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Importance of the host specificity in the selection of probiotic bacteria

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The gastrointestinal tract is a complex and dynamic ecosystem. Commensal microorganisms (C), which proliferate in the intestine from birth, are crucial for gut homeostasis while non commensal (NC) microorganisms are transient and enter the organism from the environment and foods. We studied comparatively the influence of oral administration of C and NC *Lactobacillus fermentum* and *Lactobacilus acidophilus* on the gut-associated lymphoid tissue (GALT) of conventional mice. To determine the importance of the selection of probiotic host-specificity bacteria with immunomodulating capacity, we examined the interaction with the gut by transmission electron microscopy and FITC-labelled bacteria. We compared the immunomodulation capacities of C and NC strains by studying the number of IgA secreting cells and cytokine profile. No differences were found in the number of IgA+ cells; however, the pattern of cytokine response to C and NC bacteria was different. With regard to proinflammatory cytokine (IFNγ and TNFα), we found that TNFα was mainly produced by NC bacteria, while C bacteria were able to elicit mainly IFNγ. The regulatory cytokines (IL-10 and IL-4) were induced with different patterns for both C and NC strains. No differences in the pathway of internalization to the gut between C and NC were found. In summary, we determined that C and NC bacteria interact with the intestine in the same way; both C and NC bacteria were able to reinforce the surveillance of the gut mucosal immune system. The cytokine profile showed that C bacteria would be involved in the regulation of intestinal homeostasis rather than in the immune activation as the NC bacteria.

Keywords: commensal and non commensal bacteria, gut immunity, cytokines.

The gastrointestinal tract is a complex ecosystem made up of three main components that are permanently in contact and interact with each other: host cells, nutrients and microflora. Intestinal ecology results from a dynamic equilibrium, remodelled by food intakes, between the inhabitants of the gut lumen, where more than 400 different bacterial species coexist.

Although the predominant human intestinal microflora is relatively stable, each individual harbours a specific bacterial community. In addition to individual differences, some factors such as stress or antibiotic treatments also induce variations in the gut microflora, resulting in subdominant species or even pathogens becoming more dominant (Moreau & Gaboriau-Routhiau, 2000).

The intestinal microflora can be divided in two main groups: the *autochthonous* or *commensal* (C) flora, which proliferate in the intestine from the moment of birth, becoming stable after weaning; and a second group, called *allochthone* or *non-commensal* (NC) microflora, which are temporary and consist of different microorganisms introduced by ingestion together with the food. The C intestinal microflora play an important role in maintaining the normal physiology and health of the host (Moreau & Gaboriau-Routhiau, 2000). The composition and activity of C microorganisms is responsible for three basic functions: metabolic (food breakdown and vitamin synthesis), barrier (protection against external and invading pathogens) (Raibaud, 1992) and interactions with the host

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(in particular through the immune system). Experimentally, the role of the microflora is determined by comparison between germ-free and conventional animals. Newborn germ-free animals exhibit an underdeveloped intestinal immune system which can be rapidly normalized by bacterial colonization of the intestine with faecal flora from conventional animals. Recent studies using *Bacteroides thetaiotamicron*, a prominent member of adult mice and humans, revealed that this bacterium not only modulates the expression of the genes involved in several important intestinal functions such as nutrient absorption, mucosal barrier fortification, angiogenesis and postnatal intestinal maturation, but also modifies the pattern of gut epithelium glycosylation (Bry et al. 1996; Stappenbeck et al. 2002). Postnatal colonization of the intestine 'educates' our immune system so that we become tolerant to a wide variety of microbial immunodeterminants (Macpherson & Uhr, 2004). This education appears to reduce allergic responses to food or environmental antigens (Moreau & Gaboriau-Routhiau, 2000; Braun-Fahrlander et al. 2002).

Furthermore, C bacteria play crucial roles in promoting B cell development in Peyer's patches, which are underdeveloped in germ-free animals. Consequently, animals harbouring a normal intestinal flora have 10-times more IgA-producing cells in the intestinal lamina propria than germ-free animals, showing that C flora are essential for the maturation of the immune system (Crabbe et al. 1968).

Lactic acid bacteria (LAB) are frequently used in dairy products as probiotic microorganisms because of their health-promoting effects (Lidbeck & Nord, 1993; Gibson & Wang, 1994). Perdigón et al. studied different LAB as well as the mechanisms involved in the mucosal immune system activation (Perdigón et al. 2002; Maldonado Galdeano & Perdigón, 2004) and showed that LAB can adhere to the intestinal epithelial cells, interact with and stimulate the gut immune cells to release inflammatory and regulatory cytokines, and increase the number of IgA-producing cells. This effect was dose- and strain dependent. However, much of the literature advocates the use of host-specific strains to achieve a better effect on the intestinal ecosystem, due to colonization or strain competition, mainly for use in farm animals. However, in relation to the signals induced by pathogenic bacteria on the immune cells associated with the gut, NC microorganisms should be more effective than C bacteria since the latter could induce greater tolerance.

Previous results and the above considerations led us to study comparatively, in conventional BALB/c mice, the influence of the oral administration of C and NC Gram-positive bacteria on the gut associated lymphoid tissue (GALT), and to determine their effectiveness for selection as probiotic bacteria. The conditions for the induction of the gut immune response while maintaining intestinal homeostasis, measured by the inflammatory immune response, were also addressed.

Materials and Methods

Microorganisms

Four lactobacilli strains were tested, grouped as NC or C bacteria with respect to BALB/c mice. The C bacterial species identified *Lactobacillus fermentum* CRL 1386 and *Lactobacillus acidophilus* CRL 1462, were isolated from the gut of healthy 6–8-week-old BALB/c mice, and kept in the Centro Referencia Lactobacilos (CRL) collection. The NC strains, *Lactobacillus fermentum* CRL 345 and *Lactobacillus acidophilus* A9, were isolated from a regional and commercial cheese respectively (Bioqueso, Ilolay-Vita, Argentina).

Animals

BALB/c adult mice (6–8 weeks old, weighing 25–30 g) were obtained from the close random bred colony kept in our department at CERELA. Each experimental group consisted of 20–30 mice (4–5 for each day and assay). The control group were animals without lactobacilli administration. In all assays, test and control groups were fed *ad libitum* with a conventional balanced diet.

Oral administration of lactobacillus strains

All strains were cultured in MRS broth (Britania, Buenos Aires, Argentina) overnight at 37 °C. Cells were harvested by centrifugation at 8000 ***g*** for 10 min and washed twice in phosphate-buffered saline (PBS). Cells were suspended in 10% non-fat milk and were given to the mice in the drinking water in a daily dose at a final concentration of 10^8 cells/mouse for NC strains, the same dose used for probiotics preparations, and at 10^4 cfu/mouse per day for commensal strains. This dose was selected according Vinderola et al. (2004), as that which did not induce side effects such as normal microflora translocation to the liver and /or spleen. Animals were given the cell suspension for 2, 5 or 7 consecutive days. The volume drunk, controlled daily, was 2·5–3 ml for each mouse in both experimental and control groups. This feeding did not modify the weight of the animals with respect to the control. The control was a group of three animals (for each day and assay) fed with a conventional balanced diet and water plus 10% non-fat milk.

Histological examinations

At the end of each feeding period, with the dose selected by negative translocation assays, test and control animals were killed. Small (ileum) and large (transverse, ascending and descending colon) intestine were removed and processed according to Sainte-Marie's technique for paraffin embedding (Sainte-Marie, 1962). Sections of 4 µm were cut and stained with haematoxylin-eosin for examination by light microscopy.

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Bacteria-labelling procedure

To determine the transit and interaction of the administered bacteria with the gut, bacterial pellets resulting from an overnight culture were washed twice in PBS and then resuspended in PBS solution with fluorescein isothiocyanate (FITC) (100 ug/ml) for 1 h at 37 °C in the dark. Labelled bacteria were washed three times with PBS solution to remove unincorporated FITC. No spontaneous release of the FITC label was determined after 72 h (time of experimentation). The pellet was finally resuspended in PBS to a concentration of 10^8 or 10^4 cells/ml and administered by gavage (0·2 ml) to mice.

Animals were killed by cervical dislocation after 20-min gavage. To determine the interaction capacity of the strains studied with the site of induction of an immune response, the small and large intestine were removed and washed with physiological saline solution (NaCl, 0·85%). Tissues were prepared according to Sainte-Marie's technique for paraffin embedding and examined using a fluorescence light microscope. The study of the transit and persistence of lactobacilli in the gut was also performed with these fluorescent bacteria administered by gavage as previously described. Samples from the small and large intestine were removed at 24, 48 and 72 h after the administration of FITC-labelled bacteria.

Transmission electron microscopy assays

Groups of three mice that had received 0·2 ml of each strain at doses of 10^{10} cfu/ml by gavage were killed by cervical dislocation 20 min after LAB administration. The small intestine of each mouse was removed and washed with 1 ml of physiological saline solution (NaCl, 0·85%). Tissues were fixed in formaldehyde (10%) and glutaraldehyde (2%). Specimens were then washed in PBS and fixed in 1% osmium tetraoxide, dehydrated in ethanol, cleared in propylene oxide and finally embedded in a low-viscosity medium. Sections of the small intestine were stained with saturated uranyl acetate in 50% ethanol and 4% citrate. All sections were examined by transmission electron microscopy.

Immunohistochemical detection of IgA- and cytokine-producing cells in the small and large intestine

At the end of each feeding period, animals were killed. The small and large intestine from test and control animals were removed and processed according to Saint-Marie's technique for paraffin embedding.

The number of IgA secreting cells was determined on histological slices by a direct immunofluorescence assay. After deparaffinization and rehydration in a graded series of ethanol, paraffin sections (4 μm) were incubated with a 1 : 100 dilution of α-chain monospecific antibody conjugated with FITC (Sigma, St Louis MO, USA) for 30 min and observed with a fluorescent light microscope.

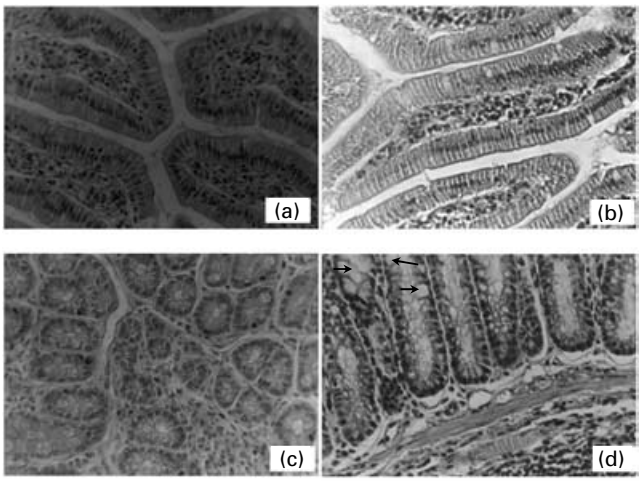

Fig. 1. Light micrograph of haematoxilin-eosin stained sections from the small and large intestine of mice fed with the assayed strains (a) Small intestine of unfed mice; (b) small intestine of mice after 7-d administration of NC *Lb. acidophilus*; (c) Large intestine of unfed mice; (d) large intestine of mice after 7-d administration of NC *Lb. acidophilus* (magnification ×400). A slight cellular infiltration and increases of goblet cells (arrows) were seen after lactobacilli administration compared to the unfed mice.

Cytokine-producing cells were studied by an indirect immunofluorescence assay. After deparaffinization and rehydration in a graded ethanol series, paraffin sections (4 μm) were incubated with a 1% blocking solution of bovine serum albumin (BSA) (Sigma) for 30 min at room temperature. They were washed three times in PBS and incubated with normal goat serum (dilution 1 : 100) for 30 min. Rabbit anti-mouse IL-2, IL-4, IL-6, IL-10, IFNγ and TNFα were applied to the sections for 60 min at room temperature. Then the slices were washed twice in PBS and incubated for 45 min with a 1 : 100 dilution of the goat anti-rabbit antibody conjugated with FITC (Jackson Immuno Research Labs Inc., West Grove PA, USA) at room temperature and washed twice in PBS.

The number of fluorescent cells was counted in 30 fields at 1000× magnification and results were expressed as the number of positive fluorescent cells per ten fields.

Statistical analysis

Statistical analyses were performed using MINITAB 14 software. A factorial distribution (5 × 3, feeding procedure × days) was used; the comparison were accomplished by an ANOVA general linear model followed by a Tukey's *post hoc* test and $P<0·05$ was considered significant. Each experiment was repeated three times and all results (from the three trials) were analysed together. No interactions between the three trials were observed when they were analysed at different times (days) or when different lactobacilli administrations were used.

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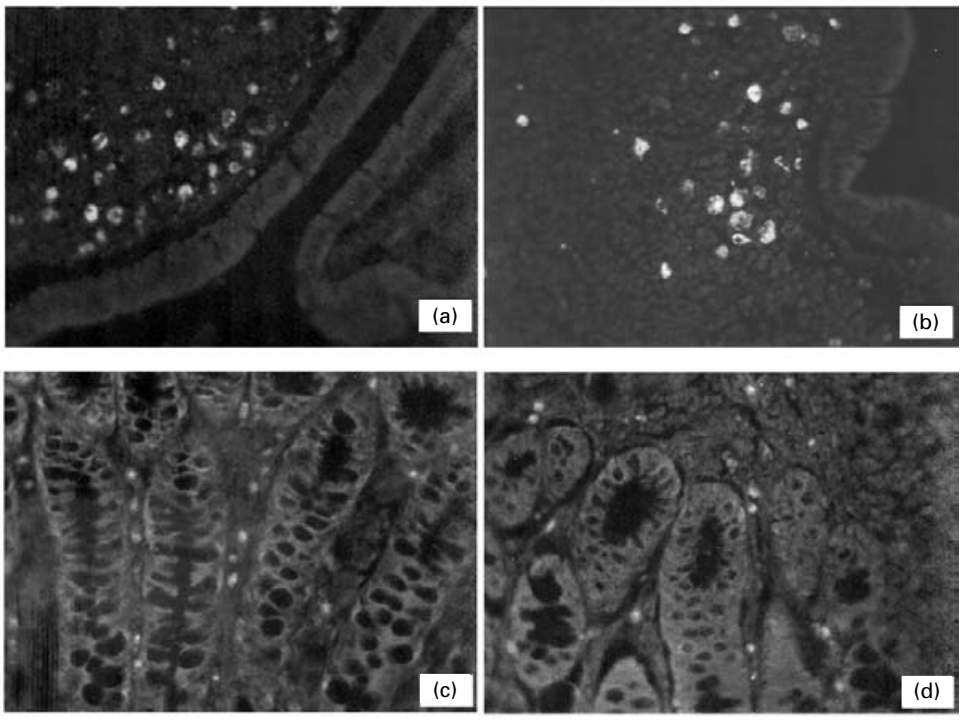

Fig. 2. Histological slices of the intestine of mice after receiving FITC-labeled bacteria. Fluorescence in Peyer's patches 20 min after the administration of C *Lb. acidophilus* (a) and NC *Lb. acidophilus* (b). Fluorescence in the crypts of the large intestine 20 min after the administration of C *Lb. acidophilus* (c) and NC *Lb. acidophilus* (d).

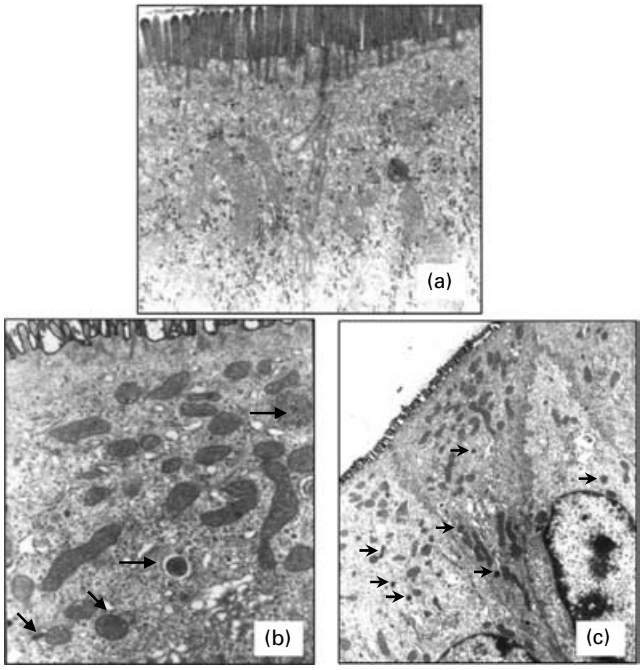

Fig. 3. Transmission electron micrographs (a) Small intestine of control mouse (magnification ×5000) (b) small intestine of mouse after receiving NC *Lb. fermentum* (magnification ×5000); (c) small intestine of mouse after receiving C *Lb. fermentum* (magnification ×3000). Intense lysosomal activity (arrows) can be seen in Figs b and c.

Results

Histological studies

No inflammatory immune response was observed for either C or NC bacteria (Fig. 1). No evidence of modification in the structure of the villi was detected in the small or large intestine of mice fed with NC or C strains. A slight cellular infiltration was seen for all the strains assayed. No differences between C and NC bacteria were determined.

Gut interaction studies

To see whether there were differences between C and NC bacteria and their capacity to interact with the gut, we used FITC labelled-bacteria. Figure 2 shows that C and NC bacteria had a similar capacity of interaction with the gut. We observed fluorescent bacterial antigens in the inductor sites of the intestinal immune system (Peyer's patches), in the immune cells associated with the lamina propria of the small intestine and in nodules and crypts of the large intestine for all the microorganisms under study.

To determine whether the time of clearance of the bacteria was similar in C and NC bacteria, we assayed the persistence of lactobacilli in the gut. We observed that fluorescent particles were cleared 72 h after the administration of FITC-labelled bacteria, in the same way as all particulate antigens, in agreement with earlier findings (Maldonado Galdeano & Perdigón, 2004)

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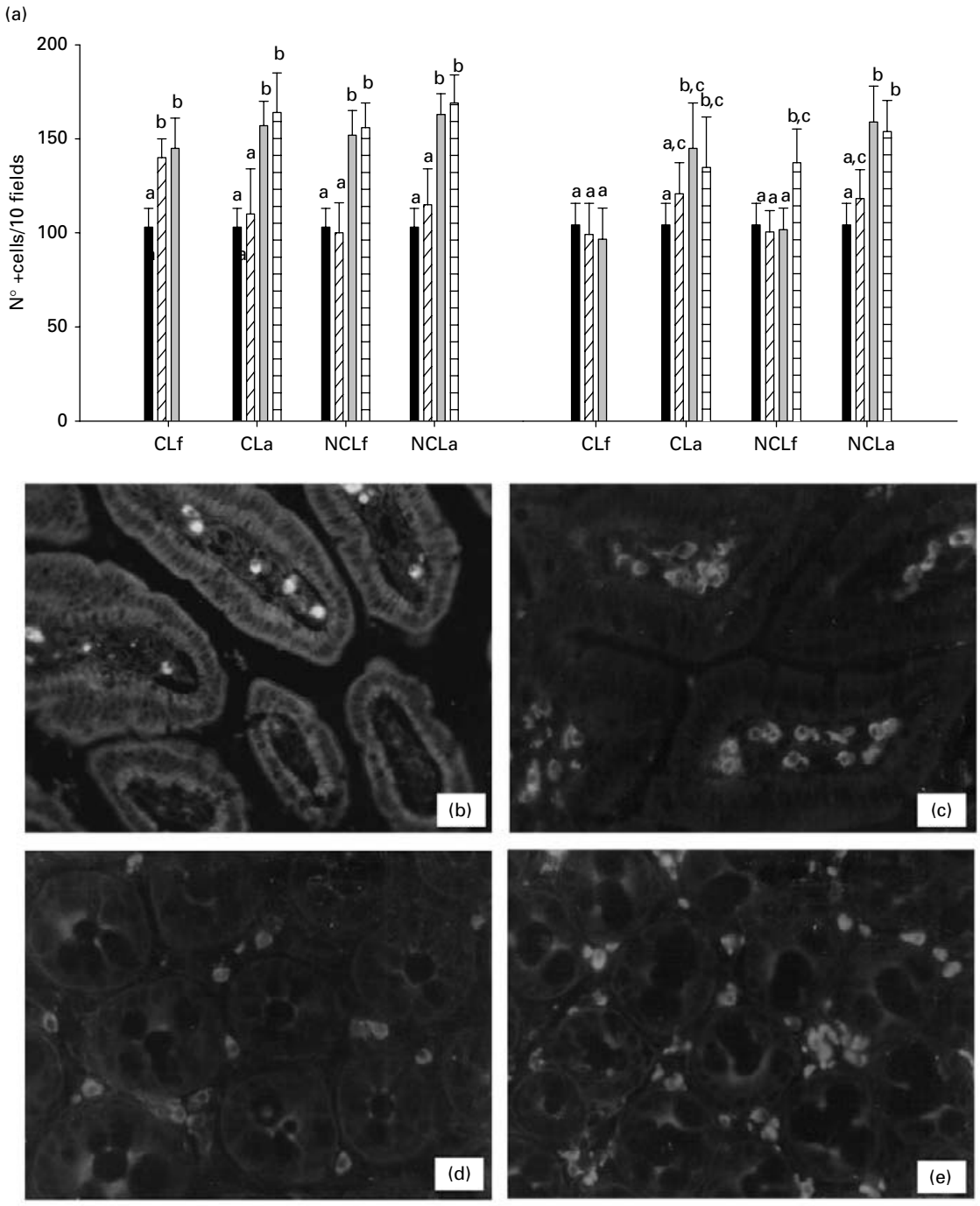

Fig. 4. Effect of C and NC lactobacilli on IgA secreting cells (a) Effect of lactobacilli administration on the number of IgA+ cells. Positive cells were counted in histological sections from small and large intestine of unfed control (black bar) 2-d group (diagonal lines), 5-d group (grey bar) and 7-d group (horizontal lines). Values are means for $n=5 \pm$ SD. Means for each value without a common letter differ significantly ($P<0{\cdot}05$). (b) Microphotography of IgA+ cells in the lamina propria of the small intestine of unfed (control) mice (c) Microphotography of IgA+ cells in the lamina propria of the small intestine of mice after 7-d administration of NC *Lb. acidophilus* (d) Microphotography of IgA+ cells in the lamina propria of the large intestine of unfed (control) mice (e) Microphotography of IgA+ cells in the lamina propria of the large intestine of mice after 7-d administration of NC *Lb. acidophilus* (magnification ×400).

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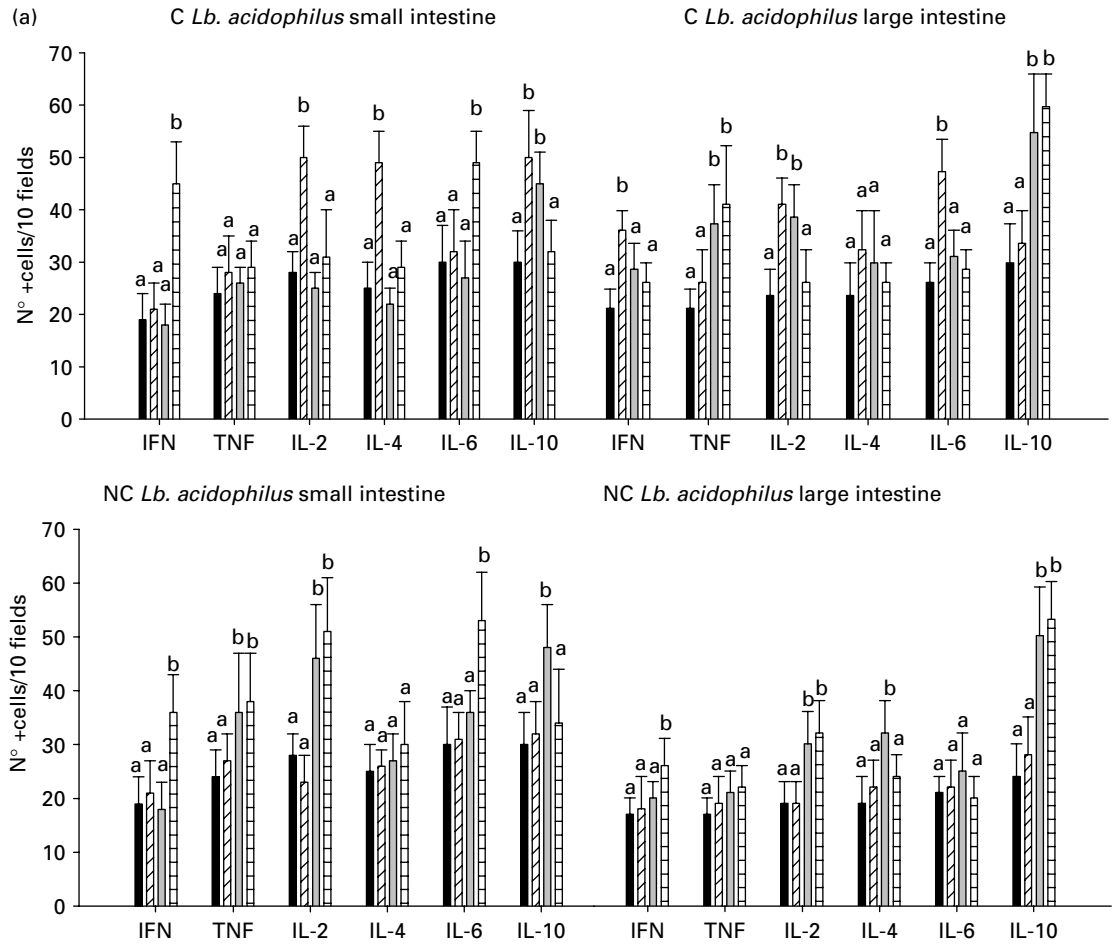

Fig. 5. (*Cont.*)

Transmission electron microscopy assays

We looked for bacteria adhered to the intestinal epithelial cells or for lysosomal activation induced by bacterial interaction 20 min after bacterial administration. No adhered bacteria were observed on the surface of the epithelial cells. No differences were seen for either strain in the epithelial cells, while an increase in the lysosomal activity of these cells was observed. These results are shown in Fig. 3.

Determination of IgA producing cells

When analysing the effect of feeding with C or NC lactobacilli on the IgA producing cells, we observed that all strains assayed induced a significant increase in these cells ($P \leqslant 0{\cdot}01$) in the small intestine after 5 and 7 d of administration, in relation to the unfed control. Only C *Lb. fermentum* was able to increase the number of IgA+ cells after 2 d of administration. In the large intestine, both C and NC *Lb. acidophilus* stimulated the production of IgA secreting cells for 5 and 7 d, while C and NC *Lb. fermentum* increased the number of IgA+ cells only after 7 d of administration. (Fig. 4a, b, c, d).

Effect of commensal and non commensal lactobacilli on the cytokine profile

We also evaluated the effect of the oral administration of C and NC lactobacilli on the activity of the immune cells associated with the intestinal mucosa by cytokine release, especially for the proinflammatory (IFNγ and TNFα) and regulatory (IL-10 and IL-4) cytokines. When we analysed comparatively the effect of C and NC *Lb. acidophilus*, we observed that the latter induced an increase in the number of TNFα+ cells in the small intestine 5 and 7 d after administration with respect to the unfed control, while no increase was observed for C *Lb. acidophilus*. Both C and NC *Lb. acidophilus* increased the number of IFNγ+ cells in the small intestine after 7 d of administration. In the small intestine, IL-2 increased in the group receiving NC *Lb. acidophilus* for 5 and 7 d while for C *Lb. acidophilus* this increase was observed only for 2 d of administration. Both strains caused a significant increase in the number of

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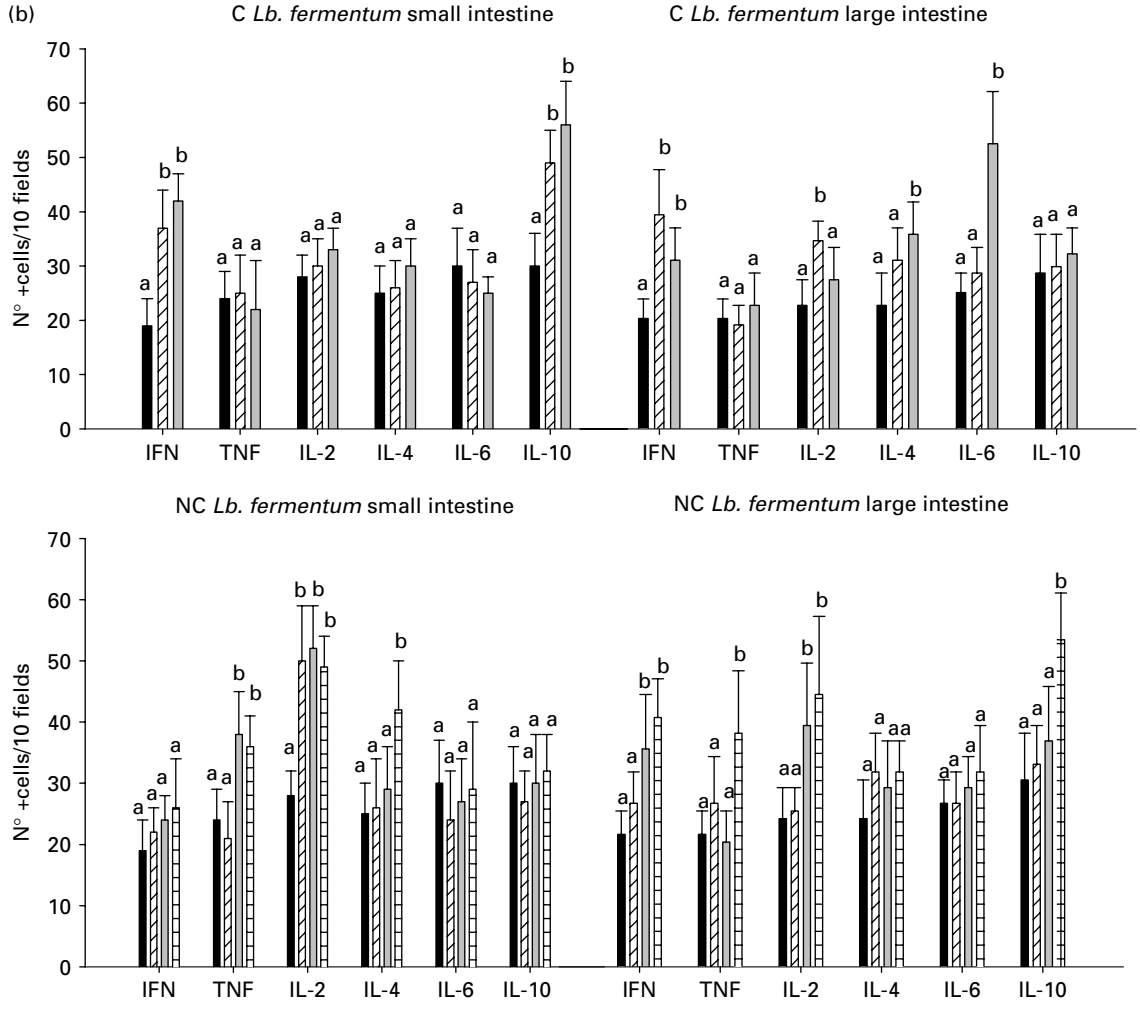

Fig. 5. (a) Effect of C and NC *Lb. acidophilus* on the cytokine profile on the small and large intestine (b) Effect of C and NC *Lb. fermentum* on the cytokine profile on the small and large intestine Positive cells for each cytokine were counted in histological sections from small and large intestine of unfed control (black bar) 2-d group (diagonal lines), 5-d group (grey bar) and 7-d group (horizontal lines). Values are means for $n=5 \pm$ SD. Means for each cytokine without a common letter differ significantly ($P<0{\cdot}05$).

IL-6+ cells for 7 d in the small intestine. Commensal *Lb. acidophilus* increased IL-4 in the small intestine for 2 d of administration. IL-10 increased with both strains in the small intestine, the effect being more relevant for C *Lb. acidophilus*. (Fig. 5a). With regard to the large intestine, C *Lb. acidophilus* induced an increase in IL-6, IL-10 and TNFα and a slight increase in IL-2 and in IFNγ positive cells. No differences with respect to the unfed control were found for IL-4.

When we analysed comparatively the results for C and NC *Lb. fermentum*, we observed that C *Lb. fermentum* increased the number of IFNγ+ cells and IL-10+ cells in the small intestine for 2 and 5 d, whereas NC *Lb. fermentum* induced the release of TNFα, IL-2 and IL-4+ cells, no increase in IL-10 or IFNγ being observed. In the large intestine, C *Lb. fermentum* induced an increase in IFNγ, IL-4 and IL-6. No increase in IL-10 was observed. IL-2 was increased only for 2 d, while NC *Lb. fermentum* increased IL-10, IFNγ, TNFα and IL-2. These results are shown in Fig. 5b.

Discussion

The C microflora that populates the intestine is in close and continuous contact with the immune cells and the stimulus induced by these microorganisms is essential for the maturation of the immune system (Moreau & Gaboriau-Routhiau, 2000). This enteric microflora maintains a chronic and balanced intestinal inflammatory response (Fiocchi, 1996; Schreiber, 1997). The maintenance of local homeostasis in the intestinal mucosa to luminal antigens requires a fine tuning of the immunological processes. It is known that there is tolerance towards the C microflora and that unbalance in the bacterial population

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may lead to intestinal inflammation (Duchmann et al. 1995). Actually it is argued that the concept of oral tolerance does not apply to C bacteria. Healthy animals are ignorant of their microflora but not tolerant (Macpherson & Uhr, 2004)

The microbes present in fermented foods could contribute to health improvement by interacting with the normal microflora and with the eukaryotic cells associated with the gut. This implies that while the established normal gut microflora are important in supporting a well-balanced mucosal immune response, this balance can be emulated by the transient selected microbes present in the food, most commonly Gram-positive ones such as LAB which are ingested as oral probiotics or in fermented foods. These non pathogenic bacteria can also influence the functioning of the gut mucosal immune system by different signals through their interaction with the epithelial and immune cells.

In the selection of these probiotic bacteria to be added to animals or human food, the claim is that these microorganisms must be species-specific (host specificity). Considering the present knowledge of the role of the C microflora (host ignorance) we might expect the behaviour of both C and NC non pathogenic bacteria to be different. The results obtained in the present study, however, in general did not show the differences we expected.

We showed here that the oral administration of NC lactobacilli induced immune signals in the gut different from those of the C lactobacilli. However, no changes in gut structure were observed with both strains in the histological studies (Fig. 1). To determine whether the interaction of both bacteria with the immune cells associated with the intestine were different, we performed experiments using FITC-labelled bacteria. We demonstrated that both C and NC microorganisms and their fragments were able to cross the epithelial barrier and interact with the immune cells of the lamina propria (Fig. 2). No fluorescent cells were found adhered to the surface of the gut enterocytes. No differences in the clearance (72 h) of these microorganisms were observed in spite of the different concentration of C (10^4 cfu/mouse per d) and NC (10^8 cfu/mouse per d) bacteria administered; both were processed as any particulate antigen. Recent knowledge that M cells are present not only in Peyer's patches but also in the epithelial cells of the villi (Jang et al. 2004) would explain the positive fluorescence observed in the lamina propria of the small and large intestine. The above observations led us to analyse by electron microscopy whether there were differences between NC and C bacteria with regard to their interaction with the epithelial cells. The epithelial surface is continuously exposed to numerous microorganisms on its apical side and in close proximity to the immune cells on its basolateral side (Raibaud, 1992). The impact of different antigens (soluble or particulate) on the epithelial cells leads to gene expression for receptors, cytokines, signals of proliferation or differentiation and to increase in their metabolic activity and in enzyme production, which are modulated by the microflora, through mechanisms not well understood. It is accepted that the C microflora do not initiate strong immunological responses such as an inflammatory response; however, C bacteria can constantly enter the gut in small numbers and survive within intestinal dendritic cells for an extended number of hours (72–96 h) and these bacteria-loaded dendritic cells do induce mucosal responses that maintain the immunological surveillance in the gut (Macpherson & Uhr, 2004a)

On the other hand, Gram-positive and Gram-negative bacteria from the microflora down-regulate the expression of special receptors, the Toll-like receptors (TLR) (Karlsson et al. 2004) which are the pattern of recognition receptors, to prevent an inflammatory response induced by the interaction of a high number of microorganisms with the epithelial cells. These receptors are up-regulated by pathogenic bacteria (Kagnoff & Eckmman, 1997). Since the first signal for immune activation takes place through the epithelial cells, when we analysed by transmission electron microscopy the adhesion of the bacteria, we noticed that neither C nor NC bacteria were found interacting with the epithelial cells. However, intense lysosomal activity with an increase in Golgi's cisterns was observed (Fig. 3). These results indicate that even when the bacteria were not found interacting with epithelial cells, they were able to induce a strong activation of these cells. We think that whole bacteria were not found because they were degraded in the intestinal lumen and that their fragments were responsible for the activation of epithelial cells.

While the complex signals generated in the gut by bacterial colonization *de novo* remain to be fully elucidated, the irrefutable fact is that orally delivered probiotic strains from lactobacilli or bifidobacteria or fermented milk with probiotic bacteria are capable of modulating the gut immune response (Perdigón et al. 2002); can activate antitumour responses to prevent or delay tumour growth (de Moreno de LeBlanc & Perdigón, 2004; de Moreno de LeBlanc et al. 2005); and can increase secretory IgA antibody production for the control of microbial pathogens (Elmer et al. 1996). In this context, we compared C and NC strains under study with respect to the number of IgA producing cells. We did not find differences in the number of IgA+ cells in the small intestine, where the pattern of increase in the number of these cells was similar for both C and NC bacteria. Differences in the number of IgA+ cells were found in the large intestine, where only a slight increase for certain doses was observed for the strains assayed (Fig. 4a). Results from the small intestine showed that both C and NC bacteria were able to reinforce the surveillance of the gut mucosal immune system, due to the multiple functions attributed to IgA (Lamm et al. 1996). When we tried to determine whether the signals through the epithelial cells or the direct interaction with the immune cells associated with the lamina propria of the intestine were translated to the cytokine release, we noticed that the pattern of cytokine response in this

complex ecosystem of conventional mice to C and NC bacteria was different (Fig. 5a, b). With regard to the proinflammatory cytokines (IFNγ or TNFα) we found that TNFα was produced mainly by NC bacteria, possibly as a way of initiating the cross talk between the immunocompetent cells associated with the lamina propria and the intestinal epithelial cells (Neutra et al. 2001). The role of TNFα in immune homeostasis, among other functions, and the control exerted by the epithelial cells to maintain immune activation without an inflammatory response have been well demonstrated (Nathens et al. 1995). Commensal bacteria are able to elicit mainly IFNγ, which is necessary for the maturation of dendritic cells and for priming them for antigen presentation. IFNγ is also able to up-regulate TLR to increase the surveillance against pathogens and to control cellular proliferation at the intestinal level (Goodrich & McGee, 1999; Becker & Neurath, 2004) to maintain homeostasis. With regard to IL-2 and IL-6 positive cells, they were mainly increased by NC bacterial stimulation. This observation agrees with the concept that heterologous antigens are potentially more antigenic than their homologous ones by the role of the C microorganisms in the maintenance of immunological surveillance. The role of IL-6 as a B cell activator to maintain the number of IgA cells in the lamina propria is also described (Haller et al. 2001). The regulatory cytokines, IL-10 and IL-4 were induced with different patterns for both C and NC bacteria. This finding agrees with the important role of IL-10 as an immune suppressor cytokine to regulate the inflammatory immune response, and with our histological studies where no alterations were found (Fig. 1). However, at the mucosal level these cytokines can induce T independent switch of IgM to IgA to maintain the number of IgA+ cells in the intestine (Macpherson & Uhr, 2004)

In summary, we showed that oral administrations of C and NC lactobacilli were able to induce activation of the immune cells associated with the gut without side effects such as an inflammatory immune response. Both NC and C lactobacilli interacted with the intestine and with the immune cells associated with it, inducing signals to increase the mechanisms of surveillance. The difference in the cytokines profile found may have a role in controlling local inflammation in the gut maintaining the number of IgA+ cells and the intestinal homeostasis. The C microorganisms would be involved in the regulation of intestinal homeostasis rather than in the immune activation as the NC lactobacilli. Even though our results are from mouse, the similarity in the behaviour of the immune system to the human one allows us to predict the immunological capacity of potentially probiotic bacteria. In our opinion, in the selection of probiotic bacteria with immunomodulatory properties for their use in human functional food, NC bacteria would be more appropriate than C ones. However, the probiotic properties are specific for each strain and should be analysed independently of the specificity of the host.

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References

Becker C & Neurath MF 2004 Lamina propria dendritic cells mediate localized mucosal immune response. *Mucosal Immunology Update* **12** 4–6

Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D & von Mutius E 2002 Environmental exposure to endotoxin and its relation to asthma in school-age children. *New England Journal of Medicine* **347** 869–877

Bry L, Falk PG, Midtvet T & Gordon J 1996 A model of host microbial interactions in an open mammalian ecosystem. *Science* **273** 1380–1383

Crabbe PA, Bazin H, Eyssen H & Heremans JF 1968 The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. The germ-free intestinal tract. *International Archives of Allergy and Applied Immunology* **34** 362–375

de Moreno de LeBlanc A, Matar C, LeBlanc N & Perdigón G 2005 Effects of milk fermented by *Lactobacillus helveticus* R389 on a murine breast cancer model. *Breast Cancer Research* **7** 477–486

de Moreno de LeBlanc A & Perdigón G 2004 Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. *Medical Science Monitor* **10** 96–104

Duchmann R, Kaiser I, Herman E, Mayer W, Ewe K & Meyer ZBK 1995 Tolerance exists toward resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clinical and Experimental Immunology* **102** 448–455

Elmer G, Surawicz C & Mac Farland L 1996 Biotherapeutic agents: a neglected modality for the prevention and treatment of selected intestinal and vaginal infections. *Journal of the American Medical Association* **275** 870–876

Fiocchi C 1996 Cytokines and intestinal inflammation. *Transplantation Proceedings* **28** 2442–2444

Gibson G & Wang X 1994 Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *Journal of Applied Bacteriology* **77** 412–420

Goodrich ME & McGee DW 1999 Effect of intestinal epithelial cells cytokines on mucosal B-cell IgA secretion: enhancing effect of epithelial-derived IL-6 but not TGF-beta on IgA+ B cells. *Immunology Letters* **67** 11–14

Haller D, Servant P, Peruisseau G, Bode C, Hammers W, Schiffrin E & Blue S 2001 IL-10 producing CD14 low monocytes inhibit lymphocyte-dependent activation of intestinal epithelial cells by commensal bacteria. *Microbiology and Immunology* **46** 195–205

Jang MH, Kweon MN, Iwatani K, Yamamoto M, Terahara K, Sasakawa C, Suzuki T, Nochi T, Yokota Y, Rennert PD, Hiroi T, Tamagawa H, Iijima H, Kunisawa J, Yuki Y & Kiyono H 2004 Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proceedings of the National Academy of Sciences of the USA* **20** 6110–6115

Kagnoff M & Eckmman L 1997 Epithelial cells as sensors for microbial infection. *Journal of Clinical Investigation* **100** 6–10

Karlsson H, Larson P, Wold A & Rudin A 2004 Pattern of cytokine responses to Gram positive and Gram negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells. *Infection and Immunity* **72** 2671–2678

Lamm ME, Nedrud JG, Kaetzel CS & Mazanec MB 1996 New insights into epithelial cell function in mucosal immunity: neutralization of intracellular pathogens and NCcretion of antigens by IgA. In: *Essentials of Mucosal Immunology*, pp. 141–148 (Eds M Kagnoff & H Kiyono). California, USA: Academic Press

Lidbeck A & Nord C 1993 Lactobacilli and the normal human anaerobic microflora. *Clinical Infectious Diseases* **16** 181–187

Macpherson AJ & Uhr T 2004 Compartmentalization of the mucosal immune responses to commensal intestinal bacteria. In *Oral Tolerance. New Insights and Prospects for Clinical Application*, pp. 36–43 (Eds H Weiner et al.). New York NY, USA: Academy of Sciences

Macpherson AJ & Uhr T 2004a Induction of protective IgA by intestinal dendritic cells carring commensal bacteria. *Science* **303** 1662–1665

Maldonado Galdeano C & Perdigón G 2004 Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *Journal of Applied Microbiology* **97** 673–681

Moreau MC & Gaboriau-Routhiau V 2000 Influence of resident intestinal microflora on the development and functions of the intestinal-associated lymphoid tissue. In: *Probiotics 3: Immunomodulation by the Gut Microflora and Probiotics*, pp. 69–104 (Eds R Fuller & G Perdigón). The Netherlands: Kluwer Academic Publishers

Nathens A, Rotstein O, Dackiw A & Marshall J 1995 Intestinal epithelial cells down-regulate macrophage tumor necrosis factor – alpha secretion: a mechanism for immune homeostasis in the gut associated lymphoid tissue. *Surgery* **118** 343–350

Neutra MR, Mantis NJ & Kraehenbuhl JP 2001 Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nature Immunology* **2** 1004–1009

Perdigón G, Maldonado Galdeano C, Valdez JC & Medici M 2002 Interaction of lactic acid bacteria with the gut immune system. *European Journal of Clinical Nutrition* **564** 21–26

Raibaud P 1992 Bacterial interactions in the gut. In *Probiotics*, pp. 9–28 (Ed. R Fuller). London: Chapman & Hall

Sainte-Marie G 1962 A paraffin embedding technique for studies employing immunofluorescence. *Journal of Histochemistry and Cytochemistry* **10** 250

Schreiber S 1997 Interleukin 10 in the intestine. *Gut* **41** 274–276

Stappenbeck T, Hooper L & Gordon J 2002 Developmental regulation of intestinal angiogenesis by commensal microbes via Paneth cells. *Proceedings of the National Academy of Sciences of the USA* **24** 15451–15455

Vinderola CG, Medici M & Perdigón G 2004 Relationship between interactions sites in the gut, hydrophobicity, mucosal immunomodulating capacities and cell wall protein profiles in indigenous and exogenous bacteria. *Journal of Applied Microbiology* **96** 230–243