Expression of luxS Gene Involved in quorum sensing in Lactobacillus acidophilus NCFM after passage through an in vitro digestion model

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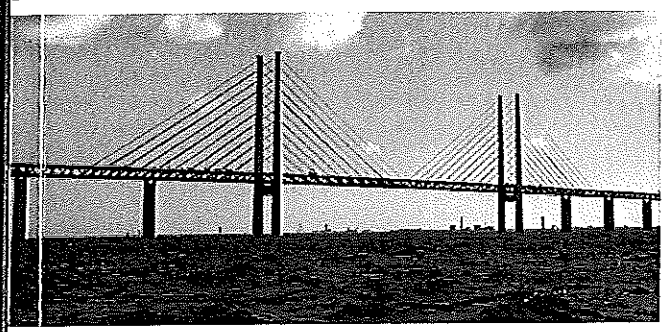

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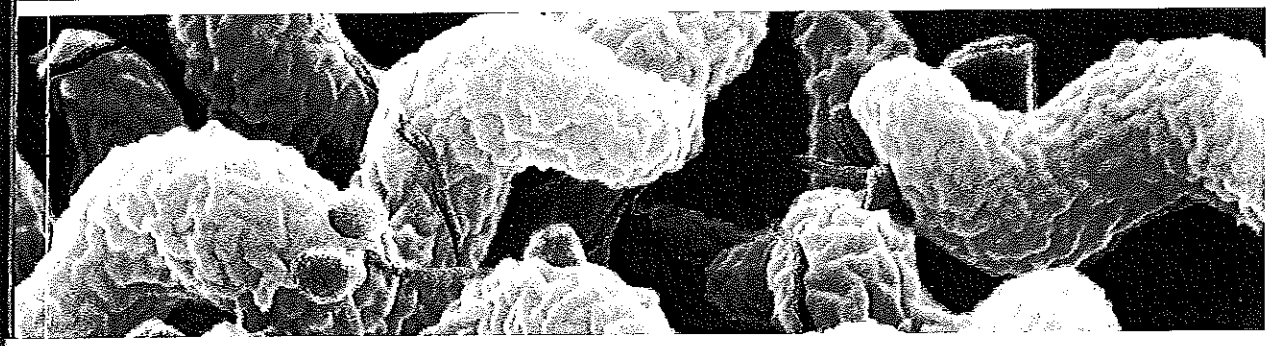

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PEE2.14 **Probiotics to young children with atopic dermatitis: a randomized placebo-controlled trial**

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Impairment of the intestinal mucosal barrier is involved in the pathogenesis of atopic dermatitis (AD). Recent studies suggest that probiotic bacteria stabilize the intestinal barrier function and decrease gastrointestinal symptoms in children with AD. The effect of probiotic bacteria *Lactobacillus acidophilus* NCFM (ATCC 700396) and *Bifidobacterium animalis* subsp. *lactis* Bi-07 (ATCC SD5220) on severity of AD and the composition of fecal microbiota was investigated in a double-blind randomized placebo-controlled intervention study. Fifty children (at the age of 7-24 months) with AD received NCFM (10^{10} CFU/day), Bi-07 (10^{10} CFU/day) or placebo for 8 weeks. Serum eosinophil cationic protein (ECP) significantly correlated with SCORing Atopic Dermatitis (SCORAD) index suggesting the use of ECP as a measure of the degree of AD in children. A post hoc analysis showed significant reduction in severity of AD concurrently with decreasing levels of IFN-γ and IL-10 in the Bi-07 group but not in the other groups. Other clinical and immunological parameters (SCORAD, faecal calprotectin, immunoglobulin IgE, ECP and regulatory cytokines IL-10, IFN- and IL-31) were not affected by intervention. The composition of the total microbiota, *Lactobacillus* group and *Bifidobacterium* in stool samples was characterized by denaturing gradient gel electrophoresis and quantitative real-time PCR. The core population of the *Lactobacillus* group was composed of *L.gasseri, L.fermentum, L.oris, Le.(pseudo-)mesenteroides*, while the bifidobacterial community included *B.adolescentis, B.bifidum, B.longum* and *B.(pseudo-)catenulatum*. The counts of lactobacilli, *L. acidophilus* and *B. lactis* increased significantly after intervention, indicating survival of the probiotics in the intestinal tract. Fecal microbiota from six children, who showed the highest reduction in the SCORAD index after intervention, was investigated by 454 FLX pyrosequencing of the V4 region of 16S rRNA gene. In total 140,000 pyrosequencing reads were assigned to five major bacterial phyla, including *Firmicutes* (56%), *Bacteroidetes* (30%), *Actinobacteria* (10%), *Proteobacteria* (2%) and *Verrucomicrobia* (1%). Administration of NCFM and Bi-07 did not show significant effects on the composition and diversity of the *Lactobacillus* group, bifidobacteria and the total fecal microbiota. *In conclusion*, the results of this study indicate the beneficial effect of *B.lactis* Bi-07 on AD. The GM composition was not affected by probiotic intake.

PEE2.15 **Expression of luxS Gene Involved in quorum sensing in *Lactobacillus acidophilus* NCFM after passage through an in vitro digestion model**

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Within recent years, there has been an increasing interest in discovering the beneficial effects of probiotics and the mechanisms by which probiotics interact with the host and the gut microbiota. One of the mechanisms that bacteria interact or communicate with each other is quorum sensing. Quorum sensing is cell-to-cell signalling through the production, secretion and detection of small signal molecules called autoinducers. The aim of the present study was to investigate the expression of the *luxS* gene involved in quorum sensing in probiotic strain *L. acidophilus* NCFM after passage through an *in vitro* digestion model, which could have effect on cell-to-cell communication between microorganisms of gut microbiota.
Overnight culture of *L. acidophilus* NCFM was inoculated (1%) to 50 ml MRS broth for 16 h at 37°C with the final pH of 4.2 and centrifuged and used for the experiments, or inoculated (10%) to 11% skim milk for 28 h at 37°C with the final pH of 4.2 and 5 g was utilized. An *in vitro* digestion model of upper gastrointestinal tract was used, including three compartments; mouth: saliva juice (6 ml, pH 6.5 for 5 min), stomach: gastric juice (12 ml, final pH 3.5 for 2 h) and small intestine: duodenal and bile juices (12 and 6 ml, respectively, final pH 6.5 for 2 h), incubation at 37°C with rotation head-over-heels. Samples were taken at t=0 and 30 min intervals for determination of survival and gene expression analysis using Real-Time PCR. 23SrRNA gene was used for normalization.
Survival of cells grown in skim milk was significantly higher after 2 h incubation in gastric juice and during incubation in the duodenal and bile juices compared to the cells grown in MRS broth. In cells grown in skim milk, the gene expression was up-regulated after 30 min incubation in the gastric juice. The expression decreased over time and increased again after 1 h incubation in duodenal and bile juices and thereafter. Cells grown in MRS without protection of food matrix were under severe stress during incubation at the harsh conditions of the digestion model, and therefore gene expression was highly unstable and analysis of the data was not possible. Conclusion: The *luxS* gene of *L. acidophilus* NCFM is up-regulated at the stress conditions of gastrointestinal tract. Food matrix has important role in the survival and in the reaction of the cells to the stress conditions.