

Immunomodulatory function and in vivo properties of *Pediococcus pentosaceus* OZF, a promising probiotic strain

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Abstract Some of the important properties of probiotics are the ability to survive during gastrointestinal transit and to modulate the immune functions. The objectives of the reported study were to assess in vivo gastrointestinal survival of orally administered *Pediococcus pentosaceus* OZF using an animal model BALB/c mice, and to examine its effects on the immune response. Following oral administration to mice, the ability of *Pediococcus pentosaceus* OZF to pass and survive through the mouse gastrointestinal system was investigated by analyzing the recovery of the strain in fecal samples. Microbiological and polymerase chain reaction (PCR) methods proved that the strain OZF could overcome specific conditions in the gastrointestinal tract of mice and reach the intestine alive after ingestion. To observe the effect of oral administration on immune response, IL-6, IL-12 and IFN- γ were measured by ELISA, and the strain OZF was found to cause increases in IL-6 synthesis in regularly fed mice. However, stimulation was carried out with various concentrations of bacterial ssDNA and heat killed cells of *Pediococcus pentosaceus* OZF. The heat killed cells of the strain OZF were shown to produce IFN- γ independently from IL-12. On the other hand, a significant difference between control and experimental group was noticed when lipopolysaccharide, a TLR4 (toll like receptor) ligand, was used. Overall, *Pediococcus pentosaceus* OZF may be a valuable probiotic strain for therapeutic uses. Nevertheless, further studies on the mechanisms of immunomodulatory effect will allow for better clarification of the immune functions of this strain.

Keywords Probiotic · Survival · Immunomodulation · In vivo

Introduction

Lactic acid bacteria (LAB) are very important in the production of many fermented foods (cheese, yogurt, etc.). Applications of these organisms are now being extended to the area of health improvement, which is known as a probiotic activity. An expert committee defined the term probiotic, popularized by Roy Fuller in 1989, as “living microorganisms, which upon ingestion in certain numbers exert health benefits to the host animal by improving its intestinal microbial balance” (Guarner and Schaafsma 1998). Several aspects, including general, functional and technological characteristics, have to be taken into consideration in the selection process of probiotic strains. The criteria for selecting a good probiotic strain have been listed comprehensively by several authors (Collins et al. 1998; Salminen et al. 1998; Deshpande et al. 2011). A successful probiotic needs to be able to reach the distal part of the intestine successfully in order to have a beneficial effect (Freter 1992; Havenaar et al. 1992; Lo Curto et al. 2011). In order to survive in and colonize the gastrointestinal tract (GIT), ingested bacteria need to express high tolerance to the enzymes in the oral cavity (e.g., lysozyme), as well as to the digestion process in the stomach (e.g., exposure to low pH) and the intestine (e.g., exposure to bile); to have the ability for adhesion to the intestinal surfaces. However, it is reported that many bacteria cannot tolerate these stresses (Fujiwara et al. 2001; Suskovic et al. 2001; Iannitti and Palmieri 2010).

In recent years, the incorporation of probiotic bacteria into foods has received increasing scientific interest for health promotion and disease prevention such as anti-infection properties (Isolauri et al. 1991), beneficial effects in intestinal inflammation (Peran et al. 2005), immunomodulatory activity

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(Olivares et al. 2006), and efficacy in the prevention of allergic diseases (Furrie 2005). Our immune system is one of the most dynamic body components in determining our state of health or disease. Therefore, the immunoregulatory effects of LAB are of primary interest and nowadays there is growing interest in research of immunobiotic LAB that have an ability to modulate or stimulate the gastrointestinal immune system (Nagao et al. 2000; Sheih et al. 2001; Lomax and Calder 2009). Many researchers actively studied the immunomodulatory function of LAB, reporting selected strains as able to prevent or reduce allergies and to preserve the host from various infectious diseases and cancer (Yasui et al. 1999; Takagi et al. 2001; Shida et al. 2002; Repa et al. 2003; Fujiwara et al. 2004; Kimoto et al. 2004). Several studies in the literature focused on the ability of selected probiotics to modulate in vitro cytokine production by human or murine cells (Niers et al. 2005; Baken et al. 2006; Helwig et al. 2006; Shida et al. 2006; Perez-Cano et al. 2010). These effects range from stimulation to inhibition of several pro-inflammatory and anti-inflammatory cytokines, as well as some chemokines, and these effects are often strain-specific and each strain appears to have its own unique immunomodulatory profile (Niers et al. 2007). The pro-inflammatory cytokines secreted by the epithelium, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, IL-8 and IL-12, are hallmarks of the inflammatory responses in the intestine (Isolauri 1999).

Recently, a new potential probiotic strain, *P. pentosaceus* OZF, has been isolated from human breast milk (Osmanagaoglu et al. 2010). The strain OZF has been shown to tolerate low pH, pepsin, bile acid and pancreatic fluid under in vitro conditions, to bind human Caco-2 cells and to exert inhibitory activity against a wide range of bacteria, including some pathogens by producing pediocin ACh/PA-1 (Osmanagaoglu et al. 2010, 2011). Since the strain was shown to fulfill in vitro probiotic selection criteria, the study was extended one step further, and this article presents results of some in vivo pre-selective studies on promising probiotic *P. pentosaceus* OZF, such as the survival in the GIT of mice and its effects on the immune system modulation.

Materials and methods

In vivo survival of *Pediococcus pentosaceus* OZF during passage through GIT of mice

Maintenance of animals Seven-week old female BALB/c mice weighing 20–22 g were purchased from Gazi University Animal Reproduction and Animal Research Center (GUDAM) and were housed in stainless-steel cages with a 12 h light/dark cycle under specific pathogen-free controlled environmental conditions (temperature 22 ± 1 °C, humidity 55 ± 5 °C). Mice were fed with an unlimited commercial

rodent diet (Harlan, Barcelona, Spain) and water ad libitum during the experimental protocol. The protocol was carried out according to the guidelines of the Helsinki declaration and was approved by the Local Ethics Committee on Animal Experiments of the Gazi University, Ankara, Turkey (G.U.E.T., Protocol No: 07/0029).

Preparation of bacterial suspension and administration to mice *Pediococcus pentosaceus* OZF was grown in tryptone-glucose-yeast extract (TGE) broth at 37 °C for 18 h. Cells were removed by centrifugation at 3,000 g for 10 min, washed twice with physiological saline solution and resuspended in the same solution to a final concentration of 10^8 CFU per 200 μ L. Mice in the experimental group ($n=7$) received a daily dose of 10^8 CFU of bacterial suspension, while the control group ($n=7$) received 200 μ L of physiological saline solution by intragastric gavage. Sera, spleen cells and peritoneal exudate cells (PECs) obtained from mice fed 30 days with *P. pentosaceus* OZF were used for detection of in vivo immunomodulatory capacity of the strain, while feces of mice fed 5 days with OZF were used in viability (survival ability in GIT) studies.

Recovery of *Pediococcus pentosaceus* OZF from feces The mice were fed with bacterial suspension regularly for 5 days. The survival ability of *P. pentosaceus* OZF through the GIT was investigated every day for 5 days, by analyzing the recovery of the strain in fecal samples as bacteriocin-producing colonies. At the end of 5 days, the feeding trial was stopped, but fecal samples were collected for 5 additional days to check the colonization ability of the strain, and subjected to the same analysis. For this aim, daily collected fecal samples were pooled, resuspended in physiological saline solution (100 mg mL^{-1}) and mechanically homogenized. Dilutions were plated onto *Pediococci* Selective Medium (PSM) agar and incubated at 37 °C for 24 h before enumeration (Simpson et al. 2006). To verify existence of *P. pentosaceus* OZF in feces, bacteriocin assay and PCR-based methods were applied before and after administration.

For bacteriocin assay, following pour plating, the plates containing ~50 to 100 colonies were overlaid with TSB (Tryptic soy broth) soft agar seeded with *Listeria monocytogenes* ATCC 7644 as an indicator. Plates were further incubated overnight at 37 °C and examined for the presence of an inhibition halo against *L. monocytogenes* ATCC 7644 (Biswas et al. 1991). The bacteriocin-producing colonies on PSM medium were counted as CFU mL^{-1} . Approximately 50 colonies passed through GIT were randomly picked from plates, observed microscopically, Gram-stained and then subjected to API kit for identification. Identification was confirmed by 16S rDNA sequencing using specific universal primers (Edwards et al. 1989; Osborne et al. 2005) as previously discussed (Osmanagaoglu et al. 2011). Amplified

PCR fragments were purified by PCR purification kit (Promega, Agarose Gel DNA Extraction Kit) and sequenced by REFGEN Biotechnology (METU Technocity, Ankara, Turkey). Basic local alignment search tool (BLAST) was used to compare the sequences with the one deposited for *P. pentosaceus* OZF (accession number 1337739, 706 bp) in National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/tr/BLAST>).

For *P. pentosaceus* OZF detection from feces by using PCR based methods, multiplex PCR was carried out first. DNA templates for PCR reactions were prepared from bacteriocin producing colonies. One colony was resuspended in 50 μL of sterilized TE (10 mM Tris–HCl, 1 mM EDTA pH 7.5) buffer and boiled for 10 min. Supernatant containing released DNA was directly used as template in PCR amplification. Target specific genes for pediococci, *P. pentosaceus* and pediocin Ach/PA-1 were amplified by using the method proposed by Suwanjinda et al. (2009). To determine whether or not the original OZF strain and the strains obtained in the feces following oral administration were the same strain, the recovered strains were further typed by RAPD profile. DNA was extracted by using Promega DNA isolation kit, and PCR was carried out with using different RAPD primers [OPO-09 (5' TCCCACGCAA 3'), OPF-14 (5' TGCTGCAGGT 3'), and OPA-07 (5' GAAACGGGTG 3')].

Immunomodulatory function of *Pediococcus pentosaceus* OZF

Culture medium, cytokines, antibodies and reagents All cell culture media components were obtained from Hyclone (USA). Cytokine ELISA assay reagents, recombinant mouse IL-6, IL-12 and IFN- γ , and their monoclonal and biotinylated antibodies were obtained from Thermo, Pierce Endogen and BD Biosciences, respectively. Streptavidin-alkaline phosphatase (SA-AKP) and p-nitrophenyl phosphate disodium salt substrate (PNPP) were purchased from Thermo. Lipopolysaccharide (LPS) and peptidoglycan (PGN), isolated from *Escherichia coli*, was obtained from Sigma. Immunosuppressive CpG oligodeoxynucleotide (ODN) K-type; K23 (12mer) and control ODN were chemically synthesized by Alpha DNA (Montreal, Canada).

Cell culture and stimulation assay BALB/c mice involved in control and experimental groups were used after a 30 day daily feeding period. Mice were euthanized by cervical dislocation. Blood, spleen cells and PECs were aseptically extracted and used for further analysis. Sera were obtained by centrifugation (1,000 g for 5 min at room temperature, RT) and stored at $-20\text{ }^{\circ}\text{C}$ until ELISA (Enzyme-linked immunosorbent assay) was carried out. Unstimulated spleen cells (4 millions of cells in 200 μL), PECs (200,000 cells in 200 μL) and serum samples of mice were used for in vivo

detection of immunomodulatory function in strain OZF. In addition, stimulated forms were used to understand the differentiation of immunostimulatory effect between the control and experimental group. For stimulation assay, spleen cells and PECs in 96 well plates were stimulated with three increasing concentrations of ss DNA (0.08, 0.8 and 8 μg in 200 μL) and heat killed cells of *P. pentosaceus* OZF (10^7 to 10^2 CFU mL^{-1}). DNA of *P. pentosaceus* OZF was extracted with Promega Wizard DNA purification kit. To avoid stimulatory effects due to contamination of bacterial proteins or LPSs, DNA preparations used in the experiments had purity values higher than 1.8 ($\text{OD}_{260/280}$). Single-stranded DNA was prepared by heat denaturing of double-stranded DNA at $95\text{ }^{\circ}\text{C}$ for 5 min, followed by cooling on ice. To obtain heat killed cells, the remaining culture fluid was heated to $121\text{ }^{\circ}\text{C}$ for 15 min, and washed three times with PBS (phosphate buffer saline pH 7.2). For positive controls, LPS (5 μg mL^{-1}), PGN (5 μg mL^{-1}), CpG-ODN K23 (1 μM) and CONTROL ODN (1 μM) were used. Stimulations were performed in duplicate wells for each indicated treatment. The cells were incubated overnight at $37\text{ }^{\circ}\text{C}$ in a 5 % CO_2 incubator and following incubation, collected supernatants were stored at $-20\text{ }^{\circ}\text{C}$ for further use.

Evaluation of cytokines production by ELISA Unstimulated and stimulated serum and cells supernatants were immediately analyzed by ELISA, to measure the IL-6, IL-12 and IFN- γ concentrations as described in Erikci et al. (2011). The optical densities of the enzymatic reaction solutions were read using an automatic ELISA plate reader (Molecular Devices, SoftmaxPro Software V5) at 405_{nm} until recombinant standards (with the starting concentrations of 4,000 ng mL^{-1} for IL-6, 100 ng mL^{-1} for IL-12 and 1,000 ng mL^{-1} for IFN- γ) reached a four parameter saturation. All ELISA assays were performed in duplicate for each group.

Statistical analysis

A student's *t* test was used to evaluate the statistical significances ($p < 0.05$) of the differences in cytokine production of mice before and after exposure to tested bacterium. Values of $p < 0.05$ were considered significant.

Results and discussion

In vivo survival of *Pediococcus pentosaceus* OZF during passage through GIT of mice

From a safety as well as a functional point of view, it is essential to determine if a strain survives in the GIT. In the present study, following oral administration to mice, the

ability of *P. pentosaceus* OZF to pass and survive through the mouse GIT was investigated by analyzing the recovery of the strain in fecal samples as bacteriocin-producing colonies. Clear inhibition zones characterized as bacteriocin production against *L. monocytogenes* ATCC 7644 were observed in all colonies recovered from fecal samples after administration with the strain OZF, and it has been suggested that ingested bacteria as probiotics cannot affect the intestinal environment unless their population reaches a certain minimum level of between 10^6 and 10^8 CFU g^{-1} in intestinal content (Marteau and Rambaud 1993). According to PSM counts, no *Pediococcus* was recorded in feces of mice belonging to control groups, highlighting the lack of this genus within the intestinal flora of mice (Data not shown). Viable cells of *P. pentosaceus* OZF administered orally to mice decreased time dependently (Fig. 1). Twelve hours after the first administration, the strain OZF appeared at a level of 10^7 CFU g^{-1} in feces of all mice in the treated group and at a level of 10^6 to 10^7 CFU g^{-1} after further 24 h. The strain OZF was detected in three of seven mice at gradually reduced levels at 48, 72, 96 and 120 h. This indicates that *P. pentosaceus* OZF administered to mice can survive in the stomach and reach the mouse intestine alive. However, the strain OZF was not detected in any mouse at 144 h (24 h after the administration had ceased). Feeding trials with the strain OZF showed that the promising probiotic strain disappears from the GIT within 1 day after the feeding was discontinued. This indicates that the strain could not get through to colonize in the mouse intestine and although it could reach the intestine alive, is eliminated from the intestine. This is because the normal intestinal microbiota provide an excellent resistance against colonization by introduced bacteria (Wells et al. 1988). On the other hand, pediococci were not detected in the control group. However, other studies showed that the number of probiotic bacteria detected 14 days after the administration of probiotic strains was lower than on the first day after administration (Murphy et al. 1999). This observation is

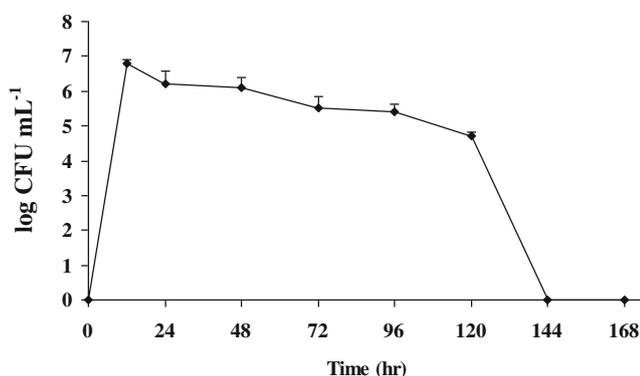


Fig. 1 Survival and time dependent changes in the fecal populations of *P. pentosaceus* OZF after oral administration to mice

supported by results of a human study completed by Goldin et al. (1992) where it was demonstrated that 60–80 % of individuals consuming *Lactobacillus rhamnosus* GG excreted the bacterium for 3–4 days, but only 33 % of the population excreted the bacterium after 7 days. In a similar study, the probiotic strain could be recovered (with no oral supplementation) from feces up to 3 days after cessation of feeding. Therefore, it appears increasingly likely that daily administration of the preferred strain is necessary for maintenance of high levels of probiotics (Murphy et al. 1999).

By using bacteriocin-producing ability, the survival and persistence of the administered strain could be monitored after transit in the GIT of mice. Almost 50 (ten for each day of administration) randomly selected bacteriocin-producing colonies were found in tetrad morphology, Gram-positive and catalase-negative. Nevertheless, to avoid the risk of mistaking colonies of *Pediococcus* spp. grown on PSM agar medium, multiplex and RAPD PCR were used. PCR was performed on the colonies grown on PSM agar to detect live bacterial cells in feces (Fig. 2). Biochemical profiles of five randomly chosen strains were obtained with API 50CH (bioMerieux) following the manufacturer's guidelines and identified as *P. pentosaceus*. Besides, 16S rRNA sequence analysis allowed us to identify the five strains as *P. pentosaceus*, and the obtained sequences were found to be the same (99 % similarity) that have already been registered for OZF strain in GenBank database system under accession number HM051378 (706 bp). Multiplex PCR profiles of 50 randomly selected colonies from the fecal samples of treated mice group were all found to be identical to the pattern of administered *P. pentosaceus* OZF. Amplification of template DNA obtained from *P. pentosaceus* OZF before and after administration, using specific primer sets, resulted in two well-differentiated PCR

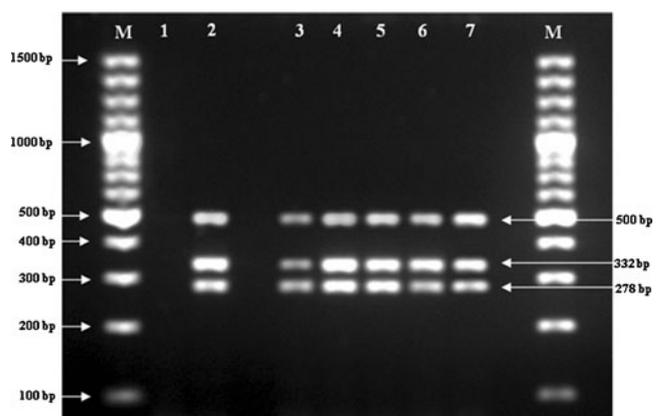


Fig. 2 Molecular identification of *Pediococcus pentosaceus* OZF in mouse feces by multiplex-PCR. Lane M, 100 bp DNA ladder (GeneRuler™ 100 bp DNA Ladder Plus, Fermentas); Lane 1, negative PCR control (no template DNA); Lane 2, *P. pentosaceus* OZF used for oral administration to mice as positive control; Lane 3–7, multiplex-PCR products derived from colonies on PSM agar after oral administration of *P. pentosaceus* OZF at 1, 2, 3, 4 and 5 days respectively

fragments with molecular weights of 500 and 278 bp specific for *P. pentosaceus*, and a 332 bp DNA fragment for pediocin AcH/PA-1 (Fig. 2). RAPD-PCR for the same randomly selected colonies generated reproducible patterns identical to fingerprint obtained for *P. pentosaceus* OZF (Fig. 3). Therefore, the one representing each day was chosen randomly and its band profiles are shown in Fig. 3. Multiplex PCR was found to be an easy, fast, reliable and reproducible method, and can be used as an alternative for verification of bacteriocin-producing pediococci strains. RAPD-PCR was also found as a rapid and reliable molecular technique to generate DNA fingerprints for each strain, and to distinguish the ingested strain from the potentially thousands of other bacterial strains that make up the gastrointestinal ecosystem.

Immunomodulatory function of *Pediococcus pentosaceus* OZF

Some LAB strains may be able to activate the immune system cells (Christensen et al. 2002; Medina et al. 2007; Vizoso Pinto et al. 2007, 2009). Inducing or enhancing the cytokine production could be a major mechanism for probiotic bacteria to exert immunomodulating activities (Marin et al. 1997). This would open up a promising use of *P. pentosaceus* OZF as an immunomodulator. Our study was performed in two stages, in vivo and in vitro. The analysis of the cytokines profiles revealed that the most remarkable effect was an increase in the IL-6 (Fig. 4), an important mediator both in humoral and cellular host defense. IL-6 plays an important role in host immune mechanism by regulating immune response and acute-phase reactions (Morita et al. 2002). In control group, IL-6 was not detected in sera, while a statistically significant increase was detected in mice regularly fed with bacterial suspension ($89.15 \pm 7.89 \text{ ng mL}^{-1}$, $p < 0.001$). On the other hand, no significant difference was recorded in IL-12 production between groups

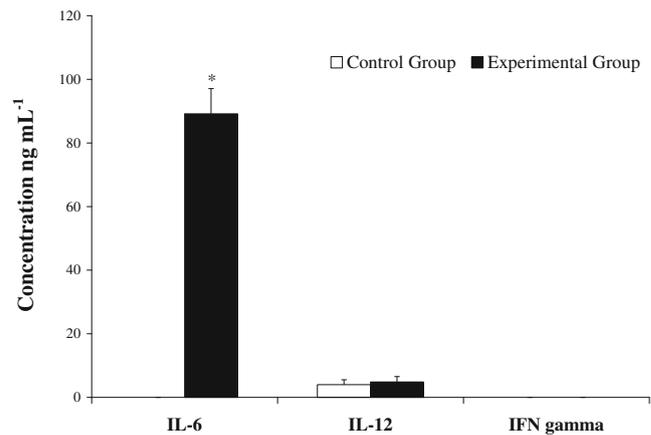
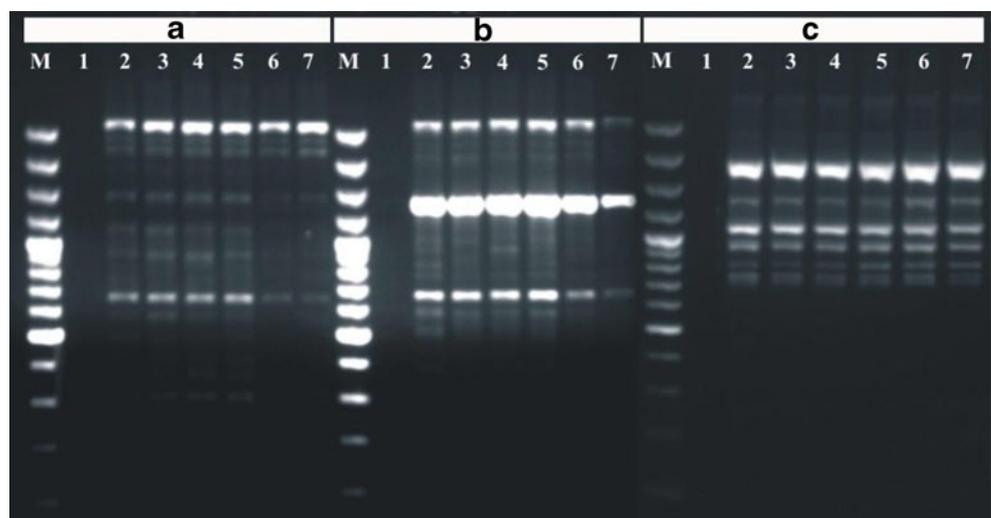


Fig. 4 IL-6, IL-12 and IFN- γ production in serum samples of BALB/c mice involved in control and experimental group after 30 days daily feeding period (*significantly difference: $p < 0.001$)

(control: $3.96 \pm 1.58 \text{ ng mL}^{-1}$; experimental: $4,82 \pm 1.72 \text{ ng mL}^{-1}$). Besides, the resulting amount of IFN gamma level was below the detection level of the method in cell supernatants including serum (Fig. 4). The increase in the production of IL-6, as a result of regular feeding with *P. pentosaceus* OZF, may create an advantage for defense of infections encountered in any way (Van Enckevort et al. 1999). Despite growing evidence for immunomodulatory capability of LAB, especially human milk-derived species, there is still little information regarding their mode of action. Several similar studies have reported the in vitro and in vivo cytokine response patterns of co-culturing cells of the innate immune defense system with different probiotic LAB for understanding their health protection mechanism. Our results are in agreement with the results obtained by Gill (1998); Morita et al. (2002); Kimura et al. (2006); Foligne et al. (2010); Perez-Cano et al. (2010) and Zhu et al. (2011). Intestinal epithelial cells from conventional mice were reported to produce IL-6 in response to the challenge in

Fig. 3 Molecular typing of *Pediococcus pentosaceus* OZF in mouse feces by RAPD PCR. Lane M, 100 bp DNA ladder (GeneRuler™ 100 bp DNA Ladder Plus, Fermentas; Marker bp, ruler was given at Fig. 2); Lane 1, negative PCR control (no template DNA); Lane 2, *P. pentosaceus* OZF used for oral administration to mice as positive control; Lane 3–7, RAPD PCR profiles derived from colonies on PSM agar after oral administration of *P. pentosaceus* OZF at 1, 2, 3, 4 and 5 day respectively. Primers used are OPA-9 (a), OPF-14 (b) and OPA-7 (c)



vitro with certain LAB strains that had also demonstrated in vivo immunomodulating capacity (Vinderola et al. 2005). Two *P. pentosaceus* strains isolated from traditional vegetable pickles were shown to have a high capacity to survive in the GIT, and have in vitro immunomodulatory and allergy inhibition effects (Jonganurakkun et al. 2008). In contrast, another *P. pentosaceus* strain, used as preservative to prevent farmer's lung pneumonitis, was shown to induce an inflammatory response in mice in the study of Duchaine et al. (1996).

In order to find out whether or not *P. pentosaceus* OZF exhibit in vitro immunostimulating capacity, ssDNA and heat killed cells of the strain were used, since it is known that cellular components of LAB such as peptidoglycans, lipoteichoic acids, cell surface protein, exopolysaccharide and DNA CpG motifs, as well as live bacteria and inactivated bacteria, may present a capacity for immune system stimulation (Lebeer et al. 2008; Ng et al. 2009). In both experimental and control groups, stimulation studies were carried out by use of both spleen cells and PECs. Following stimulation, the release of IL-6 and IL-12 was not detected in spleen cells. In PECs, when we look at the results from the perspective of IL-6 production after stimulation, the control group was found to react more than experimental group to high dosage of heat killed cells of the strain OZF ($p < 0.01$). However, when the concentration of heat killed cells was decreased (10^4 CFU mL⁻¹ and lower titrations), IL-6 production was found to be higher in the experimental group than in the control group ($p < 0.05$). When the results of heat killed bacteria stimulation are evaluated in terms of IL-12 and IFN gamma secretion, no significant difference was recorded between the groups (data not shown).

Although studies are limited in this regard, available references reported that DNA of LAB have immunostimulatory properties (Lammers et al. 2003; Iliev et al. 2005; Li et al. 2005; Medina et al. 2007; Ghadimi et al. 2008; Satokari et al. 2009; Menard et al. 2010). The present study showed that ssDNA of *P. pentosaceus* OZF induced the secretion of IL-6 and IL-12, but this was not found to be significantly different when compared to each group (data not shown). IFN- γ was not detected after stimulation with ssDNA, which could be related to the use of low dosages of ssDNA.

In conclusion, *P. pentosaceus* OZF strain proved to be able to pass live through the GIT after oral administration to mice and to stimulate an immune response. To the authors' knowledge, this is the first article dealing with the effects of a *Pediococcus* strain, isolated from human breast milk, on cytokines activation. The comparison of the stimulant effect of heat killed cells and ssDNA after co-culture with spleen cells and PECs is also novel. On the other hand, further studies are necessary to better assess the fulfill breadth of the immunomodulatory capability of our strain in the gut environment, and to understand its cellular or humoral immune

effect by measuring the inductive activity of IgA, IgG and IgM in case of usage as a oral vaccine carrier.

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References

- Baken KA, Ezendam J, Gremmer ER (2006) Evaluation of immunomodulation by *Lactobacillus casei* Shirota: immune function, auto immunity and gene expression. *Int J Food Microbiol* 112:8–18
- Biswas SR, Purbita R, Johnson MC, Ray B (1991) Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. *Appl Environ Microb* 57(4):1265–1267
- Christensen HR, Frokiaer H, Pestka JJ (2002) Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. *J Immunol* 168:171–178
- Collins JK, Thornton K, Sullivan GO (1998) Selection of probiotic strains for human applications. *Int Dairy J* 8(5):487–490
- Deshpande GC, Rao CS, Keil AD, Patole SK (2011) Evidence-based guidelines for use of probiotics in preterm neonates. *BMC Med* 9:92–98
- Duchaine C, Israel-Assayag E, Fournier M, Cormier Y (1996) Proinflammatory effect of *Pediococcus pentosaceus*, a bacterium used as preservative. *Eur Respir J* 9:2508–2512
- Edwards U, Rogall T, Blockerl H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* 17(19):7843–7853
- Erikci E, Gursel M, Gursel I (2011) Differential immune activation following encapsulation of immunostimulatory CpG oligodeoxynucleotide in nanoliposomes. *Biomaterials* 32:1715–1723
- Foligne B, Dewulf J, Breton J, Claisse O, Lonvaud-Funel A, Pot B (2010) Probiotic properties of non-conventional lactic acid bacteria: immunomodulation by *Oenococcus oeni*. *Int J Food Microbiol* 140:136–145
- Freter R (1992) Factors affecting the microecology of the gut. In: Fuller R (ed) *Probiotics*. Chapman & Hall, London, pp 111–144
- Fujiwara D, Inoue S, Wakabayashi H, Fujii T (2004) The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th₁/Th₂ cytokine expression and balance. *Int Arch Aller Imm* 135(3):205–215
- Fujiwara S, Seto Y, Kimura A, Hashiba S (2001) Intestinal transit of an orally administered streptomycin–rifampicin-resistant variant of *Bifidobacterium longum* SBT 2928: its long term survival and effect on the intestinal microflora and metabolism. *J Appl Microbiol* 90(1):43–52
- Fuller R (1989) Probiotics in man and animals. *J Appl Bacteriol* 66(5):365–378
- Furrie E (2005) Probiotics and allergy. *Proc Nutr Soc* 64:465–469
- Ghadimi D, Folster-Holst R, de Vrese M, Winkler P, Heller KJ, Schrezenmeir J (2008) Effects of probiotic bacteria and their genomic DNA on Th₁/Th₂-cytokine production by peripheral blood mononuclear cells (PBMCs) of healthy and allergic subjects. *Immunobiology* 213:677–692
- Gill HS (1998) Stimulation of the immun system by lactic cultures. *Int Dairy J* 8:535–544

- Goldin BR, Gorbuch SL, Saxelin M, Barakat S, Gualtieri L, Salminen S (1992) Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig Dis Sci* 37:121–128
- Guarner F, Schaafsma GJ (1998) Probiotics. *Int J Food Microbiol* 39(3):237–238
- Havenaar R, Brink BT, Huis in't Veld JHJ (1992) Selection of strains for probiotics use. In: Fuller R (ed) Probiotics. Chapman & Hall, London, pp 111–144
- Helwig U, Lammers KM, Rizzello F, Brigidi P, Rohleder V, Caramelli E, Gionchetti P, Schrezenmeier J, Foelsch UR, Schreiber S, Campieri M (2006) Lactobacilli, bifidobacteria and *E. coli* nissle induce pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells. *World J Gastroenterol* 12:5978–5986
- Iannitti T, Palmieri B (2010) Therapeutical use of probiotic formulations in clinical practice. *Clin Nutr* 29:701–725
- Iliev ID, Kitazawa H, Shimosato T, Katoh S, Morita H, He F, Hosoda M, Saito T (2005) Strong immunostimulation in murine immune cells by *Lactobacillus rhamnosus* GG DNA containing novel oligodeoxynucleotide pattern. *Cell Microbiol* 7:403–414
- Isolauri E (1999) Probiotics and gut inflammation. *Curr Opin Gastroenterol* 15:534–537
- Isolauri E, Juntunen M, Rautanen T, Sillanauke P, Koiuva T (1991) A human *Lactobacillus* strain (*Lactobacillus casei* sp. strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* 88:90–97
- Jonganurakkun B, Wang Q, Xu SH, Tada Y, Minamida K, Yasokawa D, Sugi M, Hara H, Asano K (2008) *Pediococcus pentosaceus* NB-17 for probiotic use. *J Biosci Bioeng* 106:69–73
- Kimoto H, Mizumachi K, Okamoto T, Kurisaki J (2004) New *Lactococcus* strain with immunomodulatory activity: enhancement of Th1-type immune response. *Microbiol Immunol* 48(2):75–82
- Kimura M, Danno K, Yasui H (2006) Immunomodulatory function and probiotic properties of lactic acid bacteria isolated from Mongolian fermented milk. *Biosci Microflora* 25(4):147–155
- Lammers KM, Brigidi P, Vitali B, Gionchetti P, Rizzello F, Caramelli E, Matteuzzi D, Campieri M (2003) Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunol Med Microbiol* 38:165–172
- Lebeer S, Vanderleyden J, De Keersmaecker SCJ (2008) Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol R* 72:728–764
- Li Y, Qu X, Yang H, Kang L, Xu Y, Bai B, Song W (2005) Bifidobacteria DNA induces murine macrophages activation *in vitro*. *Cell Mol Immunol* 2:473–478
- Lo Curto A, Pitino I, Mandalari G, Dainty JR, Faulks RM, John Wickham MS (2011) Survival of probiotic lactobacilli in the upper gastrointestinal tract using an *in vitro* gastric model of digestion. *Food Microbiol* 28(7):359–366
- Lomax AR, Calder PC (2009) Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. *Curr Pharm Des* 15:1428–1518
- Marin ML, Lee JH, Murtha J, Ustunol Z, Pestka JJ (1997) Differential cytokine production in clonal macrophage and T-cell lines cultured with bifidobacteria. *J Dairy Sci* 80:2713–2720
- Marteau P, Rambaud JC (1993) Potential of using lactic acid bacteria for therapy and immunomodulation in man. *FEMS Microbiol Rev* 12(1–3):207–220
- Medina M, Izquierdo E, Ennahar S, Sanz Y (2007) Differential immunomodulatory properties of *Bifidobacterium longum* strains: relevance to probiotic selection and clinical applications. *Clin Exp Immunol* 150:531–538
- Menard O, Gafa V, Kapel N, Rodriguez B, Butel MJ, Waligora-Dupriet AJ (2010) Characterization of immunostimulatory CpG-rich sequences from different *Bifidobacterium* species. *Appl Environ Microbiol* 76(9):2846–2855
- Morita H, He F, Fuse T, Ouwehand AC, Hashimoto H, Hosoda M, Mizumachi K, Kurisaki J (2002) Adhesion of lactic acid bacteria to CaCo-2 cells and their effect on cytokine secretion. *Microbiol Immunol* 46(4):293–297
- Murphy L, Dunne C, Kiely B, Shanahan F, O'Sullivan GC, Collins J (1999) *In vivo* assessment of potential probiotic *Lactobacillus salivarius* strains: evaluation of their establishment, persistence, and localisation in the murine gastrointestinal tract. *Microb Ecol Health D* 11:149–157
- Nagao F, Nakayama M, Muto T, Okumura K (2000) Effects of a fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. *Biosci Biotech Biochem* 64:2706–2708
- Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC (2009) Mechanisms of action of probiotics: recent advances. *Inflamm Bowel Dis* 15:300–310
- Niers LE, Timmerman HM, Rijkers GT, van Bleek GM, van Uden NO, Knol EF, Kapsenberg ML, Kimpfen JL, Hoekstra MO (2005) Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin Exp Allergy* 35:1481–1489
- Niers LE, Hoekstra MO, Timmerman HM, van Uden NO, de Graaf PM, Smits HH, Kimpfen JL, Rijkers GT (2007) Selection of probiotic bacteria for prevention of allergic diseases: immunomodulation of neonatal dendritic cells. *Clin Exp Immunol* 149:344–352
- Olivares M, Diaz-Ropero MP, Gomez N, Lara-Villoslada F, Maldonado JA, Martin R, Lopez-Huertas E, Rodriguez JM, Xaus J (2006) Oral administration of two probiotic strains, *Lactobacillus coryniformis* CECT5711 and *Lactobacillus gasseri* CECT5714, enhances the intestinal function of healthy adults. *Int J Food Microbiol* 107:104–111
- Osborne CA, Galic M, Sangwan P, Janssen PH (2005) PCR-generated artefact from 16S rRNA gene-specific primers. *FEMS Microbiol Lett* 248(2):183–187
- Osmanagaoglu O, Kiran F, Ataoglu H (2010) Evaluation of *in vitro* probiotic potential of *Pediococcus pentosaceus* OZF isolated from human breast milk. *Probiot Antimicrob Prot* 2(3):162–174
- Osmanagaoglu O, Kiran F, Nes IF (2011) A probiotic bacterium, *Pediococcus pentosaceus* OZF, isolated from human breast milk produces pediocin AcH/PA-1. *Afr J Biotechnol* 10(11):2070–2079
- Peran L, Camuesco D, Comalada M, Nieto A, Concha A, Diaz-Ropero MP, Olivares M, Xaus J, Zarzuelo A, Galvez J (2005) Preventive effects of a probiotic, *Lactobacillus salivarius* ssp. *salivarius*, in the TNBS model of rat colitis. *World J Gastroenterol* 11:5185–5192
- Perez-Cano FJ, Dong H, Yaqoob P (2010) *In vitro* immunomodulatory activity of *Lactobacillus fermentum* CECT5716 and *Lactobacillus salivarius* CECT5713: two probiotic strains isolated from human breast milk. *Immunobiology* 215(12):996–1004
- Repa A, Grangette C, Daniel C, Hochreiter R, Hoffmann-Sommergruber K, Thalhammer J, Kraft D, Breiteneder H, Mercenier A, Wiedermann U (2003) Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy. *Vaccine* 22(1):87–95
- Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fonden R, Saxelin M, Collins K, Mogensen G, Birkeland SE, Mattila-Sandholm T (1998) Demonstration of safety of probiotics - a review. *Int J Food Microbiol* 44(1–2):93–106
- Satokari R, Gronroos T, Laitinen K, Salminen S, Isolauri E (2009) *Bifidobacterium* and *Lactobacillus* DNA in the human placenta. *Lett Appl Microbiol* 48:8–12
- Sheih YH, Chiang BL, Wang LH, Liao CK, Gill HS (2001) Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. *J Am Coll Nutr* 20:149–156

- Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, Habu S, Hachimura S, Kaminogawa S (2002) *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clin Exp Allergy* 32(4):563–570
- Shida K, Suzuki T, Kiyoshima-Shibata J, Shimada S, Nanno M (2006) Essential roles of monocytes in stimulating human peripheral blood mononuclear cells with *Lactobacillus casei* to produce cytokines and augment natural killer cell activity. *Clin Vaccine Immunol* 13:997–1003
- Simpson PJ, Fitzgerald GF, Stanton C, Ross RP (2006) Enumeration and identification of pediococci in powder-based products using selective media and rapid PFGE. *J Microbiol Meth* 64(1):120–125
- Suskovic J, Kos B, Goreta J, Matosic S (2001) Role of lactic acid bacteria and bifidobacteria in synbiotic effect. *Food Technol Biotech* 39:227–235
- Suwanjinda D, Pala-Or K, Panbangred W (2009) Simultaneous detection of pediocin gene and species differentiation between *Pediococcus acidilactici* and *Pediococcus pentosaceus* in a one step multiplex-overlapping PCR methods. *Food Biotech* 23:179–189
- Takagi A, Matsuzaki T, Sato M, Nomoto K, Morotomi M, Yokokura T (2001) Enhancement of natural killer cytotoxicity delayed murine carcinogenesis by a probiotic microorganism. *Carcinogenesis* 22(4):599–605
- Van Enckevort FH, Netea MG, Hermus AR, Sweep CG, Meis JF, Van der Meer JW, Kullberg BJ (1999) Increased susceptibility to systemic candidiasis in interleukin-6 deficient mice. *Med Mycol* 37(6):419–426
- Vinderola CG, Matar C, Perdigon G (2005) Role of the epithelial cells in the immune effects mediated by gram-positive probiotic bacteria. Involvement of Toll-like receptors. *Clin Diagn Lab Immunol* 12:1075–1084
- Vizoso Pinto MG, Schuster T, Briviba K, Watzl B, Holzapfel WH, Franz CM (2007) Adhesive and chemokine stimulatory properties of potentially probiotic *Lactobacillus* strains. *J Food Prot* 70:125–134
- Vizoso Pinto MG, Gomez MR, Seifert S, Watzl B, Holzapfel WH, Franz C (2009) Lactobacilli stimulate the innate immune response and modulate the TLR expression of HT29 intestinal epithelial cells *in vitro*. *Int J Food Microbiol* 133:86–93
- Wells CL, Maddaus LA, Jechorek RP, Simmons RL (1988) Role of intestinal anaerobic bacteria in colonization resistance. *Eur J Clin Microbiol Infect Dis* 7(1):107–113
- Yasui H, Shida K, Matsuzaki T, Yokokura T (1999) Immunomodulatory function of lactic acid bacteria. *Antonie van Leeuwenhoek* 76(1–4):383–389
- Zhu J, Zhao L, Guo H, Jiang L, Ren F (2011) Immunomodulatory effects of novel *bifidobacterium* and *lactobacillus* strains on murine macrophage cells. *Afr J Microbiol Res* 5(1):8–15