Protection of *Lactobacillus reuteri* against *Trichinella spiralis* via Nrf2 Signal Pathway in Intestinal Phase

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Article

Protection of *Lactobacillus reuteri* against *Trichinella spiralis* via Nrf2 Signal Pathway in Intestinal Phase

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Simple Summary: Trichinellosis is a widespread parasitic zoonosis. Despite the multiple efforts made, while there is no effective vaccine. However, uncomplete drug treatment schemes and the rise of anthelmintic resistance makes a hidden danger for the increase of parasite infection. To deal with worrisome development, alternative treatments based on probiotics have been explored as anthelmintic therapy or as a complement to traditional anthelmintic treatments. In summary, the results of our study demonstrate that oral with *L. reuteri* protection against by invasive *T. spiralis* stimulated the intestinal immune responses in mice, thereby reducing worm burden and reducing inflammatory factors mRNA expression level following infection with *T. spiralis*, which may be attributable to Nrf2 signal pathway mediated antioxidant enzyme expression. These findings provide evidence for the efficacy of a novel potential strategy for trichinellosis.

Abstract: Trichinellosis is a major worldwide health concern caused by *T. spiralis* infection without highly efficient and safe therapeutic method so far. To solve the problem of uncomplete drug treatment schemes and the rise of anthelmintic resistance, a potential protective effect of probiotic strains against zoonotic *T. spiralis* infection was investigated in the framework of a new therapeutic strategy aimed at using probiotics to control parasitic zoonoses. The study was focused on *L. reuteri* protected against *T. spiralis* infection in intestinal phase throughout the detection of adult worm number, inflammatory factors mRNA expression, antioxidant enzyme expression and Nrf2 signal pathway protein expression. Mice were divided randomly into 3 groups: 1) Control group: no treatment; 2) Ts group: *T. spiralis* infection; 3) LAB group: *T. spiralis* infected after orally administered *L. reuteri* for 7 days. Probiotics bacteria were administered daily in a dose of 10^9 CFU/mL, and mice were infected with 400 *T. spiralis* larvae in the experimental groups. The number of adult worms was also significantly increased after administration of probiotics. Meanwhile, *L. reuteri* alleviate the inflammatory response caused by *T. spiralis* infection in intestinal has been confirmed. In addition, the promotion of antioxidant enzyme expression can be inferred that *L. reuteri* regulate Nrf2 signal pathways against *T. spiralis* infection. Our study demonstrates that *L. reuteri* provide protection against *T. spiralis* infection in intestinal. Therapeutic approaches with probiotic strains admission could help to reduce the risks of trichinellosis or complement classical anti-parasite treatments.

Keywords: *Trichinella spiralis*; *Lactobacillus reuteri*; intestinal; mice

1. Introduction

Trichinellosis is a widespread food-borne parasitic zoonosis resulted from *Trichinella spiralis* (*T. spiralis*) infection, which the pathological mechanism is associated with initial inflammatory response during the intestinal phase [1] Numerous studies demonstrate that *T. spiralis* is an intestinal nematode that can modulate the host immune system [2]. The efficacy of classic therapy with antiparasitic drugs limited due to weak activity against *T. spiralis* larvae and the emergence of antiparasitic resistance

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[3,4]. The development of alternative therapeutic approaches is essential, therefore, the utilisation of the beneficial-probiotic bacteria has been proposed [5].

Positive effects of probiotic bacteria reducing the parasite burden and pathological changes in experimental trichinellosis were described previously [6–10]. Probiotic bacteria can provide an indirect protection to the host, probably by modulation the effect on newborn and muscle *T. spiralis* larvae [11]. 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.

It is noteworthy that increasing evidence has revealed that intestinal helminth infections are closely correlated with the gastrointestinal microbiota [12–15]. Lactobacillus (LAB), a large group of autochthonous intestinal microbiota in humans and animals, are especially distinguished for their probiotic properties [16]. Probiotics contribute to regulate Nrf2 signal pathway by balancing intestinal immunological barrier，relieveing inflammatory response [17].

T. spiralis infection is determined not only by the dose of infective larvae, but also by the immune status and inflammatory responses of the host to the parasite antigens [18].The host elicits an immune response to the parasite invasion by activation of immune mechanisms at the intestinal level, the site where the interaction between the parasite and gut microflora modify each other and the host immune system. This study aimed to explore the change in intestinal flora in the intestinal during T. spiralis infection, as well as the potential effects of probiotic on parasite survival or its underlying pathogenesis.

2. Materials and Methods

2.1. Animals and Ethics Statement

Specific pathogen free (SPF) KM mice aged 6-8 weeks were purchased from the Harbin Medical University (Harbin, China), which were housed in cages with a 12 h light and 12 h dark cycle with free access to a standard diet. All animal care procedures were in accordance with the Animal Management Committee of Northeast Agricultural University (SYXK [Hei] 2016-007). Moreover, animal suffering was minimized during the experimental procedure.

2.2. Parasite and Probiotic Strain

T. spiralis (ISS533 strain) was stored in our lab and maintained in KM mice. In addition, the muscle larvae (ML) were released through pepsin-acid digestion as previously described [19], and kept in saline solution until inoculation of experimental mice.

Probiotic strains were separated, screened, identified, characterized and evaluated according to the previous studied [5]. *Lactobacillus reuteri* (*L. reuteri*)50319 possessed extraordinary probiotic properties in vivo was prepared for experiment.

2.3. Experimental Design

Mice were divided randomly into 4 groups: Control group -no treatment; Ts (n = 15)-T. *spiralis* infection without the administration of bacterial strains; LAB (n = 15)- *L. reuteri* +*T. spiralis*. Probiotic strains were administered *per os* daily at a dose of 10^9 CFU/mL in a total volume of 200 μL. Mice were infected *per os* with 400 *T. spiralis* larvae/mouse.

Five mice from each experimental group were euthanized on day 1, 4, 7 after infection, so as to collect the intestinal samples. During sampling procedure (Figure 1), make sure that samples shall not be contaminated. Additionally, Control group was euthanized on day 1, which the samples collected as the uninfected controls. All samples were preserved at -80 C for later use.

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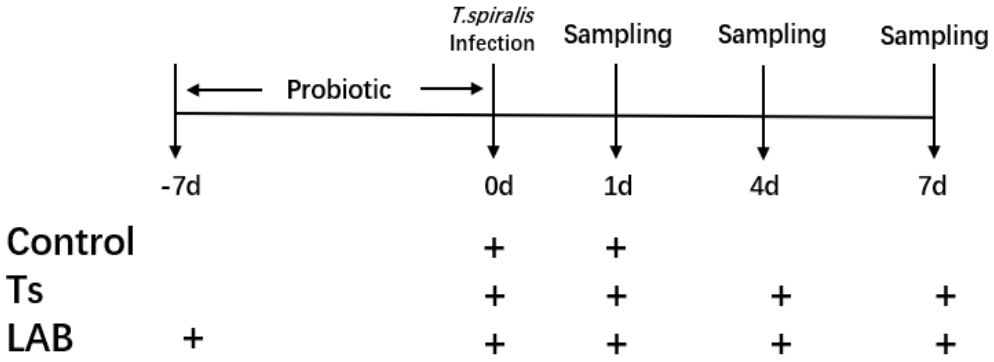

Figure 1. Schematic outline of the experimental design.

2.4. Intestinal Worm Burdens

The intestinal phase of infection was investigated on day 4, and 7. The small intestine was cut into pieces and incubated in 37 °C NaCl (0.9 % saline) overnight. The worms in the sediment were counted after incubation.

2.5. Oxidative Stress Assay

To investigate the oxidative stress of intestinal tissues, the levels of serum superoxide dismutase (SOD) and malondial dehyde (MDA) in the homogenates of intestinal tissues were measured by commercially kits on day 1, 4, and 7, according to the manufacturer's instruction. The level of SOD was measured as an indicator of cellular defense against reactive oxygen species. Also, the level of MDA was measured as an index of lipid peroxides.

2.6. Measurement of Inflammatory Cytokines

Total RNA from each tissue sample was extracted as described [20]. The RNA quantity and purity were assessed. Extracted RNA was diluted to the same concentration and the synthesis of cDNA was performed as the manufacturer's instructions. The cDNA samples were diluted used for qRT-PCR amplification. Each sample was analyzed in triplicate. For the relative quantification of the gene expression levels, the logarithmic-scaled threshold cycle(Ct) values were used in the $2^{-\Delta\Delta Ct}$ method before calculating the mean and standard error of the mean (SEM) for the references and individual targets.

The encoded gene expression levels of inflammatory response, including interleukin 4 (IL-4), interleukin 10 (IL-10), interleukin 17 (IL-17), and interferon-γ (IFN-γ) expression in the intestinal samples were investigated on day 1, 4, and 7. All oligonucleotide sequences of the forward and reverse primers used in this experiment are obtained as described [21].

2.7. Western Blot Analysis

Proteins were extracted from the intestinal tissues using protein extraction reagent on day 7.Protein concentration was determined by BCA protein assay kit. Proteins were separated by SDS PAGE and blotted onto PVDF membranes .After blockade for 2h at room temperature, the membranes were probed with primary antibodies (Nrf2, HO-1, and β-actin) at 4°C overnight. Followed by incubation with secondary antibody（peroxidase conjugated goat anti-rabbit IgG）with incubation for 2h at room temperature， proteins were visualized with ECL-chemiluminescent kit.

2.8. Statistical Analysis

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.

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3. Results

3.1. Adult Worm Reduction

As shown in Table 1, a significant reduction of adult worm in the intestine occurred in LAB group (18.24%) and treated with *L. reuteri 50319* on day 4. The highest numbers of adult worm were detected in Ts group on days 7. The probiotic treatment resulted in a significant adult worm count reduction in LAB group (44.49%). Experiment results confirmed that *L. reuteri* promote AD excretion and reduce colonization to against *T. spirali* infection.

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

	Day 4		Day 7	
	mean±SD	**reduction**	**mean±SD**	**reduction**
Ts	296±31	——	227±15	——
LAB	242±26	18.24%	126±23*	44.49%

*P<0.05 means statistically significant differences from T. spiralis infected group.

3.2. Oxidative Stress Marker Levels

As shown in Figure 2, after *T. spiralis* infection day 1, the level of MDA in Ts group was significantly increased than that of the Control group (P<0.01), as well as day 4 and day 7. While, the level of MDA in LAB group was significantly increased than that of the Control group (P<0.01) on day 1 and day 4 (P<0.05).

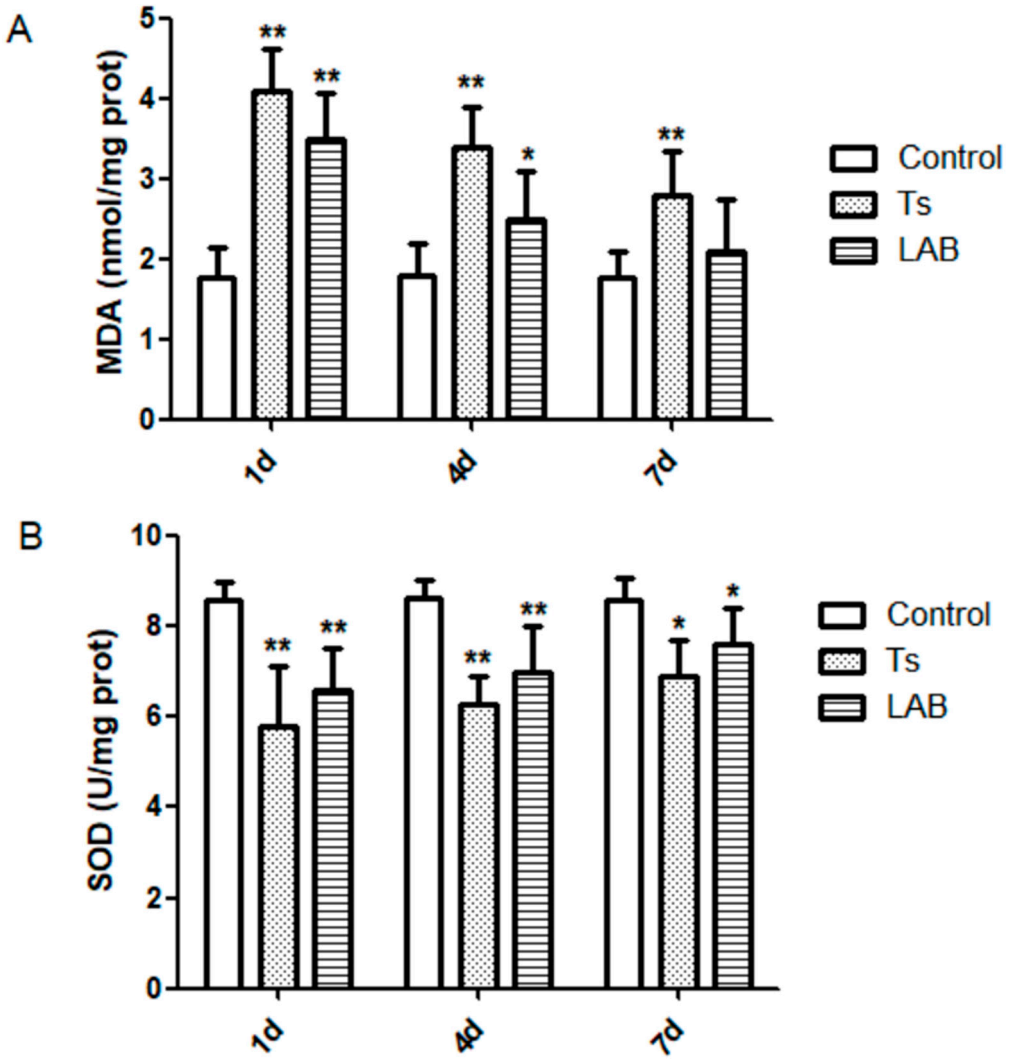

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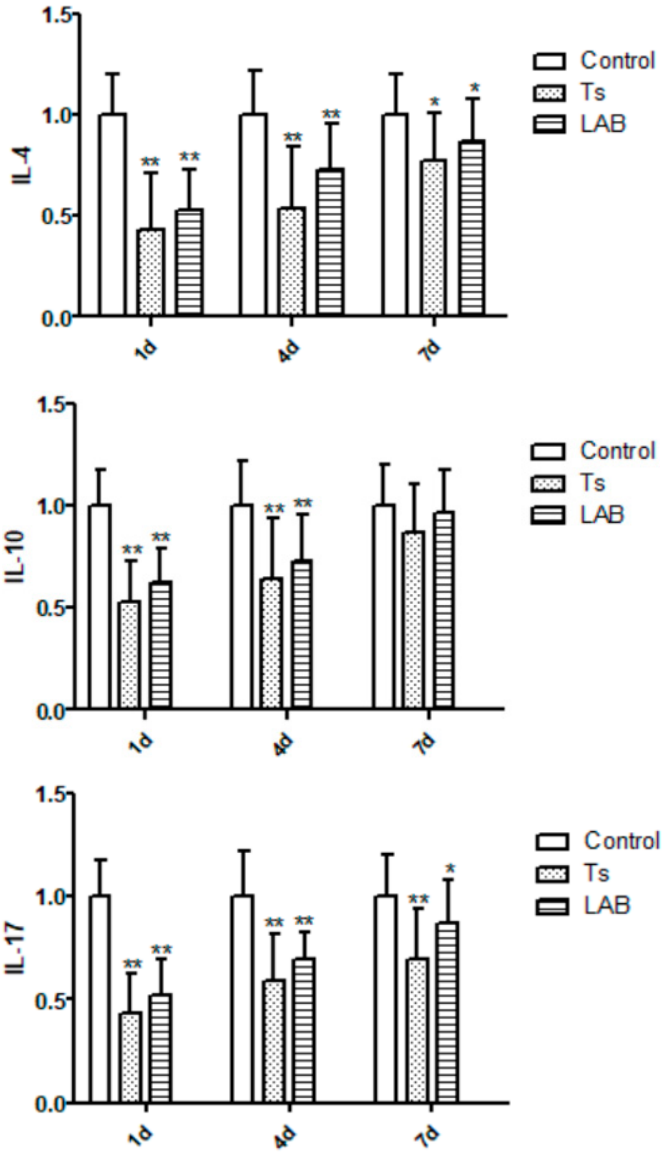
Figure 2. Oxidative stress markers detection results. *P<0.05 and **P<0.01 mean statistically significant differences from the Control group.

The level of SOD in Ts group and LAB group were significantly increased than that of the Control group (P<0.01), as well as day 4. While, on day 7 the level of MDA in Ts group and LAB group were significantly increased than that of the Control group (P<0.05).

3.3. Inflammatory Response

Statistical significances were found between Control group and experimental groups (Ts group and LAB group) throughout the entire process, as shown in Figure 3. After infection with *T. spiralis*, the IL-4 expression levels in Control group had statistically significant values for Ts group and LAB group, and LAB group were higher than Ts group. In the same instant, significantly increasing was obverved in the IL-10 expression levels between Ts group and LAB group，compared with Control group. IL-17 expression results showed a similar tendency at experimental period. On the contrary, compared with Control group, IFN-γ expression significantly increased in Ts group and LAB group. The LAB group gradually recovered to the IFN-γ expression level without no treatment, while Ts group higher than in LAB group consistently .The experiment results confirmed that *L. reuteri* could inhibit the inflammatory reaction caused by invading intestinal in mice.



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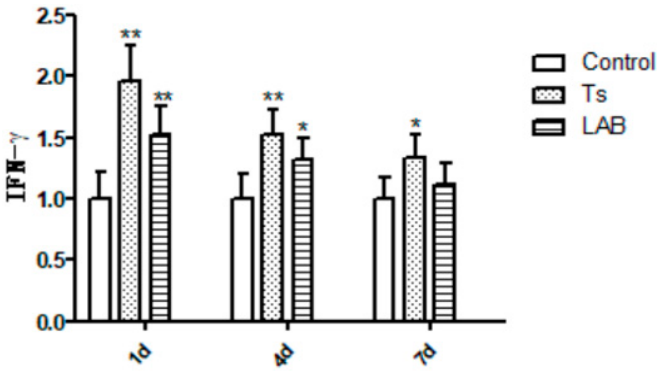

Figure 3. Inflammatory factor expression results. *P<0.05 and **P<0.01 mean statistically significant differences from the Control group.

3.4. Nrf2 and HO-1 Expression

The expression of Nrf2 and HO-1 in intestinal tissues was detected by western blot analysis, as shown in Figure 4. Compared with the control group, the mice Nrf2 and HO-1 expression level in Ts group and LAB group increases in intestinal tissue.

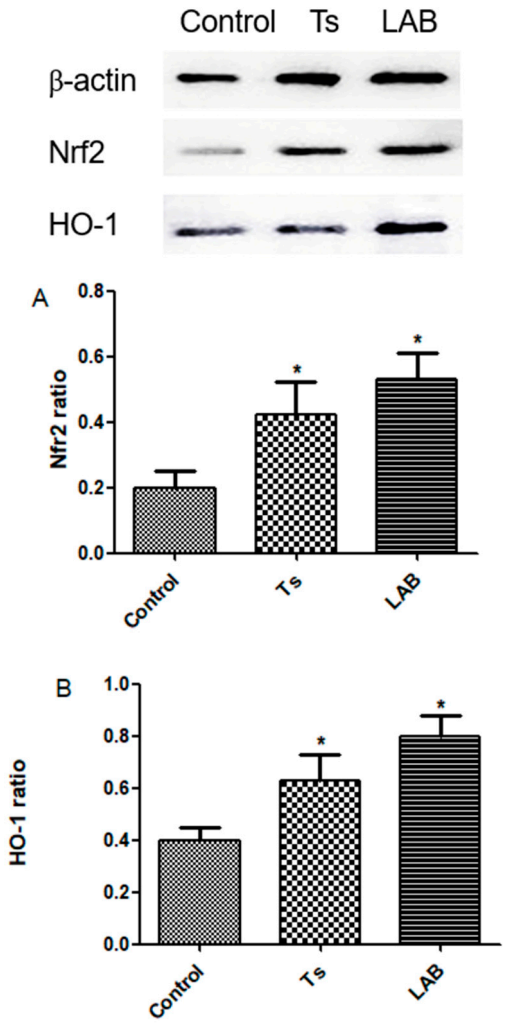

Figure 4. Nrf2 and HO-1 protein expression results. *P<0.05 means statistically significant differences from the Control group.

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4. Discussion

T. spiralis is a kind of the typical tissue-dwelling pathogen, which develops in a life cycle including muscle larva (ML), adult worm (Ad) and the newborn larva (NBL) [22]. NBL can be released into the mucosa at day 4 after infection [23,24], which represents the intestine phase of *T. spiralis* infection [25]. Significantly, *T. spiralis* is a kind of intestinal parasite that modulate the host immune system strongly.The crucial drugs currently used to control helminth infections including benzimidazoles, imidazothiazoles and macrocyclic lactones families, however, evidences have revealed increasing anthelmintic resistance in animals which a potential health problem. To deal with limitation of anthelmintic drugs, alternative treatments based on probiotics have been developed. Probiotics consumed in adequate amounts, confer benefit to the host [26]. It has been proven that probiotic microbes have the ability to exert an immunomodulatory effect in vivo [27]. As is known to all, the oral administration of probiotics contributes to controlling *T. spiralis* infections since it has been demonstrated that probiotic could stimulate intestinal immune response, among other effects on the host immune system. The present paper aimed to explore the protection of probiotics in intestinal phase during *T. spiralis* infection, as well as its potential effects on parasite survival or its underlying pathogenesis. To investigate the protection of probiotics against *T. spiralis* infection on intestinal, mice were infected with *T. spiralis* infectious larvae or probiotic by oral gavage. Previous studies demonstrate that *T. spiralis* produce diverse immunomodulatory effects at different stages. Therefore, samples were also collected at different time points of infection, so as to examine the effects of intestinal stages of *T. spiralis* infection.

As previously describe, probiotic reduced the parasite burden in experimental trichinellosis.It has been reported that the reduced parasite burden is associated with decreased fecundity induced by probiotic strains. In present paper, L. reuteri reduced adult worms after T. spiralis infection, in agreement with prior observation that LAB administered to induce resistance against T. spiralis infection in mice [28].

Host immune dependent damage to helminth parasites is mediated by the generation of oxygen-derived free radicals via nonspecific defense reaction [29]. Therefore, administered *L. reuteri* after *T. spiralis* induction, significant increases in the levels of the antioxidant enzyme SOD starting from day 1, lasting through day 7 were observed. However, significant decreases in the level of antioxidant enzyme MDA. This may be caused by LAB-mediated antioxidant protective effects. The antioxidant enzyme levels in our experimental model are consistent with the results of previous study [30].

In general terms, the immune response in helminth infections is a type 2 immune response which is characterized by the production of interleukin including IL-4, IL-5, IL-9, IL-10, and IL-13 et al [31]. Previous study has been reported that IFN-γ activated macrophages which rapidly processed *T. spiralis* antigens improving the acquired immune response against the parasite. These macrophages also produced nitric oxide (NO) and probably promoted inflammatory response in the intestine [32]. IL-17 cytokine plays an important role in the pathogenesis of various autoimmune inflammatory diseases [33]. Inflammatory response is a protective mechanism to protect against injury, infection, trauma, and noxious stimuli [34]. The purpose of this process is to eliminate injurious agents or intruder [35]. Mounting evidence suggests that LAB ameliorates inflammation during intestinal injury [36,37]. Therefore, our observation in agreement with the previous literature that LAB alleviate the inflammatory response caused by *T. spiralis* infection in intestinal.

Nrf2 is essential for the regulation of detoxifying and antioxidant genes [38]. It can activate the Nrf2 signaling pathway to regulate the expression of antioxidant enzymes, alleviate oxidative stress, and alleviate inflammatory reactions. To investigate the anti-oxidative effects of *L. reuteri* , Nrf2 signaling pathway was measured. In this study, our results showed that *L. reuteri* dependently up regulated the expression of Nrf2 and HO-1. These results suggested that *L. reuteri* inhibited *T. spiralis* induced oxidative stress by activating Nrf2 signaling pathway.

The use of probiotics as a treatment for helminth infection is an incipient research line with promising perspectives. Nonetheless, several limitations should be noted in our study. The sample size is small relatively, which may affect the statistical power. A larger sample size should be expanded in prospective studies.

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5. Conclusions

Therapeutic approaches with probiotic strains benefit to reduce the risks of trichinellosis or complement classical anti-parasite treatments. Present study demonstrates that probiotic bacteria can provide strain-specific protection against *T. spiralis* infection throughout reduced parasite burder, regulated intestinal flora and alleviated inflammatory response. Several additional mechanisms involved in the anti-parasite defense should be further studied and elucidated to justify the therapeutic use of probiotics. We look forward to more findings as a wide range of possibilities to be explored that may deliver groundbreaking treatment strategies for helminth diseases. In addition, to the best of our knowledge, this is the first experimental report on the antiparasitic effects of *L. reuteri* used as a novel treatment option against nematode *T. spiralis* infection.

Author Contributions: Conceptualization and methodology S.X. and Y.X.L.; Conduction of the experimental work and data curation, S.X.; writing—review and editing, S.X. and Y.X.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Animal Management Committee of Northeast Agricultural University (Animal Ethics Committee approval number SYXK [Hei] 2016-007).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hasby Saad, M.; Safwat, O.; El-Guindy, D.; Raafat, R.; Elgendy, D.; Hasby, E. Biomolecular Changes and Cortical Neurodegenerative Lesions in Trichinella Spiralis Infected BALB/c Mice: A Preliminary Study Elucidating a Potential Relationship Between Systemic Helminthic Infections and Idiopathic Parkinson's. *Helminthologia* **2018**, *55*, 261-274. https://doi.org/10.2478/helm-2018-0029.
2. Liu, S.; Pan, J.; Meng, X.; Zhu, J.; Zhou, J.; Zhu, X. Trichinella spiralis infection decreases the diversity of the intestinal flora in the infected mouse. *J Microbiol Immunol Infect* **2021**, *54*, 490-500. https://doi.org/10.1016/j.jmii.2019.09.009.
3. Pettersson, E.; Sjölund, M.; Wallgren, T.; Lind, E.O.; Höglund, J.; Wallgren, P. Management practices related to the control of gastrointestinal parasites on Swedish pig farms. *Porcine Health Manag* **2021**, *7*, 12. https://doi.org/10.1186/s40813-021-00193-3.
4. Zajíčková, M.; Nguyen, L.T.; Skálová, L.; Raisová Stuchlíková, L.; Matoušková, P. Anthelmintics in the future: current trends in the discovery and development of new drugs against gastrointestinal nematodes. *Drug Discov Today* **2020**, *25*, 430-437. https://doi.org/10.1016/j.drudis.2019.12.007.
5. Vargová, M.; Hurníková, Z.; Revajová, V.; Lauková, A.; Dvorožňáková, E. Probiotic Bacteria can Modulate Murine Macrophage's Superoxide Production in Trichinella Spiralis Infection. *Helminthologia* **2020**, *57*, 226-234. https://doi.org/10.2478/helm-2020-0024.
6. Bautista-Garfias, C.R.; Ixta, O.; Orduña, M.; Martínez, F.; Aguilar, B.; Cortés, A. Enhancement of resistance in mice treated with Lactobacillus casei: effect on Trichinella spiralis infection. *Vet Parasitol* **1999**, *80*, 251-260. https://doi.org/10.1016/s0304-4017(98)00210-6.
7. Martínez-Gómez, F.; Fuentes-Castro, B.E.; Bautista-Garfias, C.R. The intraperitoneal inoculation of Lactobacillus casei in mice induces total protection against Trichinella spiralis infection at low challenge doses. *Parasitol Res* **2011**, *109*, 1609-1617. https://doi.org/10.1007/s00436-011-2432-2.
8. El Temsahy, M.M.; Ibrahim, I.R.; Mossallam, S.F.; Mahrous, H.; Abdel Bary, A.; Abdel Salam, S.A. Evaluation of newly isolated probiotics in the protection against experimental intestinal trichinellosis. *Vet Parasitol* **2015**, *214*, 303-314. https://doi.org/10.1016/j.vetpar.2015.08.029.
9. Dvorožňáková, E.; Bucková, B.; Hurníková, Z.; Revajová, V.; Lauková, A. Effect of probiotic bacteria on phagocytosis and respiratory burst activity of blood polymorphonuclear leukocytes (PMNL) in mice infected with Trichinella spiralis. *Vet Parasitol* **2016**, *231*, 69-76. https://doi.org/10.1016/j.vetpar.2016.07.004.
10. Bucková, B.; Hurníková, Z.; Lauková, A.; Revajová, V.; Dvorožňáková, E. The Anti-parasitic Effect of Probiotic Bacteria via Limiting the Fecundity of Trichinella Spiralis Female Adults. *Helminthologia* **2018**, *55*, 102-111. https://doi.org/10.2478/helm-2018-0010.

11. Travers, M.A.; Florent, I.; Kohl, L.; Grellier, P. Probiotics for the control of parasites: an overview. *J Parasitol Res* **2011**, *2011*, 610769. https://doi.org/10.1155/2011/610769.
12. Harris, N.L. Intimate gut interactions: helminths and the microbiota. *Cell Res* **2016**, *26*, 861-862. https://doi.org/10.1038/cr.2016.72.
13. Gause, W.C.; Maizels, R.M. Macrobiota - helminths as active participants and partners of the microbiota in host intestinal homeostasis. *Curr Opin Microbiol* **2016**, *32*, 14-18. https://doi.org/10.1016/j.mib.2016.04.004.
14. Peachey, L.E.; Jenkins, T.P.; Cantacessi, C. This Gut Ain't Big Enough for Both of Us. Or Is It? Helminth-Microbiota Interactions in Veterinary Species. *Trends Parasitol* **2017**, *33*, 619-632. https://doi.org/10.1016/j.pt.2017.04.004.
15. Su, C.; Su, L.; Li, Y.; Long, S.R.; Chang, J.; Zhang, W.; Walker, W.A.; Xavier, R.J.; Cherayil, B.J.; Shi, H.N. Helminth-induced alterations of the gut microbiota exacerbate bacterial colitis. *Mucosal Immunol* **2018**, *11*, 144-157. https://doi.org/10.1038/mi.2017.20.
16. Walter, J. Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Appl Environ Microbiol* **2008**, *74*, 4985-4996. https://doi.org/10.1128/aem.00753-08.
17. Rheinallt, M.J.; Chirayu, D.; Trevor, M.D.; Liping, L.; Alexandra, A.W.; Christopher, D.S.; Courtney, S.A; April. R.R; Erin. S.K.; Andrew, S.N. Lactobacilli Modulate Epithelial Cytoprotection through the Nrf2 Pathway[J]. *Cell Reports* **2015**, 12, 1217-1225. https://doi.org/10.1016/j.celrep.2015.07.042.
18. Bolas-Fernández, F. Biological variation in Trichinella species and genotypes. *J Helminthol* **2003**, *77*, 111-118. https://doi.org/10.1079/joh2003170.
19. Gamble, H.R.; Bessonov, A.S.; Cuperlovic, K.; Gajadhar, A.A.; van Knapen, F.; Noeckler, K.; Schenone, H.; Zhu, X. International Commission on Trichinellosis: recommendations on methods for the control of Trichinella in domestic and wild animals intended for human consumption. *Vet Parasitol* **2000**, *93*, 393-408. https://doi.org/10.1016/s0304-4017(00)00354-x.
20. Park, I.; Lee, Y.; Goo, D.; Zimmerman, N.P.; Smith, A.H.; Rehberger, T.; Lillehoj, H.S. The effects of dietary Bacillus subtilis supplementation, as an alternative to antibiotics, on growth performance, intestinal immunity, and epithelial barrier integrity in broiler chickens infected with Eimeria maxima. *Poult Sci* **2020**, *99*, 725-733. https://doi.org/10.1016/j.psj.2019.12.002.
21. Xie, Z.; Zhang, G.; Liu, R.; Wang, Y.; Tsapieva, A.N.; Zhang, L.; Han, J. Heat-Killed Lacticaseibacillus paracasei Repairs Lipopolysaccharide-Induced Intestinal Epithelial Barrier Damage via MLCK/MLC Pathway Activation. *Nutrients* **2023**, *15*. https://doi.org/10.3390/nu15071758.
22. Pozio, E. Searching for Trichinella: not all pigs are created equal. *Trends Parasitol* **2014**, *30*, 4-11. https://doi.org/10.1016/j.pt.2013.11.001.
23. Song, Y.Y.; Zhang, Y.; Ren, H.N.; Sun, G.G.; Qi, X.; Yang, F.; Jiang, P.; Zhang, X.; Cui, J.; Wang, Z.Q. Characterization of a serine protease inhibitor from Trichinella spiralis and its participation in larval invasion of host's intestinal epithelial cells. *Parasit Vectors* **2018**, *11*, 499. https://doi.org/10.1186/s13071-018-3074-3.
24. Liu, M.Y.; Zhu, X.P.; Xu, K.C.; Lu, Q.; Boireau, P. Biological and genetic characteristics of two Trichinella isolates in China; comparison with European species. *Parasite* **2001**, *8*, S34-38. https://doi.org/10.1051/parasite/200108s2034.
25. Wakelin, D.; Goyal, P.K. Trichinella isolates: parasite variability and host responses. *Int J Parasitol* **1996**, *26*, 471-481. https://doi.org/10.1016/0020-7519(96)89377-5.
26. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S., et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* **2014**, *11*, 506-514. https://doi.org/10.1038/nrgastro.2014.66.
27. Saracino, M.P.; Vila, C.C.; Baldi, P.C.; González Maglio, D.H. Searching for the one(s): Using Probiotics as Anthelmintic Treatments. *Front Pharmacol* **2021**, *12*, 714198. https://doi.org/10.3389/fphar.2021.714198.
28. Tsai, Y.T.; Cheng, P.C.; Pan, T.M. The immunomodulatory effects of lactic acid bacteria for improving immune functions and benefits. *Appl Microbiol Biotechnol* **2012**, *96*, 853-862. https://doi.org/10.1007/s00253-012-4407-3.
29. Mido, S.; Fath, E.M.; Farid, A.S.; Nonaka, N.; Oku, Y.; Horii, Y. Trichinella spiralis : Infection changes serum paraoxonase -1 levels, lipid profile, and oxidative status in rats[J]. *Experimental Parasitology* **2012**, 131,190-194. https://doi.org/10.1016/j.exppara.2012.03.023.
30. Farid, A.S.; Mido, S.; Linh, B. K.; Hayashi, T.; Horii, Y. An atherogenic lipid profile with low serum paraoxonase-1 activity during nematode infection in rats[J]. *European Journal of Clinical Investigation* **2010**, 40(11):984-993. https://doi.org/10.1111/j.1365-2362.2010.02352.x.
31. Allen, J.E.; Maizels, R.M. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol* **2011**, *11*, 375-388. https://doi.org/10.1038/nri2992.
32. Bautista-Garfias, C.R.; Ixta-Rodríguez, O.; Martínez-Gómez, F.; López, M.G.; Aguilar-Figueroa, B.R. Effect of viable or dead Lactobacillus casei organisms administered orally to mice on resistance against Trichinella spiralis infection. *Parasite* **2001**, *8*, S226-228. https://doi.org/10.1051/parasite/200108s2226.

 doi:10.20944/preprints202406.0406.v1

33. Rudner, X.L.; Happel, K.I.; Young, E.A.; Shellito, J.E. Interleukin-23 (IL-23)-IL-17 cytokine axis in murine Pneumocystis carinii infection. *Infect Immun* **2007**, *75*, 3055-3061. https://doi.org/10.1128/iai.01329-06.
34. Choy, C.S.; Hu, C.M.; Chiu, W.T.; Lam, C.S.; Ting, Y.; Tsai, S.H.; Wang, T.C. Suppression of lipopolysaccharide-induced of inducible nitric oxide synthase and cyclooxygenase-2 by Sanguis Draconis, a dragon's blood resin, in RAW 264.7 cells. *J Ethnopharmacol* **2008**, *115*, 455-462. https://doi.org/10.1016/j.jep.2007.10.012.
35. Kanmani, P.; Kim, H. Protective Effects of Lactic Acid Bacteria Against TLR4 Induced Inflammatory Response in Hepatoma HepG2 Cells Through Modulation of Toll-Like Receptor Negative Regulators of Mitogen-Activated Protein Kinase and NF-κB Signaling. *Front Immunol* **2018**, *9*, 1537. https://doi.org/10.3389/fimmu.2018.01537.
36. Roselli, M.; Finamore, A.; Britti, M.S.; Mengheri, E. Probiotic bacteria Bifidobacterium animalis MB5 and Lactobacillus rhamnosus GG protect intestinal Caco-2 cells from the inflammation-associated response induced by enterotoxigenic Escherichia coli K88. *Br J Nutr* **2006**, *95*, 1177-1184. https://doi.org/10.1079/bjn20051681.
37. Wang, Y.; Kirpich, I.; Liu, Y.; Ma, Z.; Barve, S.; McClain, C.J.; Feng, W. Lactobacillus rhamnosus GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *Am J Pathol* **2011**, *179*, 2866-2875. https://doi.org/10.1016/j.ajpath.2011.08.039.
38. Fan, L.; Yunyi, Y.; Hui, H.; Hua, H.; Mingzhong, Y. Xanthohumol attenuates cisplatin-induced nephrotoxicity through inhibiting NF-κB and activating Nrf2 signaling pathways[J]. *International Immunopharmacology* **2018**, 61:277-282. https://doi.org/10.1016/j.intimp.2018.05.017.