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Protective effect of the probiotic *Lactobacillus acidophilus* ATCC 4356 in BALB/c mice infected with *Toxocara canis*

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ABSTRACT

Human toxocariasis consists of chronic tissue parasitosis that is difficult to treat and control. This study aimed to evaluate the action of the probiotic *Lactobacillus acidophilus* ATCC 4356 on larvae of *Toxocara canis* and the effect of IFN-γ cytokine on parasite-host *in vivo* (1.10^9 CFU) and *in vitro* (1.10^6, 1.10^7, 1.10^8, 1.10^9 CFU) interactions. Four groups of six BALB/c mice were formed: G1 - *L. acidophilus* supplementation and *T. canis* infection; G2 - *T. canis* infection; G3 - *L. acidophilus* supplementation; and G4 - PBS administration. Mice were intragastrically suplemented with probiotics for 15 days before inoculation and 48 h after inoculation with 100 *T. canis* eggs. The inoculation of T. *canis* was also perfomed intragastrically. The recovery of larvae took place through digestion of liver and lung tissues; the evaluation of IFN-γ gene transcription in leukocytes was performed by qPCR. The *in vitro* test consisted of incubating the probiotic with *T. canis* larvae. The supplementation of probiotics produced a reduction of 57.7% ($p = 0.025$) in the intensity of infection of *T. canis* larvae in mice, whereas in the *in vitro* test, there was no larvicidal effect. In addition, a decrease in the IFN-γ gene transcription was observed in both, *T. canis*-infected and uninfected mice, regardless of whether or not they received supplementation. The probiotic *L. acidophilus* ATCC 4356 reduced *T. canis* infection intensity in mice, however, the probiotic did not have a direct effect on larvae, demonstrating the need of interaction with the host for the beneficial effect of the probiotic to occur. Yet, the proinflammatory cytokine IFN-γ did not apparently contributed to the observed beneficial effect of probiotics.

KEYWORDS: Toxocariasis. Probiotic. Innate immunity. Cytokine. INF-γ.

INTRODUCTION

Human toxocariasis involves chronic tissue parasitosis, is globally distributed and is most prevalent in countries with tropical climate[1]. The main etiological agents are the nematodes *Toxocara canis* and *Toxocara cati*, intestinal parasites of dogs and cats, respectively. In humans, larvae of these nematodes are incapable of reaching maturity they undergo tissue migration affecting various organs and causing lesions of varied degrees of severity. The main form of infection in humans is through the ingestion of embryonic eggs of the parasite, but it can also occur through the ingestion of larvae present in meat or viscera of paratenic hosts, ingested raw or undercooked[2,3]. This parasitosis is considered a neglected disease that is prevalent in some developed[4] and developing countries[5].

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In the host immune response triggered by *T. canis,* the parasite switches the Th1 to Th2-type response[6]. Studies using murine models have shown that in the early phase of *T. canis* infection, there is an increase in IFN-γ, TNF-α and IL-12 cytokine levels[7,8]. During infection, *T. canis* modulates the immune response by suppressing proinflammatory cytokines production and stimulating the production of cytokines by Th2 and Treg cells[6,9]. This modulation is a form of parasite evasion from the host immune response as proinflammatory cytokines, such as IFN-γ and IL-12, are important macrophages activators that act in the defense against the nematode[8,10-12].

Human toxocariasis is a parasitosis that is difficult to treat due to the moderate effectiveness of drugs on the encysted larvae of *Toxocara* spp. in tissues, making it necessary to investigate other forms of control[13-15]. An alternative treatment is the use of probiotics, which have demonstrated potential to control murine visceral toxocariasis[16-20]. One of the modes of action of probiotics is through their capacity to counterbalance the Th1-, Th2- and Treg-type immune responses[21] and by their ability to inhibit the adhesion of pathogenic microorganisms to the intestinal epithelium through the production of antimicrobial substances such as short chain fatty acids, ammonia, hydrogen peroxide and bacteriocins[22].

Probiotics of the genus *Lactobacillus* contribute to reduce the luminal pH by stimulating the growth of epithelial cells and increasing the blood flow, modifying the intestinal motility, improving the absorption of water and minerals and increasing mucus production[23]. In addition, *in vivo* studies with *Lactobacillus* spp. have shown that these probiotics are capable of stimulating the Th1-type immune response, with increased IFN-γ, IL-12 and TNF-α cytokines production[24-29], which may also be beneficial for the control of visceral toxocariasis[8]. This study aimed to evaluate the action of the probiotic *Lactobacillus acidophilus* ATCC 4356 on the infection intensity of *T. canis* in BALB/c mice and the effect of the proinflammatory cytokine IFN-γ on the parasite-host interactions.

MATERIALS AND METHODS

Mice

Male BALB/c mice of five to seven weeks of age were maintained under controlled environmental conditions at 22 °C (± 1 °C), with a 12 h light and 12 h dark cycle, and access to food and water *ad libitum*. This project was approved by the Animal Use Ethics Committee of the Federal University of Rio Grande (P039/2016, P078/2016).

Probiotic *Lactobacillus acidophilus*

The probiotic *L. acidophilus* ATCC 4356 obtained from the Fundacao Oswaldo Cruz in Rio de Janeiro/RJ - Brazil (FIOCRUZ) was cultivated in Man, Rogosa and Sharpe (MRS) broth for 48 h at 37 °C. It was then centrifuged at 4000 x g for 10 min and resuspended in sterile phosphate-buffered saline (PBS). Next, the colony-forming units (CFU) were determined. Probiotic production, quality control, culture viability, colony counts and purity were evaluated according to the methodology employed by Walcher *et al*.[20].

Collection and incubation of the eggs and larvae of *Toxocara canis*

Five to eight weeks-old dogs naturally infected with *T. canis* were treated orally with pyrantel pamoate (15 mg/kg) to recover adult specimens of *T. canis*. The adult forms of *T. canis* were sexed and the females were submitted to hysterectomy to obtain eggs, which were incubated in 2% formalin at 28 °C, with humidity greater than 80% and oxygenation for 30 days[19]. Following the eggs embryonation, *T. canis* larvae were extracted and incubated[12,30].

In vitro evaluation of the probiotic *Lactobacillus acidophilus*

The probiotic *L. acidophilus* was tested at concentrations of 1.10^6, 1.10^7, 1.10^8 and 1.10^9 CFU and incubated at concentrations of 100 larvae/well (microculture plate TPP®) in RPMI-1640 medium supplemented with 25 mM HEPES, 1% glucose, 100 IU/mL penicillin, and 50 µg/mL streptomycin) at 37 °C with 5% CO_2 for 48 h, in triplicate[31].

After the plate incubation, 0.2% Trypan blue cell viability indicator (Vetec®) was added to all wells in the plate, and the material was incubated for an additional 30 min. Control RPMI-1640 medium with live *T. canis* larvae and control dead larvae (frozen at -20 °C/ 10 days, followed by heat shock at 60 °C) were also used. The viability evaluation was based on the following criteria: morphological integrity, motility and absence of Trypan blue staining. The evaluation was performed using an optical microscope at 100 x and 400 x magnification.

In vivo evaluation of the infection intensity

Four groups of six BALB/c mice were formed: G1 – mice were supplemented with the probiotic (1.10^9 CFU) intragastrically (IG) for 15 days before the inoculation of

100 *T. canis* eggs (IG), and the supplementation condinued for 2 days post-inoculation (P.I); G2 – IG administered with sterile PBS, for 15 days preinoculation and 48 h post-IG inoculation of 100 *T. canis* eggs; G3 – IG supplemented with probiotic for 17 days; and G4 – IG-administered sterile PBS for 17 days[20]. Mice were euthanized 2 days P.I and the experiment lasted 17 days. Euthanasia was perfomed with thiopental by intraperitoneal injections (75 mg/kg). Next, the digestion of the liver and lung tissues from animals of groups G1 and G2 was performed in 1% pepsin solution and 1% hydrochloric acid with constant shaking overnight at 37 °C[32]. Afterwards, *T. canis* larvae were recovered and quantified by optical microscopy at 100 x and 400 x magnification.

IFN-γ gene transcription

Whole-blood samples were obtained from the mice belonging to the four groups (G1, G2, G3, and G4) on the day of euthanasia using a pool of blood samples per group. Leukocytes were isolated from whole blood samples after cell lysis to eliminate red blood cells followed by washingin Hanks solution and storage in TRI Reagent® (Sigma Aldrich) at -70 °C. Subsequently, RNA extraction and cDNA synthesis were performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) as instructed by the manufacturer. Quantitative polymerase chain reactions (qPCRs) were performed in a Step One Plus apparatus (Applied Biosystems, Foster City, CA, USA) using specific primers for IFN-γ and GAPDH, the latter was included as a reference gene[8]. The qPCRs were performed with 1 µL of cDNA (synthesized from 300 ng/µL of RNA), 6.25 µL of Go Taq® qPCR Master Mix (Promega Corporation, Madison, Wisconsin, USA), 0.5 µM of each primer and 4.25 µL of RNAse-free water (Sigma Aldrich, Brasil Ltda) in a final volume of 12.5 µL. The temperatures used were as follows: an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 60 s, extension at 72 °C for 60 s and a final extension step at 72 °C for 5 min. All samples were tested in duplicate. The cycle threshold (Ct) values were used to calculate the variation in gene expression relative to GAPDH (control) expression.

Statistical analysis

Data regarding the infection intensity by *T. canis* and the IFN-γ gene transcription were compared by the Student's *t*-test, with a significance level of 0.05 (BioEstat version 5.0, AnalystSoft Inc., Walnut, CA, USA). The viability of *T. canis* larvae (*in vitro* test) was evaluated by analysis of variance (ANOVA), and the means were compared by Tukey's test, with a significance level of 0.05 (BioEstat version 5.0, AnalystSoft Inc., Walnut, CA, USA).

RESULTS

Compared to control mice (G2), animals supplemented with the probiotic *L. acidophilus* ATCC 4356 (G1) presented a 57.7% (p=0.025) reduction in the total number of larvae recovered from the liver (Table 1). Larval positivity in the liver was observed in all G1 and G2 mice, confirming the acute *T. canis* infection. No larvae were recovered from the lungs.

Table 1 - Mean number of *Toxocara canis* larvae recovered from the liver 48 h post inoculation of 100 embryonated eggs in BALB/c mice supplemented with the probiotic *Lactobacillus acidophilus* (1.10^9 CFU; n = six mice/group).

Groups	Mean (± standard deviation)	p
Control	5.2 (± 2.16)	
Lactobacillus acidophilus	2.2 (± 1.09)	**0.025**
Reduction (%)	57.7%	

(Student's *t*-test)

In the *in vitro* test, compared to the control, the different concentrations of *L. acidophilus* probiotic had no direct effect on *T. canis* larvae during the 48 h incubation period with *T. canis* larvae ($p > 0.05$). The mean percentage of larvae with morphological integrity, positive motility and no Trypan blue staining ranged from 93.8% to 95.3%. These means were similar to those of the control live larvae, of 94.6%. In the control group of dead larvae, all larvae were not viable, that is, they showed no motility and Trypan blue staining.

IFN-γ gene transcription

Compared to that in control mice supplemented with PBS (G4), IFN-γ gene transcription was reduced by three times in mice supplemented with probiotics and infected with *T. canis* (G1) and six times in mice supplemented with probiotics (G3). IFN-γ gene expression in mice infected with only *T. canis* (G2) was not significantly different from that of mice in the PBS control group (G4; Figure 1). After 48 h of *T. canis* egg inoculation, mice without supplementation had consistent IFN-γ (G2) gene transcription levels.

DISCUSSION

Several studies have already shown the potential of different *Lactobacillus* species probiotics to prevent and

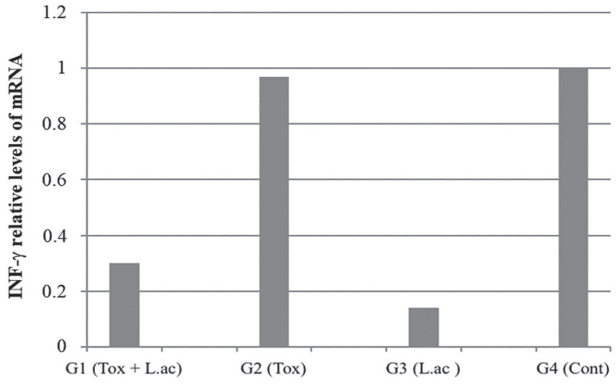

Figure 1 - IFN-γ gene transcription in Balb/c mice leukocytes (samples analyzed in one pool/group). G1 (Tox + L. ac) - infected by *Toxocara canis* and supplemented with *Lactobacillus acidophilus*; G2 (Tox) - infected by *Toxocara canis*; G3 (L. ac) - supplemented with *Lactobacillus acidophilus*; G4 (Cont) - not supplemented and not infected.

treat parasitosis, such as *Trichinella spiralis*, *Giardia lamblia* and *Toxocara canis*[20,33-35]. The results obtained in this study have shown that supplementation with *L. acidophilus* ATCC 4356 has significantly reduced the infection intensity of *Toxocara canis* larvae in experimentally infected mice. The rate of reduction in the intensity of infection induced by this probiotic was similar to that observed by Walcher *et al*.[20], who used the probiotic *L. rhamnosus* ATCC 7469 (53.3%). These results confirm the potential of *Lactobacillus* spp. to control toxocariasis under the tested conditions.

Despite the *in vivo* action of *L. acidophilus* ATCC 4356 on reducing the infection intensity, no *in vitro* direct effect on *T. canis* larvae was observed. Similar results have been shown with the probiotics *S. boulardii*[31] and *L. rhamnosus* ATCC 4356[20], which reduced the intensity of infection in mice and showed no *in vitro* effect on the larvae. In addition, our results are corroborated by those of other studies suggesting that the interaction between *S. boulardii* and the intestinal mucosa of *T. canis*-infected mice is necessary for the development of the beneficial effect of this probiotic[31,36].

To understand the mechanism underlying the action of this probiotic, the IFN-γ gene transcription was evaluated, as this is an important cytokine involved in the recruitment of macrophages during the acute phase of toxocariasis[10]. However, in this study, mice supplemented with *L. acidophilus* ATCC 4356, both infected and uninfected, had reduced IFN-γ gene transcription levels, similar to those of mice supplemented with *L. casei* ATCC 7469 and infected with *Trichinella spiralis*[33]. According to these authors, IFN-γ gene transcription levels were reduced in comparison with those of the control as IFN-γ production was poorly induced due to the small number of infective larvae, and the same phenomenon may have occurred in this study. However, animals supplemented with other species of *Lactobacillus* have shown increased levels of this proinflammatory cytokine[25,27,29], indicating that the effect obtained with one species of microorganism used as a probiotic cannot be extrapolated to other species, since closely related species can produce distinct effects or even opposite effects. Therefore, it is important that probiotics are characterized at the strain level[37].

Other hypotheses that may explain the variation in the results of different studies that evaluated the protective effect of probiotics against *T. canis* infections include the use of different probiotics (*Enterococcus faecalis* CECT 7121, *S. boulardii* and *L. rhamnosus* ATCC 7469), the dose of the administered probiotic, the species and lineage of the host and the evaluated organ[8,17-20,36].

Although no increment in the transcription of IFN-γ was observed in animals supplemented with *L. acidophilus*, the obtained results suggest that the effect of this probiotic against *T. canis* involves the innate immunity participation, as the reduction in the number of larvae occurred within 48 h, an insufficient period for the development of an adaptive immune response[8,10]. In addition, in this study, IFN-γ transcription levels were evaluated at the systemic level (blood) and the action of the probiotic can occur at the local level (intestinal mucosa)[36], and this aspect, like other pro-inflammatory and anti-inflammatory interleukins need to be evaluated in future studies.

CONCLUSION

The probiotic *Lactobacillus acidophilus* ATCC 4356 reduced the intensity of *T. canis* infection. However, the proinflammatory cytokine IFN-γ was not involved in this reduction and in the establishment of infection by *T. canis* larvae, and the probiotic did not have a direct effect on larvae, demonstrating the need of the interaction with the host for the probiotic beneficial effect to occur. Thus, it is important to evaluate other possible mechanisms of action of the probiotic, as well as other cytokines, to elucidate its protective effect.

REFERENCES

1. Ma G, Holland CV, Wang T, Hofmann A, Fan CK, Maizels RK, et al. Human toxocariasis. Lancet Infect Dis. 2018;18:e14-24.
2. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology and molecular aspects. Clin Microbiol Rev. 2003;16:265-72.
3. Nijsse R, Ploeger HW, Wagenaar JA, Mughini-Gras L. Prevalence and risk factors for patent Toxocara infections in cats and cat owners' attitude towards deworming. Parasitol Res. 2016;115:4519-25.

4. Macpherson CN. The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. Int J Parasitol. 2013;43:999-1008.
5. Fialho PM, Correa CR. A systematic review of toxocariasis: a neglected but high-prevalence disease in Brazil. Am J Trop Med Hyg. 2016;94:1193-9.
6. Dlugoz E, Wasyl K, Klockiewicz M, Wisniewski M. Toxocara canis mucins among other excretory-secretory antigens induce in vitro secretion of cytokines by mouse splenocytes. Parasitol Res. 2015;114:3365-71.
7. Maizels RM. Toxocara canis: molecular basis of immune recognition and evasion. Vet Parasitol. 2013;193:365-74.
8. Avila LF, Leon PM, Moura MQ, Berne ME, Scaini CJ, Leivas Leite FP. Modulation of IL-12 and IFN-g by probiotic supplementation promotes protection against Toxocara canis infection in mice. Parasite Immunol. 2016;38:326-30.
9. Resende NM, Gazzinelli-Guimarães PH, Barbosa FS, Oliveira LM, Nogueira DS, Gazzinelli-Guimarães AC, et al. New insights into the immunopathology of early Toxocara canis infection in mice. Parasit Vectors. 2015;8:354.
10. Kuroda E, Yoshida Y, En Shan B, Yamashita U. Suppression of macrophage interleukin-12 and tumor necrosis factor-α production in mice infected with Toxocara canis. Parasite Immunol. 2001;23:305-11.
11. Valli JL, Williamson A, Shrif S, Rice J, Shewen PE. In vitro cytokine responses of peripheral blood mononuclear cells from healthy dogs to distemper virus, Malassezia and Toxocara. Vet Immunol Immunopathol. 2010;134:218-29.
12. Maizels RM, Blaxter ML, Robertson, BD, Selkirk ME. Parasite antigens, parasite genes: a laboratory manual for molecular parasitology. Cambridge: Cambridge University Press; 1992.
13. Lescano SZ, Chieffi PP, Amato Neto V, Ikai DK, Ribeiro MC. Anti-helmínticos na toxocaríase experimental: Efeito na recuperação de larvas de Toxocara canis e na resposta humoral. J Bras Patol Med Lab. 2005;41:21-4.
14. Frazier M, Anderson ML, Sophocleuss S. Treatment of ocular toxocariasis with albendazole: a case report. Optometry. 2009;80:175-80.
15. Othman AA. Therapeutic battle against larval toxocariasis: are we still far behind? Acta Trop. 2012;124:171-8.
16. Lescano SA, Santos SV, Assis JM, Chieffi PP. Efficacy of nitazoxanide against Toxocara canis: larval recovery and humoral immune response in experimentally infected mice. Rev Inst Med Trop Sao Paulo. 2015;57:337-41.
17. Basualdo J, Sparo M, Chiodo P, Ciarmela M, Minvielle M. Oral treatment with a potential probiotic (Enterococcus faecalis CECT 7121) appears to reduce the parasite burden of mice infected with Toxocara canis. Ann Trop Med Parasitol. 2007;101:559-62.
18. Chiodo PG, Sparo MD, Pezzani BC, Minvielle MC, Basualdo JA. In vitro and in vivo effects of Enterococcus faecalis CECT7121 on Toxocara canis. Mem Inst Oswaldo Cruz. 2010;105:615-20.
19. Avila LF, Conceição FR, Telmo PL, Dutra GF, Santos DG, Martins LH, et al. Saccharomyces boulardii reduces infection intensity of mice with toxocariasis. Vet Parasitol. 2012;187:337-40
20. Walcher DL, Cruz LA, Telmo PL, Martins LH, Avila LF, Berne ME, et al. Lactobacillus rhamnosus reduces parasite load on Toxocara canis experimental infection in mice, but has no effect on the parasite in vitro. Parasitol Res. 2018;117:597-602.
21. Ghadimi D, Folster-Holst R, Vrese M, Winkler P, Heller KJ, Schrezenmeir J. 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. 2008;213:677-92.
22. O'Hara AM, O'Regan P, Fanning A, O'Mahony C, Macsharry J, Lyons A, et al. Functional modulation of human intestinal epithelial cell responses by Bifidobacterium infantis and Lactobacillus salivarius. Immunology. 2006;118:202-15.
23. Ohashi Y, Ushida K. Health-beneficial effects of probiotics: its mode of action. Anim Sci J. 2009;80:361-71.
24. Maroof H, Hassan ZM, Mobarez, AM, Mohamadabadi MA. Lactobacillus acidophilus could modulate the immune response against breast cancer in murine model. J Clin Immunol. 2012;32:1353-9.
25. Sharma R, Kapila R, Dass G, Kapila S. Improvement in Th1/Th2 immune homeostasis, antioxidative status and resistance to pathogenic E. coli on consumption of probiotic Lactobacillus rhamnosus fermented milk in aging mice. Aged (Dordr.). 2014;36:9686.
26. Su J, Li J, Zheng H, You Y, Luo X, Li Y, et al. Adjuvant effects of L. acidophilus LW1 on immune responses to the foot-and-mouth disease virus DNA vaccine in mice. PLoS One. 2014;9:e104446.
27. Ren D, Li C, Qin Y, Yin NR, Du S, Liu H, et al. Evaluation of immunomodulatory activity of two potential probiotic Lactobacillus strains by in vivo tests. Anaerobe. 2015;35:22-7.
28. Lee RM, Moore LB, Bottazzi ME, Hotez PJ. Toxocariasis in North America: a systematic review. PLoS Negl Trop Dis. 2014;8:e3116.
29. Gorska S, Schwarzer M, Srutkova D, Hermanova P, Brzozowska E, Kozakova H, et al. Polysaccharides L900/2 and L900/3 isolated from Lactobacillus rhamnosus LOCK 0900 modulate allergic sensitization to ovalbumin in a mouse model. Microb Biotechnol. 2017;10:586-93.
30. Savigny DH. In vitro maintenance of Toxocara canis larvae and a simple method for the production of Toxocara ES antigens for use in serodiagnostic tests for visceral larva migrans. J Parasitol. 1975;61:781-2.
31. Avila LF, Telmo PL, Martins LH, Glaeser TA, Conceição FR, Leite FP, et al. Protective effect of the probiotic Saccharomyces boulardii in Toxocara canis infection is not due to direct action on the larvae. Rev Inst Med Trop Sao Paulo. 2013;55:363-5.

32. Xi WG, Jin LZ. A novel method for the recovery of Toxocara canis in mice. J Helminthol. 1998;72:183-4.
33. Martínez-Gómez F, Fuentes-Castro BE, Bautista-Garfias CR. The intraperitoneal inoculation of Lactobacillus casei in mice induces total protection against Trichinella spiralis infection at low challenge doses. Parasitol Res. 2011;109:1609-17.
34. Amer EI, Mossallam SF, Mahrous H. Therapeutic enhancement of newly derived bacteriocins against Giardia lamblia. Exp Parasitol. 2014;146:52-63.
35. El Temsahy M, Ibrahim IR, Mossallam SF, Mahrous H, Bary AA, Salam SA. Evaluation of newly isolated probiotics in the protection against experimental intestinal trichinellosis. Vet Parasitol. 2015;214:303-14.
36. Moura MQ, Terto WD, Jeske ST, Castro LM, Pinto NB**,** Avila LF, et al. Evaluation of the transcription of interleukin-12 in the intestinal mucosa of mice subjected to experimental toxocariasis and supplemented with Saccharomyces boulardii. Vet Parasitol. 2017;242:59-62.
37. Azaıs-Braesco V, Bresson JL, Guarner F, Corthier G. Not all lactic acid bacteria are probiotics, but some are. Br J Nutr. 2010;103:1079-81.