

RESEARCH ON THE EFFECTS OF PROBIOTICS ON SHIGA- TOXIN PRODUCING *ESCHERICHIA COLI* (STEC) LAN / INRA (MICROBIOLOGY UNIT)

- **Effects of probiotics on digestive carriage of STEC in ruminants**

The digestive tract of ruminants is considered as an important reservoir of Shiga-toxin producing *E.coli* responsible for food-borne infections in humans. It is essential to limit digestive carriage of these potentially pathogenic strains in ruminants in order to reduce their faecal excretion and the risk of contamination of animal products (meat and dairy products). To achieve this goal, the physiological and ecological basis of STEC survival has to be investigated in the gastro-intestinal tract of ruminants, to develop strategies aimed to limit gut colonisation of the animals by these bacterial communities.

In this context we have undertaken a research program to study the role of biotic factors (barrier effect of the digestive flora, effects of probiotics – live yeasts and lactic acid bacteria-) and abiotic factors (role of the diet on physico-chemical characteristics of the ruminal or faecal environment) on the behaviour of STEC in the rumen and in the terminal part of the ruminant gastro-intestinal tract. We are also interested in acid resistance capacity of STEC, which favours the survival of these bacteria in the abomasum, and consequently favours their shedding. Moreover, this characteristic helps the bacteria to resist to food processing procedures and to acidity of human stomach.

Therefore the **current research project is focused on three aspects :**

- **physiology of STEC in ruminal and faecal environment;**
- **interactions STEC/digestive autochthonous flora ;**
- **acid resistance capacity of STEC.**

The potential effects of LAN probiotics (Levucell SC, Levucell SB, *L.acidophilus* strains) on these three aspects will be studied.

New data on the effects of LAB on survival of STEC in digestive environment are expected during the first semester 2004.

- ***In vitro* inhibition of *E.coli* EDL 933 (O157 :H7) by *Lactobacillus acidophilus* (BG2FO4), *Lactobacillus buchnerii* (40788) and *Propionibacterium freundrenchii* P63.**

The effects of different LAB strains on growth of *E.coli* EDL 933 (O157 :H7) have been studied in different conditions :

- growth on LB medium with or without filtrated culture supernatants of La, Lb or P63 (La and Lb were grown overnight at 37°C on MRS medium, P63 was grown at 32°C on sodium lactate broth) adjusted or not to pH 7 ;
- incubations of EDL 933 (final concentration 5×10^5 cfu/ml), anaerobically or aerobically, in a complex culture medium containing 40% of clarified rumen fluid and glucose (3 g/l), in absence or in presence of the different LAB strains (final concentrations of 1.9×10^7 , 2.4×10^6 , and 1.4×10^6 cfu/ml for Lb, La and P63, respectively).

Results have shown that *E.coli* growth (measured by OD600nm) on LB medium was totally inhibited in the presence of 1 ml of La supernatant, and partially inhibited in the presence of Lb or P63 supernatants (figure 1). This effect was much lower when the supernatants were diluted 10-fold or when they had been previously adjusted to pH 7.0. A control realised by adding sterile MRS medium adjusted to pH 4.0 showed a similar inhibition of bacterial growth, suggesting that strong acidification of the medium could explain the drastic effect of La.

After incubation in the presence of the different LAB strains in the complex medium, counts of EDL 933 were lower than the counts made before incubation, but the effects were modest (<one log less bacteria). Nevertheless the more important decrease was obtained with La under anaerobic conditions (figure 2) . In this case, the effect was not due to a strong acidification because pH of the culture medium was buffered by an addition of sodium bicarbonate.

These preliminary data suggest that *L.acidophilus* BG2FO4 could be very interesting for limiting the development of *E.coli* O157 :H7 (EDL 933); more experiments are needed to verify these findings.

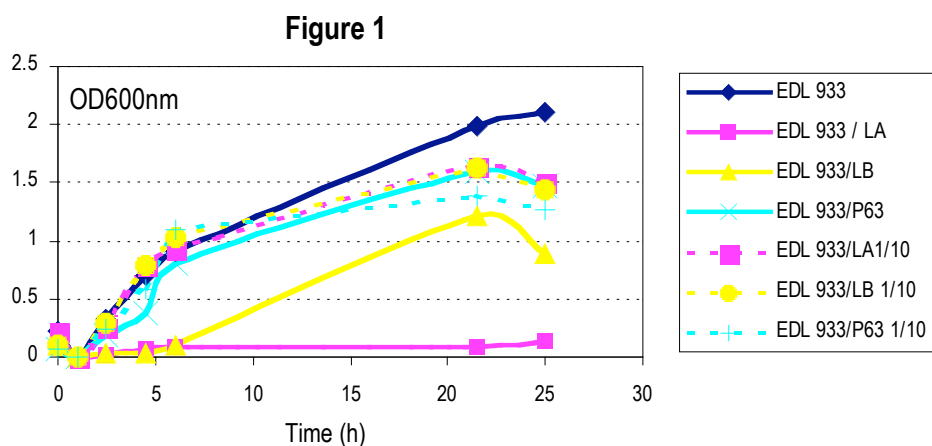


Figure 2

