was performed by exposing them to gastrointestinal juice at different pH: 2.0, 3.0 and 2.0 containing 10% skim-milk for 90 min at 37°C in aerobic conditions.

Bacterial suspensions were further centrifuged, resuspended in duodenal juice and incubated for 10 min at 37°C in an anaerobic chamber

Finally, bacterial suspensions were resuspended in intestinal juice and incubated for 180 min under anaerobic conditions

The final percentage of survival was calculated from the ratio: CFU recovered bacteria after complete GIT challenge / CFU initial bacterial

Adhesion to epithelial intestinal and vaginal cell lines

Three epithelial intestinal cell lines, Caco-2, HT-29 and HT-29-MTX, as well as the epithelial vaginal cell line HeLa were used to assess the adhesion ability of the 4 selected bacteria

The cell lines were used after reached the confluent differentiated stage (13-14 days). The strain *Lactobacillus rhamnosus* LMG18243 (also named GG) was used as positive control of well-recognised adherent LAB.

The adherence of 5 bacterial strains to cell line monolayers were expressed as the percentage of bacteria adhered with respect to the amount of bacteria added (% CFU bacteria adhered / CFU bacteria added).

CONCLUSION

If a putative probiotic strain is intended for oral delivery, it should survive the adverse conditions of the upper GIT tract stressing conditions of the human GIT tract, mainly low pH in the stomach and bile salts in the small intestine to arrive alive to the target action site, the small and large intestine.

Due to moderate viability losses at the end of the simulated GIT digestion, the selected isolates could be proposed for oral delivery as probiotics.

In general, and in comparison with the adhesion values obtained for the GG strain, the selected isolates presented lower adhesion to Caco-2 and HT-29 cell monolayers, whereas its adhesion to HT-29-MTX was similar or higher.

Similarly, *L. paracasei* isolates showed a significantly better adherence to the vaginal HeLa cell line than strain GG. In certain situations, LAB may form a biofilm on mucosa surfaces which could confer a physical barrier for non-desirable microorganisms. The good adhesion of the selected isolates, mainly *L. paracasei* UI14, to both vaginal and intestinal epithelia supports their potential use in both anatomic locations if a probiotic effect is shown.

Summarising, in the present study we have characterized 4 LAB *in vitro* to evaluate their use as potential probiotics. In general, the isolates showed good survival to the GIT transit and acceptable adhesion capability to intestinal and vaginal epithelia when compared to their respective positive controls.

The isolates could have potential application in the gut ecosystem. For vaginal applications, UI14 could be the most interesting isolate for formulation of functional probiotics intended for oral or vaginal delivery.