ECOVAG®

Scientific factsheet SF-01

In-vitro colonization of EcoVag® strains

IN VITRO Adherence of EcoVag® probiotic strains to vaginal epithelial cells (VEC) and cultured Caco-2 cells.

— Brønstad G. & Brandsborg E. ; 25.11.2002; Patent no: (WO 03/080813 A2); 02.10.2003.

CONCLUSION

In the Patent no: (WO 03/080813 A2) from 2003, the superior IN VITRO adhesion of the EcoVag® strains *Lactobacillus gasseri* (EB01-DSM14869) and *Lactobacillus ramnosus* (PB01-DSM 14870) to vaginal epithelial cells (VEC) and Caco-2 cells are illustrated.

The IN VITRO adherence of the three lactobacilli strains (which are compared) to VEC are seen in Figure 1.The values are the mean +/- SD from 5 donors. Strain *Lactobacillus ramnosus* PB01 adheres most strongly with approximately 20 bacteria per cell after 1-hour incubation. *Lactobacillus* GG has the lowest number of adhered bacteria, while strain *Lacobacillus gasseri* EB01 has intermediate values.

The IN VITRO adherence of probiotic lactobacilli strains to Caco-2are seen in Figure 2 which illustrates the adherence of the three-probiotic strains to cultured Caco-2 cells. Almost 30% of the added *Lactobacillus* PB01 strain bacteria adhered to the cultured cells during the 1-hour incubation period, in comparison to about 20 % of the EB-01 strain and only 10 % of the Lactobacillus GG strain.

FACTS

Adherence test of probiotic strains to VEC:
By this test, the adherence of probiotic Lactobacilli strains to VEC was investigated. VEC were harvested, during gynecological investigation, into sterile phosphate buffer saline (PBS) PH 7,2 . After about 20 hours, gentamicin and fetal bovine serum was added to the cells. Final concentration were 100 yg/ml gentamicin and 2% serum.

The washed probiotic strains were suspended in 10 mM lactate and 150 mM NaCl pH buffer and the optical density was adjusted to 1,00 +/- 0,03. The bacterial suspension was mixed 1:1 with VEC suspension and incubated for 2 hours at 37C. After incubation, the cells were washed 4 times using PBS buffer with 2% fetal bovine serum and finally washed once using plain PBS. After the last centrifugation, the cell pellet was suspended in a few µl of the buffer and transferred to microscope slides. The cells were then dried at 90 C. The dried spot was fixed with methanol and stained with 0 ,1% crystal violet. The stained preparation was studied using a light microscope with oil immersion at 100x. The number of bacteria was counted on 30-70 randomly chosen cells/donor.

Adherence test of probiotic strains to Caco-2 cell cultures:
By this test, the adherence of probiotic Lactobacilli strains to Caco-2 was investigated. The Caco-2 cell line (CRL-2102, ATTC, USA) was cultured in Delbecco´s modified Eagle medium (sigma-Aldric), 10% fetal calf serum (FBS)(Merck) and 1% Penicillin/streptomycin (Merck)at 37C in an atmosphere of 5% CO2/95% air. For adherence assays, Caco-2 cells were seeded at a concentration of 1,5x105 in 96 wll microtiter plates. The cell cultures were maintained for 2 weeks prior to use. The cell culture medium was changed every other day, and it was changed 2 hours before the adherence assay.

The adherence of bacterial strains to Caco-2 cell cultures were examined by adding 200 yl of radio labelled bacterial suspension to the wells. Before adding the bacteria, the wells were washed twice with MEM-Eagle (Sigma-Aldrich) supplemented with 0,5% FBS,1% L-glutamine and 0,1% non-essential amino acids. After incubation for 1 hour, the cell cultures were washed 4 times with 250yl buffered saline solution in a Multiwash Plus AR (Labsystem) and treated with 150yl of 2% SDS in 0,01M NaOH for 20 minutes to lyse the bacteria, which were measured by liquid scintillation counting. The adherence ratio (%) was calculated by comparing the radioactivity with the original bacterial suspension.

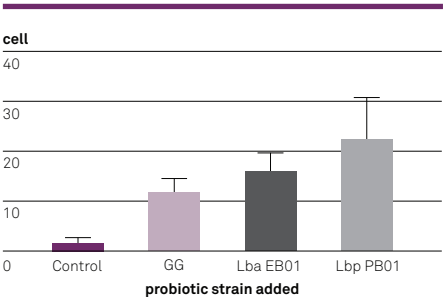

Figure 1: The adherence of the three probiotic strains to vaginal epithelal cells (bacteria pr cell)

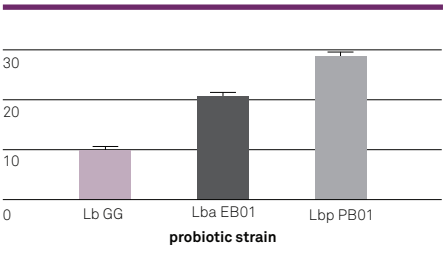

Figure 2: The adherence of the three strains to cultured Caco-2 cells in %