SUMMARY

The main objective of the present study was to assess the immunological effects of the probiotic *Enterococcus faecium* SF68 (NCIMB 10415) in piglets using two different approaches.

Firstly, we used an *in vivo* model, where piglets in a probiotic (43 piglets receiving *E. faecium* SF68 supplement) and a control group (46 piglets without the probiotic supplement) were infected with *Salmonella typhimurium* DT104. Piglets from each litter were randomly selected and sacrificed 3h, 24h and 72h and 28d post infection (*p.i*) and PBMC, CD4+ lymphocytes from spleen and discrete Peyer´s patch (PP), lymphocytes from distal continuous PP and CD8+ lymphocytes from the intraepithelial lymphocytes (IEL) of the jejunum were isolated using different protocols and a magnetic cell sorting (MACS) method. The relative percentages of CD8+ lymphocytes in the IEL of both groups as compared by FACS analysis showed, in line with previous studies, a significant decrease in CD8+ lymphocytes in the probiotic group 24h after *Salmonella* infection. The relative percentages of CD4+ cells in the discrete PP and the spleen showed a tendency towards higher percentages in piglets of the probiotic group. From all the cells isolated, RNA samples were extracted and complementary DNAs (cDNAs) were prepared. From the cDNAs of the PBMC samples and that of the distal PP samples, gene expression analysis for various cytokines/chemokines, and receptors was undertaken by real-time PCR. Analysis revealed a significant up-regulation of the genes for TLR2, IL-1α, IL-1ß and IL-8, in the PBMC and IL-1α, in the distal continuous PP of piglets of the probiotic group 71h after *Salmonella* infection. Although inflammation could not be minimized, our study is the first to report the up-regulation of the anti-inflammatory genes TLR9, CD9 and TGF-ß through the probiotic *E. faecium* SF68.

Secondly, we used an *in-vitro* model to study the immunological effect of *E. faecium* SF68 against transmissible gastroenteritis virus (TGEV) in epithelial cells. This work is the first to study the effect of probiotics against TGEV. Since probiotics act mainly in the intestine where TGEV-infection resides, establishing appropriate intestinal cell lines of porcine origin is crucial. For this purpose two previously isolated cells (type I and type II cells) from the epithelium of piglets were characterized morphologically (light and transmission electron microscopy (TEM), FACS) histochemically (FACS and fluorescence Microscopy), molecularly (RT-PCR) and with respect to their sensitivity to TGEV infection. Our results indicate that the cells are of epithelial nature and that only type II cells are sensitive to TGEV.

Type II cells were found to produce higher virus titres than the known model cells for studies on TGEV, ST cells, which do not originate from the intestine. Thus, type II could be used as appropriate porcine intestinal epithelial cell lines for *in vitro* studies with TGEV.

To study the anti-viral effect of the probiotic on TGEV, monolayers of type II cells were pre-treated with *E. faecium* SF68 before infection with the virus and cellular survival rates determined using an MTT test. It was revealed that both probiotic bacteria and their metabolic products in culture supernatant enhanced cellular survival against TGEV infection. Furthermore, we measured the viral titres of the probiotic-treated and non-treated TGEV-infected monolayers of type II cells using the TCID50 titration method on ST cells. Our results showed a decrease in the viral infectivity and viral yields as a result of *E. faecium* SF68-pretreatment. In-order to confirm the molecular out comes of the protective effect of the probiotic pre-treatment, we did real-time PCR analysis to compare the levels of expression of the genes for IL-6, IL-8 and IFN-γ between the control type II cells, the cells pre-treated with the probiotic, the cells that were infected with TGEV and the cells that were probiotic pre-treated and infected with TGEV. Our results show a highly significant up-regulation of the above genes by viral infection as compared to that attributed to the probiotic-pre-treatment. As a result of decreased virus yields, a significant decrease in the virus-induced up-regulation of the above inflammatory genes was observed after *E. faecium* SF68 pre-treatment. The results suggest a possible mechanism of beneficial effects of probiotics to intestinal infections through a reduction of inflammatory cytokines induced by infecting agents, including viruses.