Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Bifidobacteria from human origin: interaction with phagocytic cells

S.E. Assad[1], I.S. Rolny[2,3], J. Minnaard[1,3] and P.F. Pérez[1,3]

1 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Universidad Nacional de La Plata (UNLP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET- CCT La Plata) and Consejo de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), La Plata, Argentina
2 Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), UNLP, La Plata, Argentina
3 Área Microbiología e Inmunología, Departamento de Ciencias Biológicas, UNLP, La Plata, Argentina

Keywords
fermented foods, immunology, intestinal microbiology, mechanism of action, probiotics.

Correspondence
Pablo F. Pérez, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA)-Universidad Nacional de La Plata (UNLP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET- CCT La Plata) and Consejo de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Calle 47 y 116, CP 1900 La Plata, Argentina.
E-mail: pfp@biol.unlp.edu.ar

2020/1662: received 28 June 2019, revised 19 August 2020 and accepted 15 September 2020

doi:10.1111/jam.14861

Abstract

Aim of the study: Given that phagocytic cells are main players of the host immune response, we studied the interaction of bifidobacteria with monocytic THP-1 cells in nonopsonic conditions.

Methods and Results: Association/internalization, cell response (expression of HLA-DR and TLR2), M1/M2 macrophage polarization and colocalization of micro-organisms with Lysotracker or transferrin were evaluated. Screening with eight *Bifidobacterium* strains showed two patterns of interactions with THP-1 cells: high and low association and phagocytosis. Two strains with different surface properties were further studied: *B. bifidum* CIDCA 5310 and *B. adolescentis* CIDCA 5317. Strain CIDCA 5310 showed higher levels of colocalization in lysosome than strain CIDCA 5317. Both strains stimulated TLR2 expression. Strain CIDCA 5317 significantly increases HLA-DR expression, however, when cells are stimulated with IFN-γ, strain CIDCA 5310 induces the highest value of expression. Noteworthy, strain CIDCA 5310 was able to upregulate both M1 and M2 markers of macrophage polarization.

Conclusions: Our results demonstrate that bifidobacteria from human origin show different patterns of interaction with phagocytic cells thus leading to different cell responses. These findings add further insight on the mechanisms involved in the biologic effects of probiotics.

Significance and Impact of the Study: Knowledge of the interaction of bifidobacteria with key players of the host immune response is paramount for the understanding of the mechanisms involved in the beneficial effects.

Introduction

Due to their health promoting effects, bifidobacteria are often selected for the formulation of fermented foods (Herbel *et al.* 2013; Amund 2016). These health benefits are strain specific but the mechanisms behind are poorly understood (Hord 2008; Verdu 2009; Lebeer *et al.* 2010; Donkor *et al.* 2012; Lescheid 2014; Vargas García *et al.* 2015).

We have previously demonstrated that the interaction of bifidobacteria from human origin with enterocyte-like cells is strain dependent and strongly correlates with bacterial surface properties (Pérez *et al.* 1998). Further studies determined that adhesion onto cultured human epithelial cells (Caco-2) is multifactorial and involves thermolabile factors, glucidic structures, molecules non-covalently bound to the bacterial surface and also proteinaceous factors (Bibiloni *et al.* 1999).

Even though the interaction of micro-organisms with phagocytic cells has been extensively studied for bacterial pathogens (Omotade and Roy 2019), data on potentially probiotic micro-organisms are seldom found in scientific literature. Early studies reported the ability of probiotic micro-organisms of increasing phagocytic activity in

humans (Schiffrin *et al.* 1995). In addition, other reports have demonstrated that immunomodulating ability of probiotics could be related, at least partially, to the modification of the phagocytic activity (Vinderola *et al.* 2004; Yang *et al.* 2009; Núñez *et al.* 2014). In this context, it has been shown that *Bifidobacterium lactis* HN019 was able to increase activity of phagocytic cells in adult individuals that consumed milk containing this probiotic bacterium. Similar results were observed when yogurt was supplemented with *Lactobacillus acidophilus* 74-2 and *B. lactis* 420 (Klein *et al.* 2008; Ashraf and Shah 2014). In monocyte/macrophage cell lines and *ex-vivo* studies, secretion of different cytokines was reported after stimulation with *Bifidobacterium* strains (Okada *et al.* 2009; Habil *et al.* 2011; Donkor *et al.* 2012; Rodes *et al.* 2013). Macrophage activation was also observed with heat-inactivated bacteria (Mortaz *et al.* 2015; Chen *et al.* 2016) and it has been demonstrated that cellular components such as DNA from bifidobacteria are able to increase phagocytic activity of J.774.A.1 cells (Li *et al.* 2005).

Taking into account the relevance of phagocytic cells in shaping the host immune response and the limited knowledge on the interaction between bifidobacterial and professional phagocytic cells, we conducted the present study to contribute to the understanding of the mechanisms behind the probiotic effect of bifidobacteria.

Materials and methods

Bifidobacterium strains and culture conditions

Information on bifidobacteria strains is summarized in Table 1. Seven of eight strains under study (belonging to five species) were isolated from infant faeces whereas one strain (CIDCA 531) was isolated from a commercial fermented dairy product. These strains have been selected for the present study since, along with their different bacterial surface properties and ability to interact with enterocyte-like cells (Gomez Zavaglia *et al.* 1998; Pérez *et al.* 1998), they have demonstrated a wide range of features related to their probiotic potential (Trejo *et al.* 2006; Trejo *et al.* 2013). Bacteria were cultured in MRS broth (BIOKAR; Biokar Diagnostics, Beauvais, France) supplemented with cysteine (0·5 g l^{-1}) (Laboratorios ANEDRA, Argentina) for 24 h at 37°C in anaerobic conditions (Pérez *et al.* 1998; Trejo *et al.* 2006, 2013).

Table 1 Strains of bifidobacteria used in the present study

Strain*	Origin†	Feeding
B. pseudolongum CIDCA 531	Dairy product	
B. breve CIDCA 532[1]	2m	Breast + formula
B. adolescentis CIDCA 5317	14d	Breast + formula
B. infantis NCC 200‡	21d	Breast
B. bifidum NCC 235‡	2m	Breast + formula
B. bifidum CIDCA 5311[2]	6d	Breast
B. bifidum NCC 189§	1m5d	Breast
B. bifidum CIDCA 5310[2]	6d	Breast

*Same numbers indicate that strains were isolated from the same individual.
†When bifidobacteria were isolated from infant faeces, age of the individuals is indicated as days (d) or months (m).
‡Formerly CIDCA 538.
§Formerly CIDCA 533.
¶Formerly CIDCA 536.

Phagocytic cells

The human leukaemia monocytic cell line THP-1 was grown in Dubelcco's modified eagle's medium (DMEM) supplemented with inactivated foetal calf serum (10 ml 100 ml^{-1}) (FCS) (GIBCO Life Technologies, Gran Island, NY, USA), nonessential amino acids (GIBCO Life Technologies), streptomycin and penicillin (Life Technologies, Cergy, France). Cells were seeded in 24-well or 48-well tissue plates (Greiner Bio One, Frickenhausen, Germany) and incubated at 37°C for 48 h in a 5% CO_2/95 % O_2 atmosphere. Phorbol-12-myristate-13-acetate (PMA) (Sigma, St. Louis, MO) 200 nmol l^{-1} final concentration was used to differentiate cells to a macrophage-like phenotype for 48 h. For microscopic observation, cells were grown on glass coverslips (Assistant, Sondheim, Germany).

Fluorescent labelling of bacteria

Micro-organisms, grown as described above, were harvested by centrifugation at 14 000 ***g*** for 1 min, washed twice in phosphate-buffered saline (PBS) and incubated with fluorescein isothiocyanate (FITC) 0·3 mg ml^{-1} for 20 min at room temperature. Afterwards, bacteria were washed twice with PBS and then suspended in 500 µl of DMEM. Labelling was evaluated by fluorescent microscopy (Leica Microscopy Systems Ltd, Microsystems, Germany) and the bacteria concentration was assessed by determining the values of absorbance at 600 nm (METROLAB 330, Buenos Aires, Argentina).

Phagocytosis assays

In order to determine phagocytosis, bacteria were washed twice with PBS and added to THP-1 cells at different multiplicities of infection (MOI = bacteria per cell). To calculate MOI, bacterial numbers were estimated by OD readings at 600 nm (OD = 1 corresponds to 10^8 CFU per ml). Viable counts were obtained by plating serial

Table 2 Interaction of bifidobacteria with THP-1 cells after 1 h incubation. Association and internalization of bacteria at MOI 5 and 10 were evaluated by flow cytometry before and after quenching of exocellular bacteria with Trypan blue respectively

	Association*		Internalization*	
Strain	MOI 5	MOI 10	MOI 5	MOI 10
B. pseudolongum CIDCA 531	467·96 ± 260·05 ^{f}	1989·13 ± 127·23 ^{e,f}	60·38 ± 7·56 ^{k}	233·18 ± 5·04 ^{k}
B. breve CIDCA 532	903·15 ± 131·91 ^{e,f}	1576·88 ± 237·34 ^{e,f}	96·79 ± 19·18 ^{k}	184·86 ± 14·04 ^{k}
B. infantis NCC 200†	1090·27 ± 85·57 ^{e,f}	2397·03 ± 337·03 ^{d,e}	126·85 ± 5·85 ^{k}	295·16 ± 34·77 ^{k}
B. adolescentis CIDCA 5317	885·27 ± 61·56 ^{e,f}	2164·25 ± 223·65 ^{d,e,f}	929·77 ± 843·11^{i,j}	795·65 ± 72·30 ^{i,j}
B. bifidum CIDCA 5311	1048·39 ± 71·93 ^{e,f}	3879·70 ± 202·82 ^{c,d}	334·87 ± 154·13 ^{j}	1959·43 ± 1197·31 ^{h,i}
B. bifidum NCC 235‡	1722·05 ± 82·56 ^{e,f}	4988·06 ± 1063·55^{b,c}	322·62 ± 34·16 ^{j}	896·43 ± 257·79 ^{i,j}
B. bifidum NCC 189§	5736·69 ± 51·88 ^{b}	3819,93 ± 38·47 ^{c,d}	1540·08 ± 262·69^{h,l,j}	2976·63 ± 242·10 ^{g,h}
B. bifidum CIDCA 5310	5460·56 ± 555·44 $^{b,\ c}$	12669·19 ± 804·12 ^{a}	2314·52 ± 716·45^{h,i}	4440·17 ± 754·84^{g}

*Values are expressed as arbitrary units (AU) = FL1(+) cells × mean fluorescence intensity (see Materials and Methods). Different letters indicate significant differences ($P < 0·05$; $n = 3$). NCC, Nestlé Culture Collection.

†Formerly CIDCA 538.

‡Formerly CIDCA 533.

§Formerly CIDCA 536.

dilutions of bacteria suspensions onto MRS agar and incubating for 16 h at 37°C in anaerobic conditions. Number of THP-1 cells (10^6 per well) was assessed by cell count in a haemocytometer. After incubation for 1 h at 37°C, cells were washed twice with PBS and analysed by flow cytometry with a blue–green excitation light

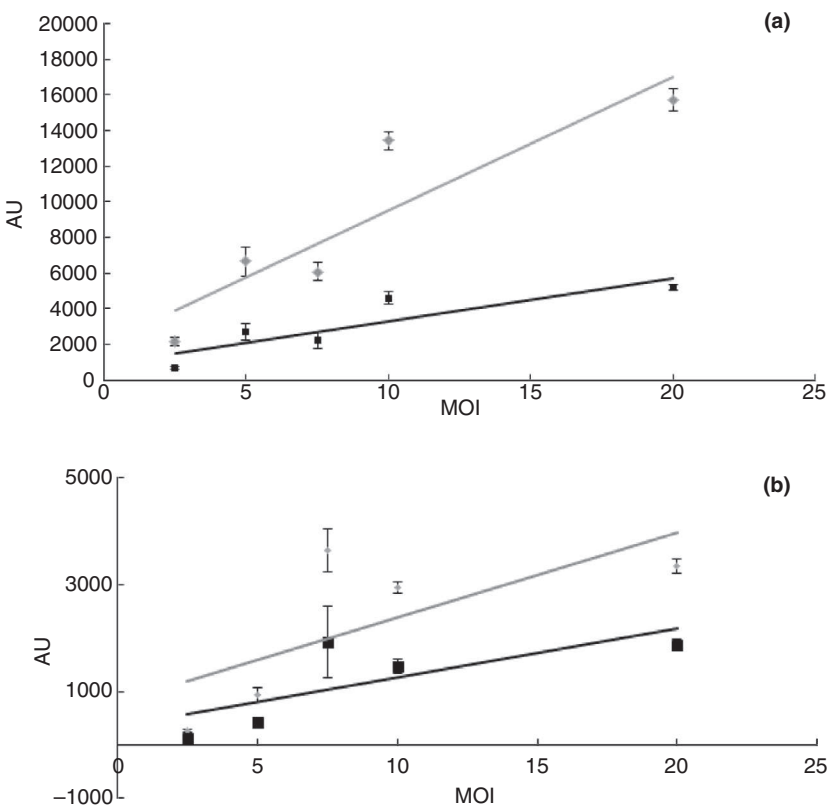

Figure 1 Effect of the multiplicity of infection (MOI) on the interaction between THP-1 cells and *Bifidobacterium* strains: CIDCA 5310 (a) and CIDCA 5317 (b). Association (◆) and internalization (■) were evaluated before and after quenching of exocellular bacteria with Trypan blue respectively. Results are expressed as arbitrary units (AU) = FL1(+) cells × mean fluorescence intensity. Results show a representative experiment from at least three independent experiments and are expressed as means ($n = 3$). Bars indicate standard deviation.

(488 nm argon-ion laser, FACSCaliburTM). Analysis was performed by using CellQuest TM software.

Gating of cells to be analysed was performed in SSC *vs* FSC scatter plots. Next, these cells were analysed according to the green fluorescence (channel FL1). Results were expressed as arbitrary units (AU) = number of FL1 (+) cells × mean fluorescence intensity. Association values include both adhered and internalized bacteria whereas internalization includes only intracellular bacteria. For quenching of noninternalized bacteria, trypan blue solution (GIBCO Invitrogen Corporation, Burlington, Ontario, Canada) was used.

Fate of internalized bacteria

Fluorescein isothiocyanate-labelled bacteria were added to THP-1 cells (MOI = 10) cultured on glass coverslips in 24-well tissue plates and incubated for 1 h at 37°C. Next, cells were stained with Lysotracker DND-99 (200 nmol l^{-1}, 5 min) (Life Technologies, Eugene, OR, USA) or with Transferrin-Alexa-594 (10 μg ml^{-1}, 10 min) (Molecular Probes, Eugene, OR, USA) as a marker of the endosomal recycling pathway. Afterwards, macrophages were washed twice with PBS and fixed with paraformaldehyde (PFA) (3 ml 100 ml^{-1}) for 1 h at 4°C in the dark. Mounting medium (DakoCytomation, Glostrup, Hovedstaden) was used as anti-fading reagent.

Samples were analysed with a confocal laser-scanning microscope (Leica TCS SP5; Leica Microsystems, Wetzlar, Germany) at excitation wavelengths of 488 nm (argon laser) and 594 nm (helium–neon laser) with a HCX PL APO CS 63·0 × 1·40 OIL UV objective (Leica Microsystems). The resolution of the resulting images was 8 bits (1024 × 1024 pixels). Image analysis was performed with the Leica LAS AF Lite software. At least 200 cells per sample were analysed. The percentage of colocalization was calculated as the number of cells with at least one bacteria colocalized with the selected marker, referred to the total number of cells with internalized bacteria.

Expression of cell surface markers

The expression of HLA-DR, toll-like receptor 2 (TLR2), CD16, CD64, CD163 and CD206 was assessed after incubation of PMA-differentiated THP-1 cells with the strains CIDCA 5310 or CIDCA 5317 at MOI 10 for 18 h at 37°C (5% CO_2/95% O_2). Stimulation with IFN-γ (375 ng ml^{-1}) final concentration (Thermo Scientific, Rockford, IL, USA) or heat killed (80°C for 1 min) *Staphylococcus aureus* were used as positive controls for

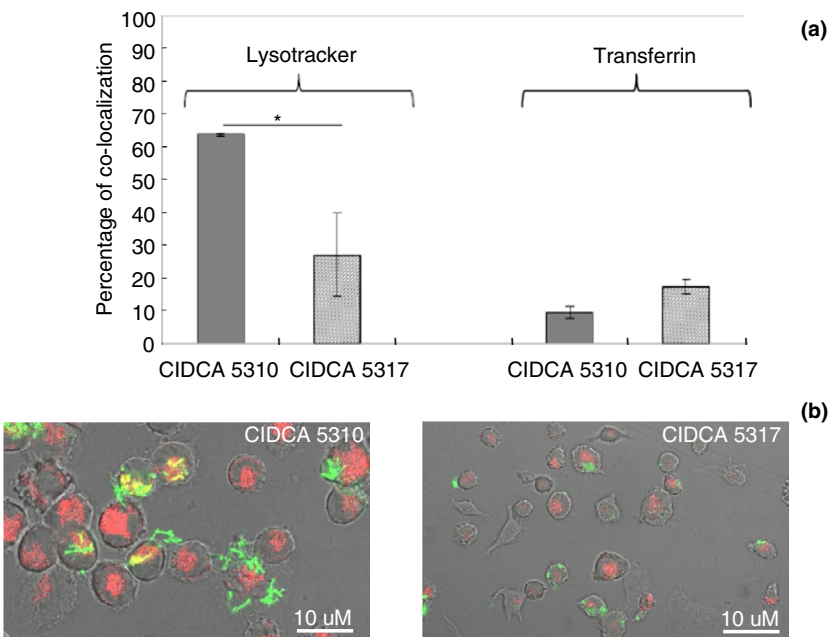

Figure 2 Intracellular trafficking of strains CIDCA 5310 and CIDCA 5317 in THP-1 cells after 1 h incubation. Colocalization with Lysotraker or transferrin with FITC-labelled bacteria was evaluated by confocal laser scanning microscopy. Results are expressed as percentage of internalized bacteria colocalizing with each marker (a) and a representative image with yellow areas indicating colocalization of FITC-labelled bacteria (green) and the lysosomal marker Lysotracker (red) (b). Results show a representative experiment from at least three independent experiments and are expressed as means (n = 3). Bars indicate standard deviation. *P < 0·05.

HLA-DR and TLR-2 expression respectively. For CD16 and CD64, IFN-γ (375 ng ml^{-1}) plus LPS (0·5 μg ml^{-1}) (Sigma-Aldrich, EEUU) was used as positive control. Positive controls for CD163 and CD206 expression were stimulated with IL-4 (10 ng ml^{-1}). Unstimulated macrophages were used as controls of basal expression.

After incubation, cells were washed with PBS and non-specific binding of antibodies was blocked with PBS supplemented with inactivated human serum (20 ml 100 ml^{-1}). Staining with PE-conjugated anti-human HLA, FITC-conjugated anti-human TLR2, FITC-conjugated anti-human CD16, PE-conjugated anti-human CD64, FITC-conjugated anti-human CD163 and FITC-conjugated anti-human CD206 (BD Pharmigen, San Jose, CA, USA) was performed for 20 min at 4°C in the dark.

After being labelled, samples were washed twice and analysed by flow cytometry. The expression index (EI) was calculated as per cent of positive cells x mean fluorescence intensity. Appropriate isotype controls were included.

Data analysis

Results of association/internalization and expression of cell surface markers were analysed by ANOVA and two-tail Student's paired *t* test at 0·05 level of significance. To support the use of these parametric statistical approaches, normality was tested by means of the modified Shapiro–Wilks method and by quantile–quantile (Q–Q) plots of residuals. To assess dose–response relationship between association/invasion and multiplicity of infection a linear regression analysis was performed.

For the analysis of colocalization the number of cells showing at least one bacteria colocalizing with the selected marker (i.e. Lysotracker or transferrin) was referred to the total number of cells of the microscopic field. These ratios

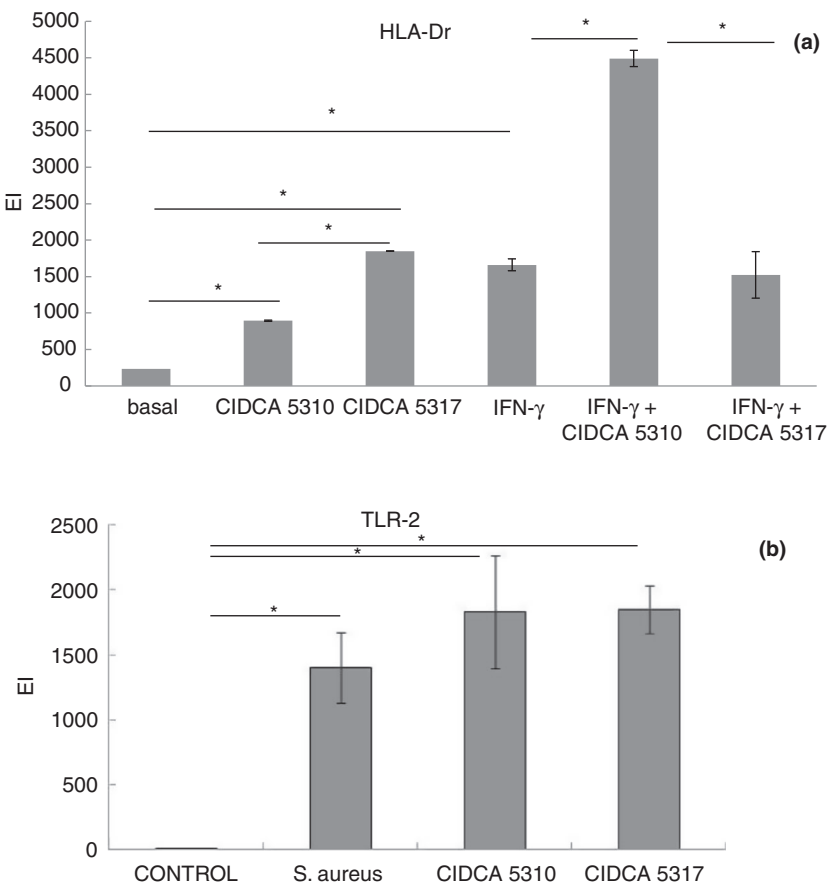

Figure 3 Expression of HLA-DR (a) and TLR2 (b) in THP-1 cells co-incubated with strains CIDCA 5310 or CIDCA 5317 at MOI 10 for 18 h. Values are expressed as expression index (EI). IFN-γ and heat-inactivated *Staphylococcus aureus* were used as a positive control of HLA-DR and TLR2 expression respectively. Results show a representative experiment from at least three independent experiments and are expressed as means ($n = 3$). Bars indicate standard deviation. *$P < 0·05$.

of colocalization were compared by means of the Fisher's exact test at the 0·05 level of significance.

All statistical tests were conducted by using INFOSTAT software (InfoStat ver. 2020; Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

Results

Association and phagocytosis

The analysis of association/internalization of bifidobacteria by flow cytometry showed that two groups of microorganisms can be defined: those showing low values of interaction with phagocytic cells; that is, *B. pseudolongum* CIDCA 531, *B. breve* CIDCA 532, *B. infantis* NCC 200, *B. bifidum* CIDCA 5311, *B. adolescentis* CIDCA 5317 and *B. bifidum* NCC 235, and those with high values of internalization: for example, *B. bifidum* NCC 189 and *B. bifidum* CIDCA 5310 (Table 2).

As shown in Fig. 1, a linear dose–response relationship was observed between MOI and association and internalization values.

Both association and internalization values were higher for strain CIDCA 5310 than for strain CIDCA 5317.

It is important to note that at MOI higher than 10 bacteria per cell, no further increase of neither association nor invasion was observed. Therefore, further experiments were conducted at MOI 10.

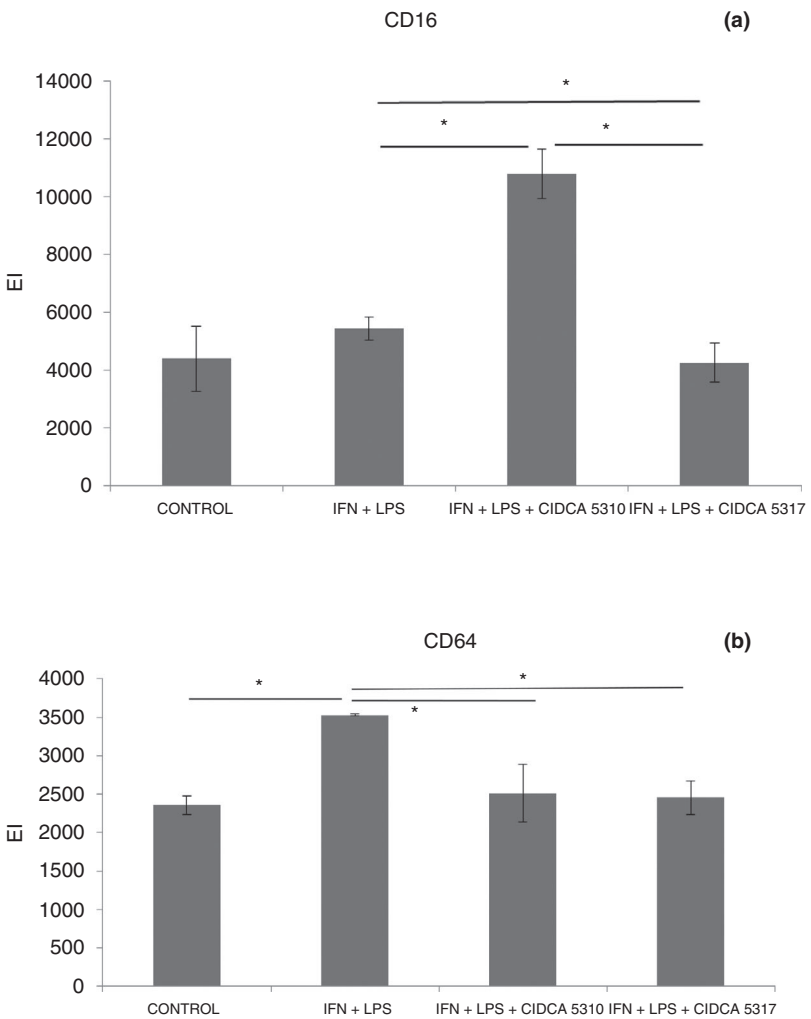

Figure 4 Polarization phenotypes of THP-1 cells stimulated with bifidobacteria (MOI = 10) in the presence of IFN-γ + LPS (M1 stimulus) for 18 h at 37°C (5% CO_2). After stimulation, expression of CD16 and CD64 was evaluated by flow cytometry. Control cells were incubated in the absence of any stimulus. Results show a representative experiment from at least three independent experiments and are expressed as means (n = 3). Bars indicate standard deviation. *P < 0·05.

Fate of internalized bacteria

After 1 h incubation, values of colocalization with Lysotracker were significantly higher for the strain CIDCA 5310 as compared with the strain CIDCA 5317 (Fig. 2a).

Assessment of bacteria colocalization with transferrin indicates a low rate of routing to recycling endosomes for both strains under study (Fig. 2a). Bacteria in acidic (lysosomal) compartments can be seen as yellow zones in a representative confocal fluorescence image of Lysotracker (red) colocalization with FITC-labelled strain CIDCA 5310 (green) (Fig. 2b). Differences in routing to acidic compartments for the strains CIDCA 5310 (Fig. 2b left) and CIDCA 5317 (Fig. 2b right) are evident.

Expression of cell surface markers

The different patterns of interaction between bifidobacteria and phagocytic cells prompted us to study the expression of cell surface markers in THP-1 cells co-incubated with bacteria.

The expression of HLA-DR in THP-1 cells stimulated by the strains CIDCA 5310 and CIDCA 5317 were significantly higher ($P < 0{\cdot}05$) than unstimulated controls (Fig. 3a). As expected, IFN-γ-stimulated HLA-DR expression even in cells incubated without bacteria. When stimulation with IFN-γ was performed in the presence of the strain CIDCA 5317, values of HLA-DR expression were similar to those without bacteria (Fig. 3a). In contrast, the presence of the strain CIDCA 5310 in addition to

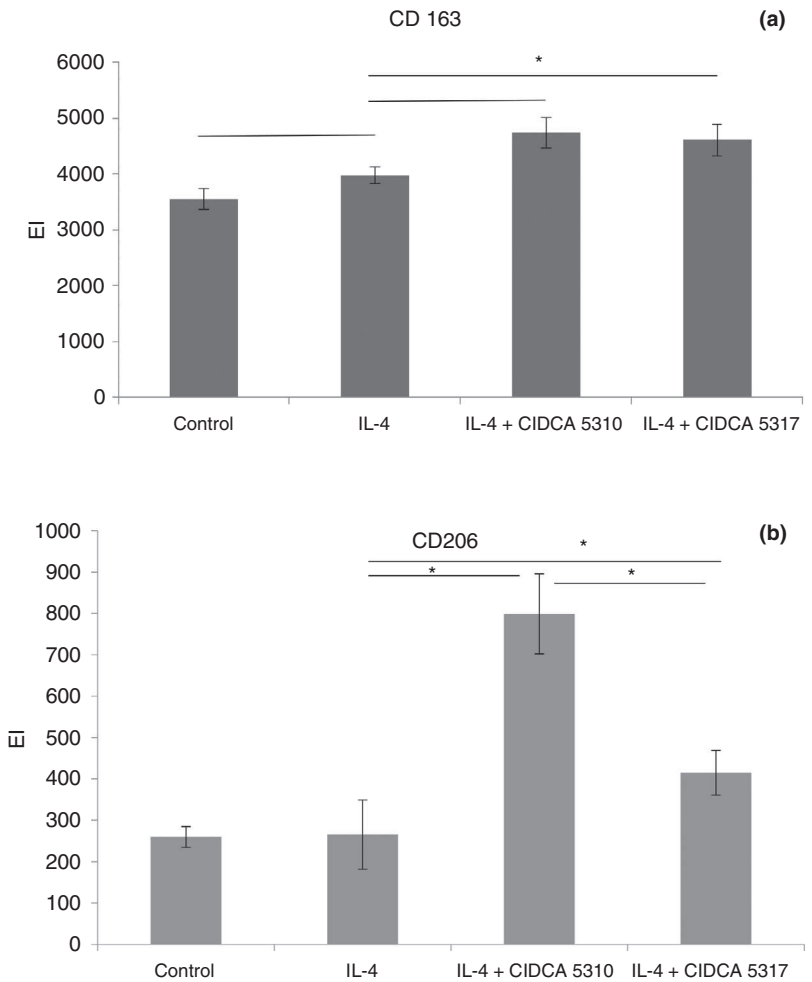

Figure 5 Polarization phenotypes of THP-1 cells stimulated with bifidobacteria (MOI = 10) in the presence of IL-4 (M2 stimulus) for 18 h at 37°C (5% CO_2). After incubation, expression of CD163 and CD206 was evaluated by flow cytometry. Control cells were incubated in absence of any stimulus. Results show a representative experiment from at least three independent experiments and are expressed as means ($n = 3$). Bars indicate standard deviation. *$P < 0{\cdot}05$.

IFN-γ led to higher values of HLA-DR expression (Fig. 3a).

Cells stimulated with bifidobacteria expressed increased levels of TLR2 although no differences were found between strains. These values were not significantly different from those showed by cells stimulated with heat-killed *S. aureus* (Fig. 3b).

As shown in Fig. 4a, in cells stimulated with IFN-γ plus LPS, the presence of strain CIDCA 5310 enhanced expression of CD16, a marker of M1 profile, as compared with control cells stimulated only with IFN-γ plus LPS. Expression of CD64, another M1 marker, was downregulated by the presence of both strains under study (Fig. 4b).

As depicted in Fig. 5a,b, both strains potentiate the expression of CD163 (Fig. 5a) and strain CIDCA 5310 showed high stimulatory effect on the expression of CD206 (Fig. 5b).

Discussion

The present study focuses on bifidobacteria as key players of intestinal microbiology and gut immunology. By using eight strains belonging to five different species, we demonstrate here that bifidobacteria have different patterns of interaction with cultured human phagocytic cells (Table 2) thus leading to differences in phagocytosis and cell response.

Our results show that, even in nonopsonic conditions, bifidobacteria route to lysosomal compartments (Fig. 2). This routing to degradative compartments is expected for a nonpathogenic micro-organism that is efficiently controlled by the immune system. However, colocalization of the strain CIDCA 5310 with the lysosomal marker Lysotracker® is higher than that of the strain CIDCA 5317 (Fig. 2). These findings suggest that kinetics of routing to lysosomal compartments is different in these two strains. This correlates with a trend of strain CIDCA 5317 to higher trafficking to recycling endosomes (Fig. 2).

Concerning cellular response following phagocytosis, it has been demonstrated that routing of the pathogen *Bacillus cereus* to acidic compartments correlated with MHCII expression in RAW 264.7 cells (Rolny *et al.* 2017). In contrast, in the present paper, we found that the less phagocytosed strain CIDCA 5317 stimulates expression of HLA-DR (the human equivalent of murine MHCII) by THP-1 cells in a greater extent than the strain CIDCA 5310 (Fig. 3), which showed a better interaction with phagocytic cells and high ratio of traffic to lysosomal compartments (Table 1, Figs 1 and 2). As it was reported for lactobacilli, cellular response is dramatically different depending on the internalization pathway, that is, receptor-induced macropinocytosis, Syk-dependent phagocytosis or simple constitutive macropinocytosis (Boye *et al.* 2016). Noteworthy, in a study with dendritic cells from patients affected by Crohn's disease or ulcerative colitis, a commercial product containing three bifidobacteria species (*B. longum*, *B. breve* and *B. infantis*) stimulated phagocytosis but failed to induce the expression of HLA-DR and CD86 (Strisciuglio *et al.* 2015). It has been proposed that there is a species-dependent stimulatory ability in the *Bifidobacterium* genus: the *B. longum/B. animalis* group triggers a Th1 skewed profile whereas the *B. bifidum* led to a Th17 profile (Ruiz *et al.* 2017).

The relationship between phagocytosis and immunomodulating capacity has been demonstrated for lactobacilli. Indeed, one strain of *L. jensenii* with high immunomodulating capacity was more phagocytosed by porcine mononuclear phagocytes from Peyer's patches than a nonimmunomodulatory strain (Tsukida *et al.* 2016). This phagocytosis partially depends on TLR2 (Tsukida *et al.* 2016). Although we evaluated only expression of exocellular TLR2, the participation of receptors located in endosomes cannot be ruled out. In this context, *L. acidophilus* induced IFN-β production after endocytosis and endosomal signalling through a MyD88-dependent mechanism. Since this effect can be inhibited by TLR2 signalling from the plasma membrane it is thought that receptors situated in the plasma membrane and those in endosomal compartments compete in recruiting MyD88 (Boye *et al.* 2016).

It is accepted that polarization profiles of macrophages range from M1 to M2 phenotypes. However, the actual situation is an intermediate state situated elsewhere between these two extreme profiles (Mantovani *et al.* 2004; Italiani and Boraschi 2014).

We show that strain CIDCA 5310 can upregulate expression of the opsonic receptor CD16 (M1 marker) in cells previously differentiated with PMA and stimulated with IFN-γ plus LPS (Fig. 4). Although this M1 skewed phenotype is expected in PMA-differentiated THP-1 cells (Lund *et al.* 2016), strain CIDCA 5310 was able to increase the expression of the nonopsonic receptors CD163 and CD206 (M2 markers) in the presence of IL-4 (Fig. 5). Concerning CD64 (M1 marker) both strains downregulated expression (Fig. 4b) as compared with cells stimulated with IFN-γ plus LPS. Interestingly, in cocultures of intestinal epithelial cells and THP-1 cells, stimulation of enterocytes with strain CIDCA 5310 in the presence of LPS leads to the production of IL-6 and TNF-α, typical pattern of a M1-skewed response (data not shown). This complex scenario agrees with the variability in differentiation of macrophages. Indeed, whereas there is a consensus on the stimulus leading to M1 phenotype (i.e. IFN-γ plus LPS), there are different

differentiation protocols for M2 phenotype (Mantovani *et al.* 2004) and there are a wide range of differentiation markers associated with each phenotype. Noteworthy, even canonical stimuli fail to lead to the expected expression pattern (Shiratori *et al.* 2017). These unexpected expression profiles probably arise from the diversity of both genetic and transcriptomic backgrounds that are evident even when THP-1 cells from different biorepositories are considered (Noronha *et al.* 2020).

Bifidobacteria isolated from different human populations differ in immunomodulating motifs (Nicola *et al.* 2016; Ruiz *et al.* 2017; Delgado *et al.* 2020). Interestingly, the effect of bifidobacteria on eukaryotic cells depends on the interplay between TLR2 and NOD2 signalling. If the strength of the interaction of lipoproteins with TLR2 is strong enough, it will abrogate the activation signals derived from NOD2 pathway triggered by interaction with peptidoglycan and lipoteichoic acids. This scenario will lead to an anti-inflammatory response with high levels of IL-10 and low levels of both IL-12 and TNF-α (Zeuthen *et al.* 2008). In addition, bifidobacterial genomic DNA is relatively rich in GC thus leading to different CpG sequences that in turn are associated with the interspecies variability of biological effects (Ménard *et al.* 2010). In addition, genes coding for serpin (a serine protease inhibitor with immunomodulating activity) are common in the *B. longum* group but is lacking in *B. adolescentis, B. catenulatum* and *B. bifidum* (Turroni *et al.* 2010). Interestingly, strains CIDCA 5310 and CIDCA 5317 belong to this group but present different behaviour concerning interaction with phagocytic cells.

Our finding that bifidobacteria can interact differentially with monocyte-derived macrophages and that these interactions impact on both intracellular fate and cellular response add new insights on the characteristics to be considered to select micro-organisms for the formulation of functional foods. In particular, the finding that interaction of bifidobacterial with phagocytic cells modifies the balance between opsonic (M1) and nonopsonic (M2) response of macrophages could contribute to the understanding of the role of probiotic micro-organisms in shaping mucosal immune response.

Acknowledgements

The present study was financed by the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), (Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de La Plata (Argentina). S.E.A. is fellow of the CONICET, I.S.R. is Professional of the CONICET, J.M. and P.F.P. are Research Scientists of the CONICET and Professors at the Departamento de Ciencias Biológicas of the Facultad de Ciencias Exactas (UNLP).

Conflict of Interest

The authors have no conflict of interest in publishing this article.

References

Amund, O.D. (2016) Exploring the relationship between exposure to technological and gastrointestinal stress and probiotic functional properties of lactobacilli and bifidobacteria. *Can J Microbiol* **62**, 715–725.

Ashraf, R. and Shah, N.P. (2014) Immune system stimulation by probiotic microorganisms. *Crit Rev Food Sci Nutr* **54**, 938–956.

Bibiloni, R., Pérez, P. and De Antoni, G. (1999) Factors involved in adhesion of bifidobacterial strains to epithelial cells in culture. *Anaerobe* **5**, 483–485.

Boye, L., Welsby, I., Lund, L.D., Goriely, S. and Frokiaer, H. (2016) Plasma membrane toll-like receptor activation increases bacterial uptake but abrogates endosomal *Lactobacillus acidophilus* induction of interferon-B. *Immunology* **149**, 329–342.

Chen, K., Shanmugam, N.K.N., Pazos, M.A., Hurley, B.P. and Cherayil, B.J. (2016) Commensal bacteria-induced inflammasome activation in mouse and human macrophages is dependent on potassium efflux but does not require phagocytosis or bacterial viability. *PLoS One* **11**, 1–20.

Delgado, S., Sánchez, B., Margolles, A., Ruas-Madiedo, P. and Ruiz, L. (2020) Molecules produced by probiotics and intestinal microorganisms with immunomodulatory activity. *Nutrients* **12**, 1–14.

Donkor, O.N., Ravikumar, M., Proudfoot, O., Day, S.L., Apostolopoulos, V., Paukovics, G., Vasiljevic, T., Nutt, S.L. *et al.* (2012) Cytokine profile and induction of T helper type 17 and regulatory T cells by human peripheral mononuclear cells after microbial exposure. *Clin Exp Immunol* **167**, 282–295.

Gomez Zavaglia, A., Kociubinski, G., Pérez, P. and De Antoni, G. (1998) Isolation and characterization of *Bifidobacterium* strains for probiotic formulation. *J Food Prot* **61**, 865–873.

Habil, N., Al-Murrani, W., Beal, J. and Foey, A.D. (2011) Probiotic bacterial strains differentially modulate macrophage cytokine production in a strain-dependent and cell subset-specific manner. *Benef Microbes* **2**, 283–293.

Herbel, S.R., Vahjen, W., Wieler, L.H. and Guenther, S. (2013) Timely approaches to identify probiotic species of the genus *Lactobacillus*. *Gut Pathog* **5**, 27.

Hord, N.G. (2008) Eukaryotic-microbiota crosstalk: potential mechanisms for health benefits of prebiotics and probiotics. *Annu Rev Nutr* **28**, 215–231.

Italiani, P. and Boraschi, D. (2014) From monocytes to M1/M2 macrophages: Phenotypical vs. Functional differentiation. *Front Immunol* **5**, 1–22.

Klein, A., Friedrich, U., Vogelsang, H. and Jahreis, G. (2008) *Lactobacillus acidophilus* 74–2 and *Bifidobacterium animalis* subsp *lactis* DGCC 420 modulate unspecific cellular immune response in healthy adults. *Eur J Clin Nutr* **62**, 584–593.

Lebeer, S., Vanderleyden, J. and De Keersmaecker, S.C.J. (2010) Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat Rev Microbiol* **8**, 171–184.

Lescheid, D.W. (2014) Probiotics as regulators of inflammation: a review. *Funct Foods Heal Dis* **4**, 299–311.

Li, Y., Qu, X., Yang, H., Kang, L., Xu, Y., Bai, B. and Song, W. (2005) Bifidobacteria DNA induces murine macrophages activation in vitro. *Cell Mol Immunol* **2**, 473–478.

Lund, M.E., To, J., O'Brien, B.A. and Donnelly, S. (2016) The choice of phorbol 12-myristate 13-acetate differentiation protocol influences the response of THP-1 macrophages to a pro-inflammatory stimulus. *J Immunol Methods* **430**, 64–70.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A. and Locati, M. (2004) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* **25**, 677–686.

Ménard, O., Gafa, V., Kapel, N., Rodriguez, B., Butel, M.J. and Waligora-Dupriet, A.J. (2010) Characterization of immunostimulatory CpG-rich sequences from different *Bifidobacterium* species. *Appl Environ Microbiol* **76**, 2846–2855.

Mortaz, E., Adcock, I.M., Ricciardolo, F.L.M., Varahram, M., Jamaati, H., Velayati, A.A., Folkerts, G. and Garssen, J. (2015) Anti-inflammatory effects of *Lactobacillus rahmnosus* and Bifidobacterium breve on cigarette smoke activated human macrophages. *PLoS One* **10**, 1–17.

Nicola, S., Amoruso, A., Deidda, F., Pane, M., Allesina, S., Mogna, L., Del Piano, M. and Mogna, G. (2016) Searching for the perfect homeostasis five strains of *Bifidobacterium longum* from centenarians have a similar behavior in the production of cytokines. *J Clin Gastroenterol* **50**, S126–S130.

Noronha, N., Ehx, G., Meunier, M.C., Laverdure, J.P., Thériault, C. and Perreault, C. (2020) Major multilevel molecular divergence between THP-1 cells from different biorepositories. *Int J Cancer* **147**, 2000–2006.

Núñez, I.N., Galdeano, C.M., de Moreno de LeBlanc, A. and Perdigón, G. (2014) Evaluation of immune response, microbiota, and blood markers after probiotic bacteria administration in obese mice induced by a high-fat diet. *Nutrition* **30**, 1423–1432.

Okada, Y., Tsuzuki, Y., Hokari, R., Komoto, S., Kurihara, C., Kawaguchi, A., Nagao, S. and Miura, S. (2009) Anti-inflammatory effects of the genus Bifidobacterium on macrophages by modification of phospho-IkB and SOCS gene expression. *Int J Exp Pathol* **90**, 131–140.

Omotade, T.O. and Roy, C.R. (2019) Manipulation of host cell organelles by intracellular pathogens. *Microbiol Spectr* **7**, 1–18.

Pérez, P.F., Minnaard, Y., Disalvo, E.A. and De Antoni, G.L. (1998) Surface properties of bifidobacterial strains of human origin. *Appl Environ Microbiol* **64**, 21–26.

Rodes, L., Khan, A., Paul, A., Coussa-Charley, M., Marinescu, D., Tomaro-Duchesneau, C., Shao, W., Kahouli, I. *et al.* (2013) Effect of probiotics Lactobacillus and Bifidobacterium on gut-derived lipopolysaccharides and inflammatory cytokines: an in vitro study using a human colonic microbiota model. *J Microbiol Biotechnol* **23**, 518–526.

Rolny, I.S., Racedo, S.M. and Pérez, P.F. (2017) Fate of *Bacillus cereus* within phagocytic cells. *Int Microbiol* **20**, 27–29.

Ruiz, L., Delgado, S., Ruas-Madiedo, P., Sánchez, B. and Margolles, A. (2017) Bifidobacteria and their molecular communication with the immune system. *Front Microbiol* **8**, 1–9.

Schiffrin, E.J., Rochat, F., Link-Amster, H., Aeschlimann, J.M. and Donnet-Hughes, A. (1995) Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J Dairy Sci* **78**, 491–497.

Shiratori, H., Feinweber, C., Luckhardt, S., Linke, B., Resch, E., Geisslinger, G., Weigert, A. and Parnham, M.J. (2017) THP-1 and human peripheral blood mononuclear cell-derived macrophages differ in their capacity to polarize in vitro. *Mol Immunol* **88**, 58–68.

Strisciuglio, C., Miele, E., Giugliano, F.P., Vitale, S., Andreozzi, M., Vitale, A., Catania, M.R., Staiano, A. *et al.* (2015) Bifidobacteria enhance antigen sampling and processing by dendritic cells in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* **21**, 1491–1498.

Trejo, F.M., De Antoni, G.L. and Pérez, P.F. (2013) Protective effect of bifidobacteria in an experimental model of *Clostridium difficile* associated colitis. *J Dairy Res* **80**, 263–269.

Trejo, F.M., Minnaard, J., Perez, P.F. and De Antoni, G.L. (2006) Inhibition of Clostridium difficile growth and adhesion to enterocytes by Bifidobacterium supernatants. *Anaerobe* **12**, 186–193.

Tsukida, K., Takahashi, T., Iida, H., Kanmani, P., Suda, Y., Nochi, T., Ohwada, S., Aso, H. *et al.* (2016) Immunoregulatory effects triggered by immunobiotic *Lactobacillus jensenii* TL2937 strain involve efficient phagocytosis in porcine antigen presenting cells. *BMC Immunol* **17**, 1–12.

Turroni, F., Foroni, E., Motherway, M.O.C., Bottacini, F., Giubellini, V., Zomer, A., Ferrarini, A., Delledonne, M. *et al.* (2010) Characterization of the serpin-encoding gene of bifidobacterium breve 210B. *Appl Environ Microbiol* **76**, 3206–3219.

Vargas García, C.E., Petrova, M., Claes, I.J.J., De Boeck, I., Verhoeven, T.L.A., Dilissen, E., von Ossowski, I., Palva, A. *et al.* (2015) Piliation of *Lactobacillus rhamnosus* GG promotes adhesion, phagocytosis, and cytokine modulation in macrophages. *Appl Environ Microbiol* **81**, 2050–2062.

Verdu, E.F. (2009) Probiotics effects on gastrointestinal function: beyond the gut? *Neurogastroenterol Motil* **21**, 477–480.

Vinderola, C.G., Medici, M. and Perdigón, G. (2004) Relationship between interaction sites in the gut, hydrophobicity, mucosal immunomodulating capacities and cell wall protein profiles in indigenous and exogenous bacteria. *J Appl Microbiol* **96**, 230–243.

Yang, H.-Y., Liu, S.-L., Ibrahim, S.A., Zhao, L., Jiang, J.-L., Sun, W.-F. and Ren, F.-Z. (2009) Oral administration of live *Bifidobacterium* substrains isolated from healthy centenarians enhanced immune function in BALB/c mice. *Nutr Res* **29**, 281–289.

Zeuthen, L.H., Fink, L.N. and Frøkiær, H. (2008) Toll-like receptor 2 and nucleotide-binding oligomerization domain-2 play divergent roles in the recognition of gut-derived lactobacilli and bifidobacteria in dendritic cells. *Immunology* **124**, 489–502.