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Effect of Acid Lactic Probiotics Against Endometrial Infection by Escherichia Coli

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Introduction
Postpartum bacterial contamination of uterine environment might reach 80-100% of cows in dairy herds. Some studies indicate that uterine infection predominated by *Escherichia coli* in the first week postpartum is associated with endometritis, a chronic inflammation in which cows fail to completely clear bacterial contaminants.

It has been recently described that administration of intravaginal lactic acid bacteria (LAB) lowers the incidence of purulent vaginal discharge associated with endometrial infections. The aim of this study was to evaluate the potential of four LAB (*Lactobacillus rhamnosus, Pediococcus acidilactici, Lactobacillus reuteri* and *Lactobacillus sakei*) to modulate the internalization and the inflammation response against an *Escherichia coli* infection in primary endometrial cells.

Methods
Primary endometrial epithelial cells were isolated from fresh endometrium of a healthy cow and cultured at $8 \cdot 10^4$ cells/well in 24 well-plates to evaluate the effects of LAB at three different doses. The doses (MOI) were established using lactate dehydrogenase as an indicator of endometrial cell viability. Cultures treated with LAB or control media (n=6) were incubated overnight at 37° and 5% CO_2. Then, cells were infected for 6 h with $4 \cdot 10^6$ cfu/well of a *fimH*[+] *Escherichia coli* strain, isolated from a metritic cow. Cell extracts were obtained with TriZol to analyze the pro-inflammatory status by qPCR or with Triton 0.1% to further enumerate the internalized *E. coli* on McConkey agar plates. Data were normalized by log or root transformation and analyzed by an ANOVA.

Results
Pediococcus acidilactici clearly decreased ($P<0.05$) *E. coli* internalization at the three tested doses (MOI 1, 25, 50) in comparison with infected control cells ($2.27 \cdot 10^4 \pm 0.174$ cfu/ml, $2.40 \cdot 10^4 \pm 0.174$ cfu/ml and $9.81 \cdot 10^4 \pm 0.174$ cfu/ml versus $1.37 \cdot 10^5 \pm 0.174$ cfu/ml) achieving in the best case a 83% reduction. *Lactobacillus sakei* counteracted ($P<0.05$) *E. coli* internalization at MOI 10 and 50 ($4.77 \cdot 10^3 \pm 0.206$ cfu/ml and $4.72 \cdot 10^3 \pm 0.206$ cfu/ml versus $3.61 \cdot 10^4 \pm 0.253$ cfu/ml), reducing up to 86% the bacterial infection. *Lactobacillus reuteri* reduced ($P<0.05$) up to 78% *E. coli* internalization at MOI 2 and 100 ($5.53 \cdot 10^3 \pm 0.122$ cfu/ml and $7.75 \cdot 10^3 \pm 0.122$ cfu/ml versus $2.49 \cdot 10^4 \pm 0.1217$ cfu/ml), whereas *Lactobacillus rhamnosus* induced a reduction ($P<0.05$) of 41% at MOI 50 and 100 ($9.50 \cdot 10^3 \pm 2.8 \cdot 10^3$ cfu/ml, $9.92 \cdot 10^3 \pm 2.8 \cdot 10^3$ cfu/ml versus $1.61 \cdot 10^4$ cfu/ml). Reduction of *E. coli* internalization by *L. sakei* and *P. acidilactici* tended ($P=0.08$) to correlate significantly with a decrease in the pro-inflammatory status, as indicated by a reduction in the expression of IL-8 in comparison with control infected cells (Relative Quantification (RQ), 16.89 ± 0.151 versus 32.32 ± 0.165 and 4.02 ± 0.114 versus 7.66 ± 0.139) respectively. Last, the measure of lactate dehydrogenase activity as a cytotoxicity marker corroborates that cell toxicity was reduced ($P<0.05$) by *P. acidilactici, L. rhamnosus* and *L. reuteri*.

Discussion
In conclusion, these results demonstrate a clear potential of LAB probiotics at fighting endometrial *E. coli* infections, with *L. sakei* and *P. acidilactici* being the two probiotics showing best results at preventing pathogen internalization and pro-inflammatory status regulation.

Keywords: Bovine Endometritis, Escherichia Coli, Lactic Acid Bacteria

Probiotic Potential of Sorghum and Pearl Millet of The Semi-arid Tropics

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Introduction
Cereals such as rice and wheat are the predominant staple food for millions across the world that lead not only to an array of emerging life style diseases but also challenges human health and nutrition. Hence, there is an urgent need for identifying and recommending diversity of other cereals which can enhance the nutrition of the mal-nourished population of the world.

Two of ICRISAT's mandate crops, sorghum and pearl millet, can serve this purpose as these can be the source of prebiotics (Total dietary fiber, resistant starch, total oligosaccharides, β-glucan, etc.) for functional food.The main objective of the present investigation is to understand the probiotic potential of sorghum and pearl millet which is grown extensively in the Semi-Arid Tropics of the world.

Materials and methods

Four varieties of sorghum grown under control and drought stress conditions (K359 control, K359 stress, R16 control, R16 stress, K648 control, K648 stress, 6040 control and 6040 stress) and two varieties pearl millet (dual purpose hybrid and high Fe hybrid) were collected from Gene bank of ICRISAT, Patancheru and milled.

Probiotic microorganisms (including bacteria, actinomycetes and yeast) of native floor and fermented samples of sorghum and pearl millet were isolated using selective mediums (including plate count agar [PCA], yeast glucose chloramphenicol agar [YGCA], actinomycetes isolation agar [AIA], lactic agar [LA] and bifido bacteria agar [BBA]) and further characterized for their morphological and biochemical parameters including urease, catalase, oxidase, starch hydrolysis, gelatin liquefaction, nitrate reduction, hydrogen sulfide, carbohydrate utilization [glucose, lactose and sucrose] and indole, methyl red, Voges-Proskauer and citrate (IMVIC) tests and acid tolerance *in vitro*. The promising isolates with acid and bile tolerance were further characterized and identified by 16S rDNA analysis.

Results

A total of 34 bacteria (19 from PCA and 15 from BBA), 30 actinomycetes (from AIA) and 30 yeasts (from LA and YGCA) were isolated from the four sorghum (control and drought stress conditions) and two pearl millet varieties based on morphological traits including color, size, form, surface, texture, elevation and margins of the colonies in the Petri plates. Based on the acid tolerance and biochemical characterization, we have narrowed down the probiotic bacteria to 16, actinomycetes to 14 and yeasts to 14. The 44 probiotic microbes (16 bacteria + 14 each of actinomycetes and yeast) are now further characterized for bile tolerance and 16S rDNA analysis (for identification of the organisms).

Discussion

The identified isolates will be analyzed for antimicrobial (against human pathogens and plant pathogens), antibiotic resistance and transit tolerance under simulated conditions. This work shall explore ways to use these crops for development of stable and functional commercial prebiotic and probiotic products, thus providing healthy food alternatives to the society. The creation of demand for these crops shall also benefit the farmers of the semi-arid tropics.

Keywords: Sorghum, Pearl Millet, Probiotics

Expression of Fluorescent Proteins in Bifidobacteria for Analysis of Host-microbe Interaction

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Abstract

Bifidobacteria are an important component of the human gastrointestinal microbiota and are frequently used as probiotics. The genetic inaccessibility and lack of molecular tools commonly used in other bacteria has hampered a detailed analysis of the factors and mechanisms of bifidobacteria involved in adaptation to, colonization of, and interaction with the host. In the present study, a range of molecular tools were developed that will allow to close some of the gaps in functional analysis of bifidobacteria.

A number of promoters were tested for transcriptional activity in *B. bifidum* S17 using pMDY23, a previously published promoter probe vector. The promoter of the *gap* gene (P_{gap}) of *B. bifidum* S17 yielded the highest reporter gene activities among the tested promoters.

Thus, P_{gap} and the pMDY23 backbone were used to construct a range of vectors for expression of different fluorescent proteins (FPs). Successful expression of green, cyan, yellow and red FPs was successfully shown for three strains representing three different *Bifidobacterium sp.*. The red fluorescent *B. bifidum* S17 pVG-mCherry was further used to demonstrate application of fluorescent bifidobacteria for adhesion assays. Furthermore, this strain was successfully detected in human primary macrophages generated by *ex vivo* differentiation of monocytes. This demonstrates that detection of bifidobacteria inside relevant host cell populations is possible.