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CONFERENCE PROCEEDINGS

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Among those, enterococci, in particular vancomycin-resistant enterococci (VRE), are a leading cause of health-care associated and community-acquired infections with life threatening issues and increased hospital costs. Enterococci are natural inhabitants of the subdominant gut microbiota and become dominant upon antibiotic treatments favoring systemic infections and transmission. Reduction of intestinal colonization or carriage after antibiotic treatment could limit the risks of VRE infections and dissemination. In this study, we investigated the effects of two *Lactobacillus* probiotic strains on intestinal VRE carriage and clearance after antibiotic treatment.

Methods

We developed an intestinal colonization mouse model based on a microbiota dysbiosis induced by clindamycin to mimic enterococci overgrowth and VRE establishment. Mice received subcutaneous clindamycin for 3 days before orogastric inoculation with *Enterococcus faecalis* VRE strain. Using this model, probiotic strains were daily orally administered to mice starting 1 week before antibiotic treatment until two weeks after arrest of antibiotic treatment and inoculation of VRE. Kinetics of establishment and clearance of VRE as well of indigenous enterococci population levels were monitored by selective plating. In parallel, fecal samples were collected for 16S rRNA gene survey analysis of the whole microbiota.

Results

In control experiments, transient increase of indigenous enterococci was concomitant with clindamycin treatment. Enterococci population reached the highest level one day after the arrest of antibiotic treatment and then decreased progressively to the initial level 4 to 5 days later. After inoculation, the *E. faecalis* VRE strain paralleled indigenous enterococci and persisted at detectable level at least up to 11 days post-gavage. Overgrowth of indigenous enterococci correlated with decreased microbiota diversity resulting from antibiotic treatment. The administration of the probiotic strains had no effect on enterococci overgrowth. Remarkably, administration of *Lactobacillus paracasei* significantly decreased *E. faecalis* persistence in the gut. VRE were only detected in half of the mice receiving *L. paracasei* 11 days after the arrest of the antibiotic treatment and VRE level was significantly decreased in the other half compared to control mice. No significant difference of VRE population level was observed in *L. rhamnosus* treated mice.

Discussion

Altogether, these results identify the *L. paracasei* strain as a promising candidate to promote VRE clearance. Impact of probiotics on the gut microbiota will be assessed by 16S rRNA gene analysis. Ultimately, this work will contribute to propose non-antibiotic prophylactic strategies against intestinal opportunistic pathogens after antibiotic dysbiosis.

Keywords: Probiotics, Lactobacillus, Enterococcus Faecalis, Clearance

Assessing the Prebiotic Diversity of Sorghum and Pearl Millet

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Introduction

International Crops Research Institute for the Semi-Arid tropics (ICRISAT), India is a major repository for world Sorghum (*Sorghum bicolor* (L.) Moench) and Pearl millet (*Pennisetum glaucum* [L.] R. Br.) germplasm. ICRISAT's gene bank consists of 37,904 accessions of Sorghum from 91 countries and 21,594 accessions of Pearl millet from 50 countries. In order to enhance the use of germplasm for screening of specific traits core and mini core collections, representing the diversity of the entire collection have been developed by ICRISAT, for both these cereals. However, these germplasm accessions of Sorghum and Pearl millet have not yet been profiled for their prebiotic diversity. Hence profiling of accessions, of the germplasm collections of Sorghum and Pearl millet, with the aim to identify accessions with desirable prebiotic traits for development of functional (probiotic) foods is being undertaken.

Methods

Representative sorghum lines (each grown under normal and drought stress conditions) and pearl millet lines from Pearl Millet Inbred Germplasm association panel (PMiGAP), grown at ICRISAT, India were assessed for total dietary fiber (TDF) and resistant starch (RS) using optimised AOAC protocols. An LC-MS/MS method was also optimised for the detection of total oligosaccharides (TOS).

Results

Measurable variations were observed in the TDF, RS, and TOS contents among the Sorghum and Pearl millet lines studied. TDF content was found to vary between 12.16 to 15.12% in case of the Sorghum lines and between 12.09 and 14.61% in case of pearl millet lines. Further, the four Sorghum lines evaluated for their RS contents, show that the RS values range between 0.6% and 2.0%. It was also observed that compared to the control lines, the drought stress lines of sorghum tend to show higher amount of TDF and RS. RS data obtained in case of the Pearl Millet show that the RS values range between 0.77% and 2.4%. Two lines each of Sorghum and Pearl Millet were used to standardise extraction and resolution of oligosaccharides using LC-MS/MS. All samples showed presence of Malto-oligosaccharides between DP3 and DP5, with varying concentrations based on the cultivar. Preliminary data show DP3 concentration to be higher in pearl millet as compared to sorghum.

Discussion

The data obtained indicate the potential of identifying lines of both sorghum and pearl millet having variable prebiotic potential, by further screening of ICRISAT's Sorghum and Pearl Millet Germplasm collection, using the optimised testing protocols. Based on this preliminary screening it is also seen that it is possible to enhance the level of certain prebiotic components by growing these crops under drought stress conditions. Presence of Malto-oligosaccharides also indicates that these cereals have the potential to be used in development of infant food formulations.

Keywords: Sorghum, Pearl Millet, Prebiotics, Malto-oligosaccharide, Drought

Characterization of a *Bifidobacterium Pseudocatenulatum* Strain Capable of Bioconverting Flavonoids in Soya and Bean Derived Milks

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Introduction

Plants are dietary sources of several phytochemicals. Among them, flavonoids are secondary metabolites derived from the phenylpropanoid pathway in higher plants. Recently their importance as health-promoting compounds in preventing hormone dependent cancers, cardiovascular diseases, osteoporosis, and menopausal symptoms have been highlighted. Flavonoids are mainly present in plants as *O*-glycosides. They are hydrolyzed by gut microbial enzymes to their aglycons, which represent the bioavailable and bioactive form. The main objective of the present work was to investigate potential applications of the strain *Bifidobacterium pseudocatenulatum* B7003, previously selected as capable of bioconverting several flavonoids to their aglycone form, as a probiotic starter culture to obtain fermented legume milks with an increased concentration of bioavailable flavonoids.

Methods

The strains used in this work were *B. pseudocatenulatum* B7003 and, as a negative control, *Bifidobacterium longum* B7254 (a strain not possessing detectable beta-glucosidase activity). *Phaseolus vulgaris* L. seeds of the cultivar "Verdone", *P. vulgaris* L. landrace "Zolfino del Pratomagno", the soybean (*Glycine max* L.) cultivar "Aires" were used in the study to prepare milks. A commercial soymilk (Valsoia) was also employed. Whole seeds of soybean and common bean were washed, crumbled in a blender using distilled water and the resultant slurry was filtered to obtain soymilk or beanmilk, which were used in the fermentation experiments. Traditional TPY medium (TPY) was used as growth control. Flavonoids were extracted with a commercial cartridge and analyzed by HPLC-DAD.

Results

B. pseudocatenulatum B7003 strain could grow effectively in soymilks: exponential growth on the commercial soymilk was observed, reaching a 3 Log increase. Growth on soya Aires milk was of less extent but comparable to that on TPY medium. *B. longum* B7254 strain evidenced a capability of growing on both soymilks similar to the B7003 strain, but the start of growth was slower. The B7003 strain showed a very good capability of growing on the milks obtained from the two *Phaseolus vulgaris* cultivars. In the initial 24 h of incubation, growth was even faster and of great extent with respect to the TPY medium.