

Probiotic bacteria can modulate murine macrophage's superoxide production in *Trichinella spiralis* infection

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Summary

The effect of probiotic strains (*Enterococcus faecium* EF55, *E. faecium* CCM7420, *E. faecium* CCM8558, *E. durans* ED26E/7, *Lactobacillus fermentum* CCM7421, *L. plantarum* 17L/1) on the production of superoxide anion (O₂⁻) in peritoneal macrophages of *Trichinella spiralis* infected mice was examined. *E. faecium* EF55 and *E. faecium* CCM8558 strains increased the O₂⁻ production prior to parasitic infection, at the day 7 of application. A significant inhibition of the O₂⁻ production caused by *T. spiralis* infection on day 5 *post* infection (p.i.) was prevented by all examined strains. Lactobacilli stimulated metabolic activity of macrophages during intestinal and early muscular phase (from day 5 to 25 p.i.) of trichinellosis. Enterococci increased the O₂⁻ production in early intestinal phase (day 5 p.i.) and during the muscular phase of trichinellosis (days 25 and 32 p.i.). Respected increase in macrophage's metabolic activity induced by probiotic treatment in the intestinal phase of trichinellosis augmented the host anti-parasite defence (damage and killing of newborn larvae with reactive oxygen species from macrophages).

Keywords: probiotic strains; *Trichinella spiralis*; mice; macrophages; superoxide anion

Introduction

Trichinellosis is a serious food-borne parasitic zoonosis caused by the nematode of the genus *Trichinella*, which is characterized by an extremely wide host range and worldwide distribution (Bruschi, 2012; Goździk *et al.*, 2017). The pathology of trichinellosis is associated with initial inflammatory response during the intestinal phase, and subsequent allergic and inflammatory responses during larval migration and invasion of the host muscles (Bruschi & Chiumiento, 2011; Hasby Saad *et al.*, 2018). The efficacy of classic therapy with benzimidazoles is limited due to weak activity against encapsulated larvae, low water solubility, anthelmintic resistance, contraindication in children and pregnancy (Yadav & Temjenmongla, 2012). The anti-parasitic potential of alternative therapeutic approaches is therefore increasing in recent years. The need for

development of a new methods to control this disease is necessary where the utilisation of the beneficial – probiotic bacteria has been proposed. In general, probiotics may be useful in prevention and treatment in various health conditions and diseases (Singh *et al.*, 2013). It is supposed that similar benefits will occur during the treatment of parasitic infections affecting gastrointestinal tract. The identification of factors mediating beneficial effects of probiotics presents an opportunity not only to understand their fine mechanisms of action, but also to develop the effective pharmacological strategies that could participate in treatments for various pathogens. Positive effects of probiotic bacteria reducing the parasite burden and pathological changes in experimental trichinellosis were previously described (Bautista-Garfias *et al.*, 1999, 2001; Martínez-Gómez *et al.*, 2009, 2011; El Temsahy *et al.*, 2015; Dvorožňáková *et al.*, 2016; Bucková *et al.*, 2018).

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Gut microbiota plays a crucial role in completing the life cycle of the parasite in the intestine, in both enabling the development into adults and their reproduction and modulating the host immune response. Probiotic bacteria, which confer a health benefit on the host (FAO/WHO, 2002), may strongly interfere with the pathophysiology of parasitic infections, and determine the parasite survival along with the outcome of parasitic infections (Berrilli *et al.*, 2012). Nematode *Trichinella spiralis* causes an intestinal and tissue disease – trichinellosis (Bruschi, 2002) characterized by the enteritis (induced by adult worms) and the inflammation with degenerative changes in the skeletal muscles (induced by larvae). Probiotic bacteria can provide an indirect protection to the host, probably by modulation the effect on newborn and muscle *T. spiralis* larvae (Travers *et al.*, 2011; Dvorožňáková *et al.*, 2016; Bucková *et al.*, 2018). The main mechanisms of probiotic actions include enhancement of the gut epithelial barrier, increase of adhesion to the intestinal mucosa and simultaneous inhibition of pathogen adhesion, competitive elimination of pathogens, production of anti-microbial molecules, and modulation of the immune system (Goudarzi *et al.*, 2014). They are able to modulate both, the innate and adaptive immune responses. In the context of non-specific immunity, their immunomodulatory activity is performed through modulation of inflammatory response, stimulation of phagocytic activity and activation of antigen-presenting cells (Tsai *et al.*, 2012).

Macrophages, as part of the innate immune response, play a key role in resistance to a helminth *Trichinella spiralis* (Wing *et al.*, 1979; Kołodziej-Sobocińska *et al.*, 2007; Bruschi & Chiumiento, 2011). Macrophages belong to the antigen-presenting cells and are essential in initiation and modulation of the host immune response (phagocytosis, release cytokines, and inflammation) to parasite infection (Bai *et al.*, 2012a; Franken *et al.*, 2016). Excretory-secretory products of each *T. spiralis* stage modulate macrophage function through toll-like receptors expression and signalling pathways in macrophages (Han *et al.*, 2018; 2019). Macrophages prevent the invasion of pathogens by release of cytotoxic molecules such as reactive oxygen species (ROS) and by secretion of proinflammatory cytokines (Franken *et al.*, 2016). Macrophages express conserved pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), which recognize microbe-associated molecular patterns (MAMPs) expressed on the cell surface of probiotic bacteria and thus can mediate the host immune response (Lebeer *et al.*, 2010). The interaction between beneficial microbiota, macrophages and parasite in the host represent very active system where all components play a relevant role in modulating each other and in the maintenance of homeostasis important for the health of the host. Immunomodulatory activity of probiotic bacteria on macrophage's function can uncover their potential role in control of trichinellosis.

The present study was designed to examine the effect of probiotic and bacteriocin-producing bacterial strains on the production of superoxide anion (O_2^-) in the peritoneal macrophages of mice infected with *T. spiralis*.

Materials and Methods

Probiotic strains

The effects of the following bacteria were tested: bacteriocin-producing strains with probiotic properties (*Enterococcus faecium* EF55, *Enterococcus faecium* 2019 = CCM7420, *Enterococcus faecium* AL41 = CCM8558, *Enterococcus durans* ED26E/7, and *Lactobacillus plantarum* 17L/1) and probiotic strain *Lactobacillus fermentum* AD1 = CCM7421. All used strains are original isolates (Bucková *et al.*, 2018), not previously used for this purpose.

Enterococcus faecium EF55 was isolated from the chicken crop and characterized at the Institute of Animal Physiology CBs SAS – IAP CBs SAS, Košice, Slovakia. The strain produces a thermo-stable bacteriocin EF55.

Enterococcus faecium 2019 = CCM7420 is a rabbit-derived strain with probiotic properties, which produces enterocin 2019 (Ent 2019). It was isolated and characterized at IAP CBs SAS, Košice, Slovakia and deposited in the Czech Culture Collection of Microorganisms, Brno, Czech Republic – CCM7420.

Enterococcus faecium AL41 = CCM8558 (isolated and characterized at IAP CBs SAS, Košice, Slovakia and deposited in the Czech Culture Collection of Microorganisms, Brno, Czech Republic – CCM8558) is an environment-derived strain. The strain produces enterocin M with a wide antimicrobial inhibitory spectrum and possesses probiotic properties.

Enterococcus durans ED26E/7 was isolated from traditional ewes milk lump cheese at the Research Dairy Institute, Žilina – RDI, Žilina, Slovakia; but identified, characterized and prepared for experiment at IAP CBs SAS, Košice, Slovakia.

Lactobacillus plantarum 17L/1 was isolated from stored ewes cheese (RDI, Žilina, Slovakia) but identified, characterized and prepared for experiment at IAP CBs SAS, Košice, Slovakia.

Lactobacillus fermentum AD1 = CCM7421 was isolated and characterized at IAP CBs SAS, Košice, Slovakia and deposited in the Czech Culture Collection of Microorganisms, Brno, Czech Republic – CCM7421. It is a canine-derived strain possessing probiotic properties.

All used strains were evaluated according to the EFSA rules (Piskoríková, 2010). For the experiment they were cultivated in MRS broth (Merck, Eppelheim, Germany) at 37 °C for 24 h. Broth cultures were centrifuged (30 min at 10,000 x g) and the cells were resuspended in Ringer solution (Merck, pH 7.0) to a concentration of 10⁹ colony forming units per ml (CFU/ml). The purity of the strains was checked by the standard microbiological method (ISO-International Organization for Standardization) by spreading dilutions in Ringer solution (pH 7.0, Merck, Eppelheim, Germany) onto the selective medium *M-Enterococcus* agar (ISO-15214, Difco, Detroit, USA) and/or MRS agar (Merck, Eppelheim, Germany). The cultures for application were stored at 4 °C.

Parasite

The reference isolate of *Trichinella spiralis* (ISS 004) (obtained

and assigned codes from the Trichinella Reference Centre in Rome) was maintained by serial passages on ICR mice at the Institute of Parasitology SAS, and used for the infection. Larvae were released by artificial digestion (1 % pepsin, 1 % HCl for 4 h at 37 °C; both from Sigma-Aldrich, Hamburg, Germany) of tissue following the Kapel and Gamble protocol (2000) and kept in saline solution until inoculation of experimental mice.

Experimental design

The experiment was performed on pathogen-free eight week old male BALB/c mice (VELAZ, Prague, Czech Republic; n = 147) weighting 18 – 20 g. Mice were kept under a 12-h light/dark regime at room temperature (22 – 24 °C) with 56 % humidity and kept on a commercial diet and water available without restrictions. Animals were divided randomly into 7 groups: Control (n = 21) – *T. spiralis* infection without the administration of bacterial strains; Group 1 (n = 21) – *E. faecium* EF55 + *T. spiralis*; Group 2 (n = 21) – *E. faecium* CCM7420 + *T. spiralis*; Group 3 (n = 21) – *E. faecium* CCM8558 + *T. spiralis*; Group 4 (n = 21) – *E. durans* ED26E/7 + *T. spiralis*; Group 5 (n = 21) – *L. plantarum* 17L/1 + *T. spiralis*; Group 6 (n = 21) – *L. fermentum* CCM7421 + *T. spiralis*. Probiotic strains were administered *per os* daily at a dose of 10⁹ CFU/ml in a total volume of 100 µl. Mice were infected *per os* with 400 *T. spiralis* larvae/mouse on day 7 of treatment. Samples of the peritoneal macrophages were obtained on days: -7, 0, 5, 11, 18, 25 and 32 post infection (p.i.).

Intestinal worm burdens

The intestinal phase of infection was investigated on days 5, 11 and 18 p.i. The small intestine was cut into 5 – 10 cm long pieces, placed into a sieve and incubated in conical pilsner glasses in 37 °C NaCl (0.9 % saline) overnight. After incubation, gut pieces were discarded and the worms in the sediment were counted under stereomicroscope at 60 x magnification (Leica S8APO, Leica Microsystems, Germany).

Isolation of muscle larvae

The muscle phase of infection was examined on days 18, 25 and 32 p.i. Whole eviscerated carcasses were minced and artificially digested (1 % pepsin HCl for 4h at 37 °C; both from Sigma-Aldrich, Germany) following the protocol of Kapel and Gamble (2000). Samples were allowed to settle for 20 min before the supernatant was discarded and the sediment was poured through a 180 µm sieve into a conical glass and washed with tap water. The sediment was finally transferred to a gridded Petri dish and counted under stereomicroscope at 40 x magnification (Leica S8APO, Leica Microsystems, Germany). Depending on the density of larvae either a sub-sample or the whole sample was counted.

Superoxide anion assay

Generation of extracellular superoxide anion (O₂⁻) from peritoneal macrophages was assayed as superoxide dismutase (SOD)-in-

hibitible reduction of ferricytochrome C with or without stimulation with phorbol myristate acetate (PMA) (Dvorožňáková *et al.*, 2008). Peritoneal cells were aseptically harvested in RPMI 1640 (Sigma-Aldrich, Hamburg, Germany) to a final concentration of 1x10⁶ cells/ml. One ml of cell suspension was adhered to each well using 24-well plates (Falcon, France) and incubated at 37 °C in 5 % CO₂ and 85 % humidity for 2 h. The reaction was carried out in 0.3 ml/well of 160 µM ferricytochrome C (Sigma-Aldrich, Hamburg, Germany) in Earl's balanced salt solution (EBSS) (pH 7.2). For control the reaction was blocked by 300 µg SOD/10 µl in EBSS (Sigma-Aldrich, Hamburg, Germany). 10 µl of PMA (Sigma-Aldrich, Hamburg, Germany) in ethanol was used for the stimulation of cells for respiratory burst. Cells were incubated at 37 °C in 5 % CO₂ and 85 % humidity for 2 h and the optical density (OD) of supernatant was measured at 550 nm. The amount of O₂⁻ produced was calculated from the difference between OD in reactions blocked by SOD and without SOD. As the resulting value, the nanomols (nmol) of produced O₂⁻ were calculated according to the formula: nmol O₂⁻ = (OD/6.3) x 100 and determined for 1 mg of cell proteins.

Statistical evaluation

Statistical differences were assessed using one-way ANOVA, followed by post hoc Tukey's test (a value of P<0.05 was considered significant), which allowed comparison between each two groups at each time point. The analyses were performed using the Statistica 6.0 (Stat Soft, Tulsa, USA) statistical package.

Ethical Approval and/or Informed Consent

The research related to animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals. The experimental protocol was in compliance with current Slovak ethical rules for animal handling and it was approved by the Animal Care Committee of the Institute of Parasitology SAS and the State Veterinary and Food Administration of the Slovak Republic (Ro-3184/14-221).

Results

Parasite burden – numbers of adults and muscle larvae

A significant reduction of parasites in the gut occurred on day 11 p.i. (Table 1) in mice treated with strains *E. faecium* CCM8558 (53 %), *E. faecium* CCM7420(51 %), *E. durans* ED26E/7 (38 %), and *E. faecium* EF55 (30 %). Mice with this probiotic treatment absolutely eliminated adults from the small intestine till day 18 p.i. Lactobacilli did not affect the presence of adults in the intestine. The highest numbers of muscle larvae were detected in untreated mice (Table 1) on days 25 and 32 p.i. The probiotic treatment resulted in a significant larval count reduction on day 25 p.i., as follows: *E. faecium* EF55 (44 %), *E. faecium* CCM8558 (74 %), *E. faecium* CCM7420 (26 %), *E. durans* ED26E/7 (54 %), *L. fermentum* CCM7421(64 %) and *L. plantarum* 17L/1(69 %).

Table 1. Parasite burden in mice treated with probiotic bacteria and infected with *T. spiralis*.

Intestinal phase (numbers of adults)	Days post infection					
	5		11		18	
	(mean ± S.D.)	reduction (%)	(mean ± S.D.)	reduction (%)	(mean ± S.D.)	reduction (%)
<i>T.spiralis</i> (control)	295 ± 24		229 ± 37		2 ± 2	
<i>E.faecium</i> EF55 + <i>T.spiralis</i>	293 ± 38	0.7	160 ± 39	30.1	0 ± 0	100
<i>E.faecium</i> CCM8558 + <i>T.spiralis</i>	244 ± 25	17.3	*107 ± 25	53.3	0 ± 0	100
<i>E.faecium</i> CCM7420 + <i>T.spiralis</i>	235 ± 35	20.3	*112 ± 14	51.1	0 ± 0	100
<i>E.durans</i> ED26E/7 + <i>T.spiralis</i>	256 ± 7	13.2	*142 ± 29	38	1 ± 2	50
<i>L.fermentum</i> CCM7421 + <i>T.spiralis</i>	209 ± 21	29.2	210 ± 27	8.3	40 ± 12	0
<i>L.plantarum</i> 17LL1 + <i>T.spiralis</i>	326 ± 48	0	192 ± 8	16.2	1 ± 2	0
Muscle phase (numbers of larvae)						
	18		25		32	
	(mean ± S.D.)	reduction (%)	(mean ± S.D.)	reduction (%)	(mean ± S.D.)	reduction (%)
<i>T.spiralis</i> (control)	2 ± 4		50,080 ± 4,931		54,069 ± 8,020	
<i>E.faecium</i> EF55 + <i>T.spiralis</i>	40 ± 9	0	37,060 ± 4,150	26	42,580 ± 7,750	21.2
<i>E.faecium</i> CCM8558 + <i>T.spiralis</i>	47 ± 2	0	**13,220 ± 1,842	73.6	*23,810 ± 799	56
<i>E.faecium</i> CCM7420 + <i>T.spiralis</i>	24 ± 6	0	27,970 ± 7,212	44.1	46,950 ± 3,818	13.2
<i>E.durans</i> ED26E/7 + <i>T.spiralis</i>	28 ± 16	0	*23,250 ± 5,938	53.6	**29,080 ± 2,204	46.2
<i>L.fermentum</i> CCM7421 + <i>T.spiralis</i>	4 ± 2	0	**18,380 ± 2,039	63.7	26,272 ± 2,566	51.4
<i>L.plantarum</i> 17LL1 + <i>T.spiralis</i>	65 ± 6	0	**15,540 ± 2,191	69	32,070 ± 6,463	40.7

*P<0.05; **P<0.01 – statistically significant differences from *T. spiralis* infected group without treatment

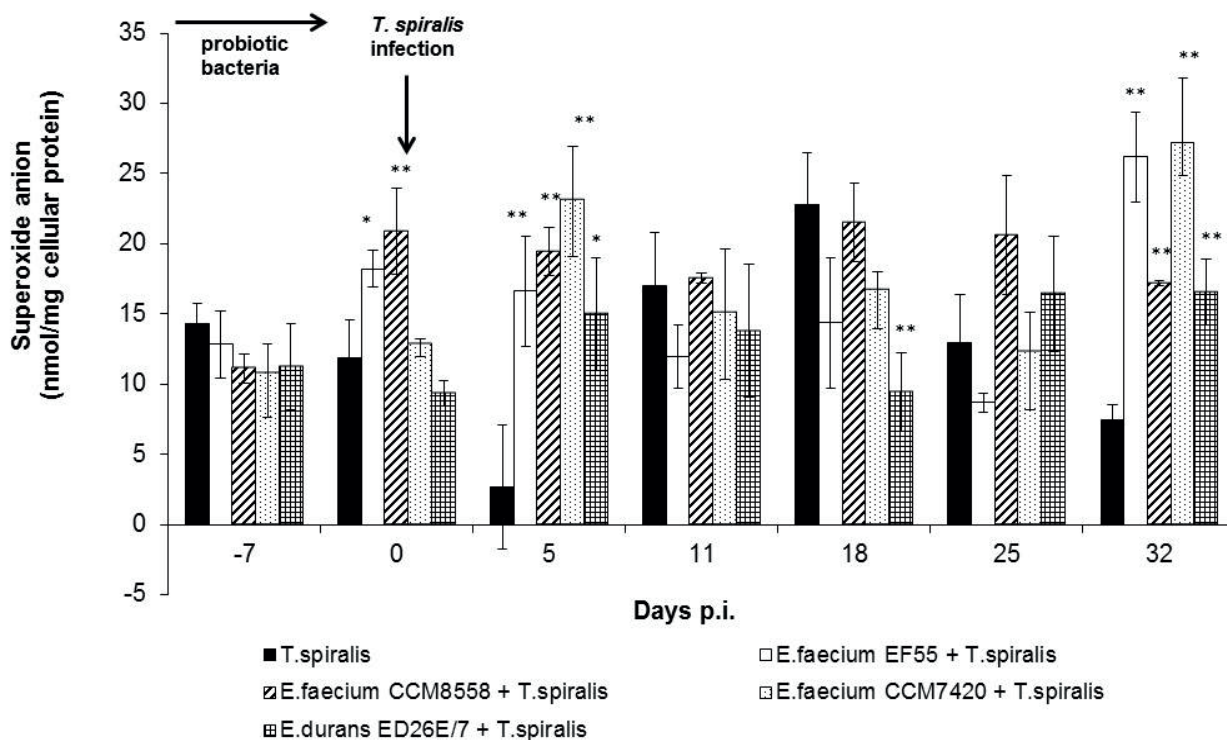


Fig. 1 Production of superoxide anion by peritoneal macrophages from mice treated with probiotic bacteria (enterococci) and infected with *T. spiralis*. * $P < 0.05$; ** $P < 0.01$ —statistically significant differences from *T. spiralis* infected group without probiotic treatment (control).

Superoxide anion production

T. spiralis infection in untreated mice (control) induced a great suppression of the metabolic activity of peritoneal macrophages on day 5 p.i. (Fig. 1). The generation of O_2^- was restored and stimulated on days 11 and 18 p.i. During the muscle phase of trichinellosis the O_2^- production in untreated and infected mice (control) was decreasing till the end of the experiment (day 32 p.i.).

The administration of *E. faecium* EF55 and *E. faecium* CCM8558 significantly ($P < 0.05$; $P < 0.01$) increased metabolic activity of macrophages before the parasite infection. This happened on day 0, e.i. after one week of the treatment (Fig. 1). All enterococci prevented the strong inhibition of the O_2^- production on day 5 p.i. caused by *T. spiralis*, and significantly ($P < 0.05$; $P < 0.01$) stimulated its generation. The increased metabolic activity induced by enterococci was also recorded in the muscle phase on days 25 (*E. faecium* CCM8558) and 32 p.i. ($P < 0.01$; *E. faecium* EF55, *E. faecium* CCM7420, *E. faecium* CCM8558, and *E. durans* ED26E/7).

Similarly, lactobacilli stimulated the O_2^- production during the acute phase of trichinellosis, on day 5 p.i. ($P < 0.05$), and prevented the strong inhibitory effect of *T. spiralis* infection (Fig. 2). The stimulative effect on macrophage's metabolic activity after treatment with *L. plantarum* 17L/1 and *L. fermentum* CCM7421 lasted throughout the entire experiment, where a significant increase on days 11, 25 and 32 p.i. ($P < 0.05$; $P < 0.01$) was observed.

Discussion

Our study revealed that the examined probiotic strains may influence macrophage's oxidative metabolism in *T. spiralis* infection and their effect is strain-specific. Macrophages are important cells involved in initiation and modulation of the host immune response to the helminth parasite. After a contact with the antigen they stimulate and produce reactive oxygen species such as superoxide anion (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxyxynitrite (ONOO⁻) that are highly toxic to the parasites (James, 1995; Martinez *et al.*, 2004).

In our study, *T. spiralis* infection in mice without probiotic treatment induced a significant suppression of superoxide anion (O_2^-) production in peritoneal macrophages in the first week of infection, what was followed by an increase in the third week of infection, i.e. at the time of newborn larvae migration into the host muscles. *Trichinella* adults and muscle larvae are more resistant to the superoxide anion because they contain 3 to 5 times higher amount of antagonist enzyme superoxide dismutase (SOD) when compared to newborn larvae that are highly susceptible to the oxygen radicals (Kazura & Meshnick, 1984). The SOD plays an important role in parasite-host interaction by modulating the reactions of these host cells. This enzyme may act as a necessary defensive mechanism of the parasite against the highly destructive superoxide radical of the host's immune system.

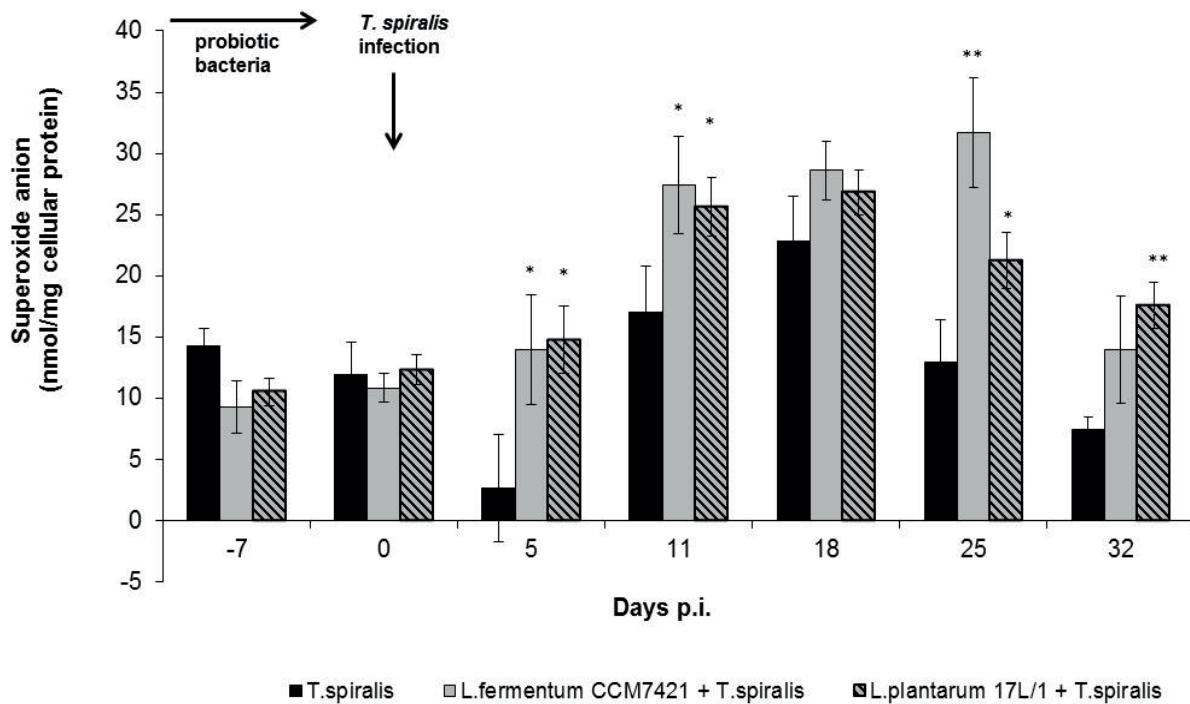


Fig. 2 Production of superoxide anion by peritoneal macrophages from mice treated with probiotic bacteria (lactobacilli) and infected with *T. spiralis*. *P<0.05; **P<0.01—statistically significant differences from *T. spiralis* infected group without probiotic treatment (control).

In the muscle phase of trichinellosis, the O_2^- generation was decreased, probably because of the Th2 response dominance. An induction of the host immunosuppression represents an important parasite evasive strategy, in context of which *Trichinella* spp. is characterized by an extremely strong ability to induce suppression of the host immune system (Gruden-Movsesijan *et al.*, 2011; Aranzamendi *et al.*, 2012). *Trichinella* excretory-secretory proteins (ESP) induce strong immunosuppression during the first 2 weeks of infection, what is followed by a Th2 polarized immune response with alternatively activated M2 macrophages (Ilic *et al.*, 2012). Larval ESP significantly inhibit the activity of M1 macrophages, which play a key role in the host immune response against various pathogens (Bai *et al.*, 2012b). Within this scope, Zhao *et al.* (2017) found that recombinant *T. spiralis* calreticulin (the surface protein produced by adults and muscle larvae) binding complement C1q reduced monocyte and macrophage adhesion to NBL mediated by complement, and also reduced the release of reactive oxygen products in the macrophages. Hence, the above studies demonstrated that larval ES products affect both classical and alternative activation of macrophages.

Wang *et al.* (2019) summarized the ability of probiotic bacteria to regulate macrophage polarization. Probiotic bacteria may induce different macrophage phenotypes depending on physiological and pathological conditions. Some probiotic strains activate the M1 macrophages to exert pro-inflammatory effect (Th1 immunity, increased oxidative metabolism), whereas a variety of the other probiotics can induce anti-inflammatory M2 macrophages. The

interactions between probiotics, macrophages and pathogens are described particularly on bacterial and protozoan infections (Vitetta *et al.*, 2016; Wang *et al.*, 2019). Nevertheless, data from helminth infections are missing. In our experiment, the probiotic strains *E. faecium* EF55 and *E. faecium* CCM8558 increased the production of the superoxide anion prior the infection on day 0 of the experiment (i.e. after 7 days of application). Similarly, *Lactobacillus paracasei* isolated from fermented milk stimulated the formation of reactive nitrogen in macrophages (Kapila *et al.*, 2013). A significant increase in the production of oxygen radicals in blood leukocytes has also been reported in humans with probiotic diets of *Enterococcus faecium* M-74 or *Lactobacillus johnsonii* (Mikeš *et al.*, 1995; Donnet-Hughes *et al.*, 1999). In our study, enterococcal strains stimulated the O_2^- synthesis during the acute phase of *T. spiralis* infection on day 5 p.i. Lactobacilli showed a weaker stimulating effect on macrophage's activity on day 5 p.i. in comparison with enterococci, but prevented the suppression caused by parasite in untreated mice. However, in the developed intestinal phase of the infection (from day 11 p.i.) lactobacilli increased the O_2^- production with greater intensity than enterococci. Probably as a result of antiparasitic defence against longer persisting worms in the gut of infected mice treated with lactobacilli (till day 18 p.i.). In our experiment, the application of *Enterococcus* strains resulted in a significant reduction of the number of adults on day 11 p.i. (30–53%), which might be associated with the early activation of pro-inflammatory M1 macrophages induced by bacterial products (Rolot & Dewals, 2018). The M1 macrophages are involved in mucin hy-

perproduction (Martinez & Gordon, 2014) that helps to remove the parasite from the intestine (Miller, 1987). In addition, as compared to lactobacilli, enterococci are better adhered to the intestinal mucosa (Lauková *et al.*, 2004), thus preventing the parasite penetration into the intestinal villi and crypts. *L. plantarum* 17L/1 and *L. fermentum* CCM7421 did not affect the number of adults in the intestine. Probiotic treatment by all strains did not have a significant effect on peritoneal macrophages on day 18 p.i. Assuming that the parasites were not present for a longer time in the host gut. *E. durans* ED26E/7 has even significantly reduced the formation of this oxygen radical on day 18 p.i. This might be explained by the fact that mice modulated by this strain showed low numbers of muscle larvae on day 18 p.i. This was due to the significantly increased enzymatic activity of blood phagocytes (Dvorožňáková *et al.*, 2016), which could contribute to the elimination of newborn larvae (NBL) and prevent their migration into the muscles. During the muscular phase, the “nurse cell” is an immunologically protected site for *T. spiralis* where larval E/S products maintain long-term communication with the host (Sofronic-Milosavljevic *et al.*, 2015). The E/S products of muscle larvae contain various proteases and protease inhibitors including serine, cysteine, metalloproteases, serpine and cystatin that can modulate the immune response of the host leading to chronic, long-term survival of *Trichinella* larvae in muscle cells (Ashour, 2013). In the present study, we have detected a significant reduction of larval burden in muscles of *T. spiralis*-infected mice and treated with probiotic strains on day 25 p.i. (26 – 74 %). Nevertheless, the production of superoxide anion in peritoneal macrophages was also stimulated during the muscle phase. In mice with probiotic treatment, despite the significant reduction compared to control on day 32 p.i. (13 – 56 %), an increase of 5,000 – 19,000 larvae/mouse between days 25 and 32 p.i. was observed. This growth points to the fact that probiotic strains have slowed down migration of NBL to the target tissues - muscles. Oxygen radicals from macrophages, production of which was stimulated and peaked on day 25 p.i. after the treatment with strains *L. fermentum* CCM7421, *L. plantarum* 17L/1, *E. faecium* CCM8558, and on day 32 p.i. after the administration of *E. faecium* E55 and *E. faecium* CCM7420, could be involved in the damage and reduction of larval infectivity. *E. durans* ED26E/7 induced the balanced production of O₂⁻ during the muscle phase. Our results suggest that probiotic strains induced the classical activation of M1 macrophages during the NBL migration. Despite alternative macrophage phenotype induced by the Th2 immune response is being activated in helminthoses (Rodríguez-Sosa *et al.*, 2002), the work of Ilic *et al.* (2011) documented that the soluble NBL antigen induces the mixed Th1/Th2 polarization. Alternatively activated macrophages (AAM) are identified as regulatory cells able to act against proinflammatory and cellular immune effector mechanisms (Martinez & Gordon, 2014). They also play an important role in the regeneration of skeletal muscle *in vivo* and have beneficial effects on myogenic growth and cell differentiation (Arnold *et al.*, 2007). We assume that alternatively activated mac-

rophages are abundantly present in muscle tissue and blood in the course of trichinellosis. In addition to AAM, NBL are also able to induce classical activation of pulmonary macrophages during migration (Falduto *et al.*, 2016). It is noteworthy that in our study the metabolic activity of peritoneal macrophages, which are associated with NBL migration from the intestine to other host tissues, was induced by probiotic therapy, that might have contributed to the NBL damage and reduced the intensity of infection in the host muscles. Probiotic treatment has also stimulated the oxidative metabolism of peritoneal macrophages during the muscle phase, which can result from the activation of immune memory cells but also can be influenced by low levels of the antagonistic enzyme superoxide dismutase due to the low number of its producers – muscle larvae.

Our experimental model study showed that probiotic strains have the ability to decrease the intensity of parasitic infection by affecting important components of the innate immune system such as phagocytosis (Dvorožňáková *et al.*, 2016) or macrophage's production of cytotoxic superoxide anion. The best protective effect against *T. spiralis* infection associated with the increased oxidative metabolism of peritoneal macrophages was exhibited by *L. fermentum* CCM7421 and *L. plantarum* 17L/1, which activated the metabolic activity of macrophages during the migration of newborn larvae (from day 5 to 25 p.i.). Enterococci efficiently supported the metabolic activity of macrophages in the anti-parasitic defence at the early phase of trichinellosis (day 5 p.i.). The obtained results confirmed the impact of the used probiotic strains as effective mediators to regulate macrophage's oxidative metabolism in *T. spiralis* infection. These are promising data which link use of probiotic with their prophylactic and therapeutic properties in trichinellosis treatment and prevention.

Conflict of Interest

The authors declare there is no conflict of interest relating to the information presented in this manuscript.

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