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Improvement of Male Mice Fecundity by Using Two New Lactobacillus Strains

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Abstract

Fertility is the most important factor to increase productivity either at the level of human or animal. Probiotics are organisms that have aspect roles in facing diseases and improving the health of the host. So, the goal of this study was to assess two new strains of *Lactobacillus plantarum* pro1 and *Lactobacillus rhamnosus* pro2 as enhancers to male fertility. A total of forty male BALB/c mice were separated into four groups: the control group was fed a standard diet, pro1, pro2, and mixed group were given drinking water containing *L. plantarum*, *L. rhamnosus*, and two of the above strains, respectively. Sexual hormones, and testicular histopathology, and sperm parameters were analyzed. The expression of the Sycp3, Vegfa, and Wtl genes was assessed using RT-PCR. The results indicated a significant increase in FSH and LH hormone levels in the pro1 and mix groups compared with the control group. The structure of seminiferous tubules was normal in all groups but with a number low in the Pro2 group compared to the other groups. The mixture of two strains recorded the highest count and motility of sperm, and the lowest sperm head and tail abnormality. The results of RT-PCR showed that Sycp3, Vegfa genes were high significant ($P \leq 0.01$) upregulated in the mix group, and significant upregulated ($P \leq 0.05$) in the pro1 group compared with the control, respectively. When compared to the control, the expression of Wtl gene was substantially elevated ($P \leq 0.01$) in the Pro1 and mixed group, and insignificantly reduced in the pro2 groups. When compared to the control, the expression of Sycp3 and Vegfa genes was insignificantly elevated in the pro2 group. As a result, this study found that a combination of two strains or Pro1 alone is effective in enhancing male fertility.

Keywords: Male fecundity, probiotics and gene expression

Introduction

Infertility is defined as a lack of pregnancy during one year of unprotected sexual encounters, according to current criteria [1]. This is one of the health issues that affect patients on an individual, societal, and financial level [2]. Male factors are responsible for 50% of infertility issues. Male infertility is mostly caused by a deficiency in spermatogenesis, which includes sperm dysfunctions, sperm count reductions, sperm maturation, and sperm motility [3]. In many situations, the specific mechanism of the impairment in sperm function is unknown, which is why they are referred to as idiopathic reasons. Oligozoospermia, asthenozoospermia, teratozoospermia, and their mixtures are all kinds of sperm abnormalities described by the World Health Organization (WHO). The concentration of sperm, the ratio of normal sperm morphology, and the percentage of sperm

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motility are all lower than WHO recommendations in asthenozoospermia [4].

Probiotics are intestinal-based nutritional bacteria that modulate regional immunity in the gastrointestinal tract and hence have a broad impact on metabolic pathways. They can stimulate metabolic pathways, restore cell hemostasis, and improve general health [5]. The removal of infections, as well as the synthesis of inhibitors such as antibacterial and organic acids, has all been hypothesized as explanations for how probiotics operate [6]. Several studies also demonstrated probiotics' powerful antioxidant benefits. Probiotics have been demonstrated to have an antioxidant impact in mice, reducing the amounts of nitric oxide and malondialdehyde while also regulating the activity of free radicals and increasing the activity of antioxidant enzymes [7]. The most often utilized probiotics, which have significant antioxidant effects, are lactic acid bacteria (LAB) and bifidobacteria. Through neutralizing free radicals, these metabolic antioxidant properties may be attributed to ROS hunting, enzyme inhibition or reduction, and prevention of ascorbate autoxidation in the gut [8].

In addition, microbial alteration by probiotics was already demonstrated to relieve gastrointestinal symptoms and inflammation in rheumatoid arthritis, ulcerative colitis, and multiple sclerosis patients in many randomized controlled trials [9]. Furthermore, the intake of probiotics simulates crucial elements of microbial symbiosis, improving mammalian reproductive performance [10]. Various benefits of probiotic supplements have long been reported, however, research on the influence of probiotic supplements on male reproductive potential, particularly the influence on gene expression relevant to fertility, is still insufficient.

Experimental

A total of 40 male mice BALB/c divided into four groups as 10 mice per cage. Mice weighing 23-27g at 15 days of age were kept at 22 ± 2 °C with 50% ± 5% humidity and a 12-hour light/dark cycle. For 60 days, the control group was fed a normal diet and only drank water, the pro1 group was fed a normal diet and drinking water containing 2.5 10^6/ml *L. plantarum*, the pro2 group was fed a normal diet and drinking water containing 2.5 10^6/ml *L. rhamnosus*, and the mix group was fed a normal diet and drinking water containing 2.5 10^6/ml mixture of pro1 and pro2 strains. The testicular tissues of mice were collected at the end of the assessment and preserved in 10% formalin for histological evaluation or stored at -80ºC for qRT-PCR.

Table 1: Sequence of primers

Measurement of sexual hormones

Blood samples were collected from all mice of four groups, and then centrifuged at 5000 rpm for 10 min to collect serum. Free testosterone, luteinizing hormone (LH) and Follicle- stimulating hormone (FSH) levels were determined using the competitive immunoassay technique [11].

Testicular histopathology

Testicular tissue samples were taken from all mice of two groups. The samples were fixed in 10% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E). Histopathological studies are undertaken through light microscopy and photomicrographs were made [12].

Sperm parameter

At the end of the experiment and after killing mice by neck vertebra luxation, the epididymides were removed from each mouse, and then sperm was collected in 1ml of phosphate-buffered saline at 37°C through cutting cauda of epididymides in a Petri dish. Using a hemocytometer and a spot of sperm spread on a cleaned slide, Motility and spermatozoa number were calculated. These slides were dried and coded before being stained with 0.05 percent aqueous Eosin Y. For each mouse, about 1000 sperm were evaluated for morphological abnormalities in the sperm head and tail [13].

Gene expression

TRIZOl reagent (thermos) was used to suspend and homogenize the testicular tissues to extract total RNA for Rq-PCR. The cDNA synthesis was performed using the COSMO cDNA synthesis kit, Willowfort (WF10205002). Real-time PCR was done using EvaGreen plus (ROX) (Solis BioDyne) and the specific primers (Macrogen Co. Ltd., Korea) (Table 1). Gene expression of Sycp3, Vegfa, and Wtl genes was normalized to mice β-Actin and analyzed using Agilent Technologies Stratagene Mx3000P Real-Time PCR System USA.

Gene	Accession no.	Nucleotide sequence 5′–3′	Size(bp)
Sycp3	NM_011517.2	F-GACAGCGACAGCTCACCGG R-GGTGGCTTCCCAGATTTCCCAGA	90
Vegfa	NM_001025257.3	F-TGCTCTCTTGGGTGCACTGGAC R-GACGGCAGTAGCTTCGCTGGT	147
Wtl	NM_144783.2	F-GGCGCTTTGAGGGGTCCGAC R-AAAGTGGGCGGAGCACCGAC	205
βactin	NM_007393.5	F 5'-GGCACCACACCTTCTACAATG-3' R 5'-GGGGTGTTGAAGGTCTCAAAC-3'	133

Results

Sexual hormones

As shown at the table (2), level of sexual hormones at normal values in all groups but it was noted a high significant increase in the level of FT hormone in pro1 and mix group compared with control. Significant increase at the level of LH in pro1 and mix group compared with control, and non-significant differences in the level of FSH hormone between all groups compared with control.

Table 2: Levels of the sexual hormones in mice treated with probiotic

	Control group	Pro2 group	Pro1 group	Mix group
FT	30^{c}±0.05	32^{c}±0.06	45^{b}±0.08	77^{a}±0.09
FSH	1±0.01	1±0.01	0.9±0.02	1.1±0.03
LH	1^{b}±0.02	0.9^{b}±0.02	1.4^{a}±0.03	1.5^{a}±0.04

Histopathological results

In microscopic examination of section of control mice showed normal histological architecture of testicular tissue which composed of seminiferous tubules (Fig 1a) lined with spermatogenic cells, spermatocyte, spermatids and Sertoli cells. Interstitial tissue with leydig cells (Fig.1b).

No histological changes observed in testes mice treated with probiotic (P1) which revealed some improvement in testicular tissue represented in high density of spermatognic cells, as well as, spermatid, while signs of degeneration in interstitial tissue could be noted (Fig1c).

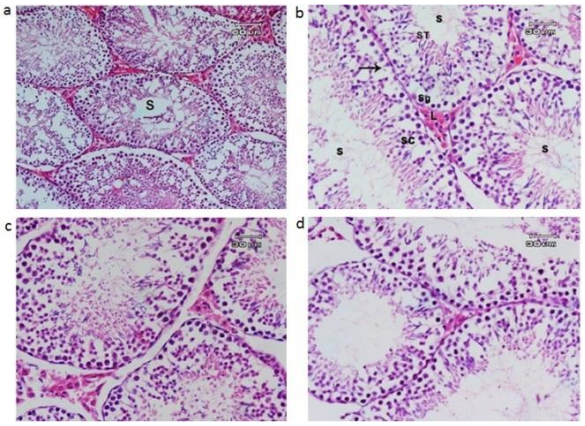

Fig 1: (a) Section of testis of control group showing normal structure of testicular tissue ,seminiferous tubules (S)and interstitial tissue (Hx&E x200), (b): High power of section of testis of control group showing different layers of spermatogenic cells, spermatocyte (sc), spermatids (st) attached to Sertoli cells (arrow). and Leydig cells (L)in interstitial tissue, (c): Section of testis of Pro1 group showing some improvement in testicular tissue represented in high density of spermatognic cells, as well as, spermatid, while signs of degeneration in interstitial tissue, (d): Section of testis of Pro2 group showing normal architecture of testicular tissue, slight disturbance of arrangement of spermatogenic cells in some tubule, low density of spermatozoa, as well as, slight vacuolation in the interstitial tissue could be observed (Hx&E x400).

The treatment testis with probiotic 2 (P2) showed normal architecture of testicular tissue, slight disturbance of arrangement of spermatogenic cells in some tubule, low density of spermatozoa, as well as, slight vacuolation in the interstitial tissue could be observed (Fig.1d). The testis of the Mix group showed more improvement represented in arrangement of spermatognic cells, density of spermatid, spermatozo and dividing spermatocytes are more prominent than other treatment (Fig 2).

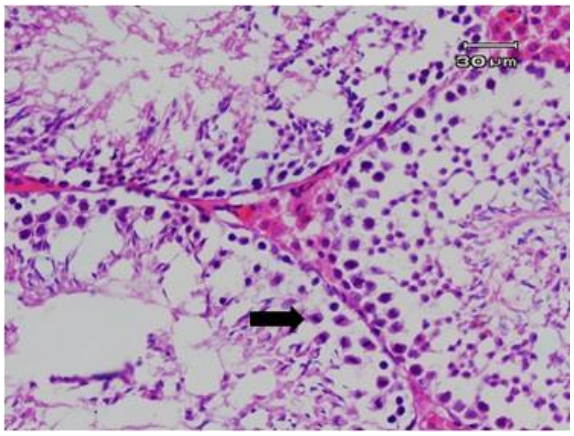

Fig (2): Section of testis of Mixed group showing more improvement represented in arrangement of spermatognic cells, density of spematid and spermatozo and dividing spermatocytes(arrow) are more prominent than other treatment (Hx&E x400).

Sperm parameters

Sperm count in all groups was at normal levels as shown in Table (3) but it was a significant increase in all groups supplemented by probiotics compared with control. The Mix group recorded the lowest value in all observed abnormalities compared with the other groups. Significant reduction in the abnormal shape of sperm; tail (coiled) and head (amorphous, without hock, small and triangle) in Pro1 and Mix groups compared with control. Amorphous head and coiled tail abnormalities increase in Pro2 group compared with the control group. Also, mean percentage of sperm abnormalities significant increase in Pro2 group while significant reduce in the Pro1 and Mix groups compared with the control group (Table 3 and Figure3).

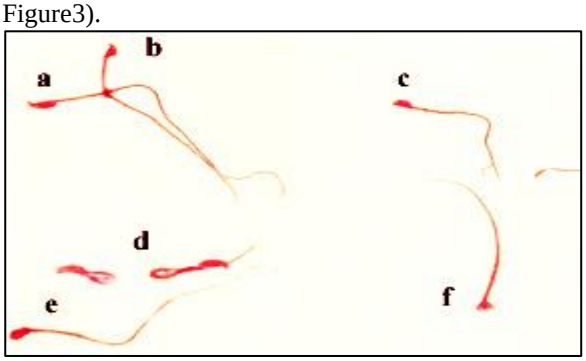

Fig. 3 show sperm morphology, a) normal sperm, b) small head, c) without hock, d) coiled tail, e) amorphous head, and f) triangle head

Gene expression

SYCP3, VEGFA and WT1 genes were significant upregulated in the pro1 and mix group compared with the control group. The highest upregulation of SYCP3, VEGFA and WT1 genes was recorded by the mix group. There are non-differences between Pro 2 and control group in expression of SYCP3, VEGFA and WT1 genes. Wt1 gene was insignificant downregulated in Pro2 compared with control group (Figure 4).

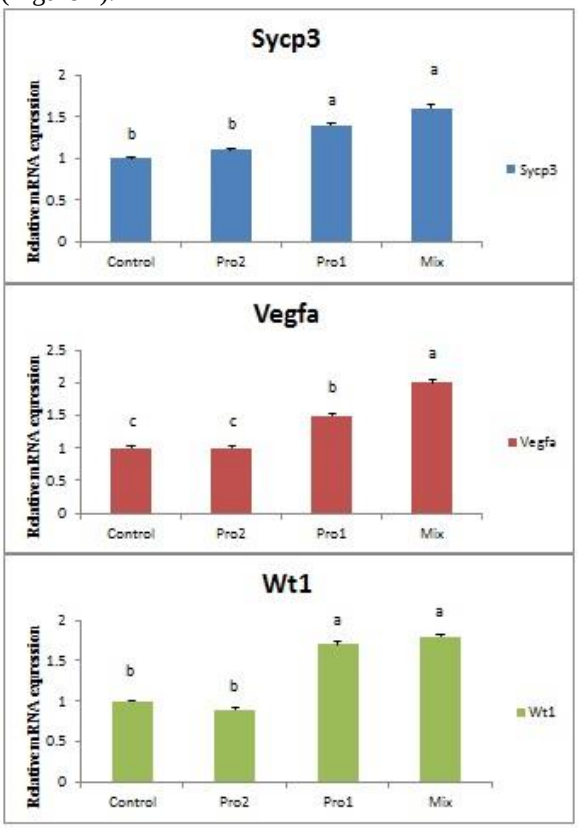

Fig. 4 Expression of Sycp3, Vegfa, and Wt1 genes under different probiotic treatments

Discussion

Low production in animals and the poor psychological state of humans is mainly due to infertility. So, increasing fertility will solve many problems in the world. The intestinal bacteria govern a variety of physiological activities and are crucial to the health of the host. The gut microbiome was recently discovered to play a role in influencing BTB permeability and controlling testicular endocrine activities [14]. By competitive suppression of epithelial and mucosal adhesion, probiotics maintain gut microbiota balance, modulate the immune response, and limit pathogenic organism colonization [15]. *Lactobacillus* is the most common organism found in a healthy human vaginal environment, accounting for over 70% of the total number of organisms extracted [16]. Lactobacilli are beneficial bacteria that live in the gastrointestinal, urinary, and vaginal tracts [17]. They generate compounds like H_2O_2, lactic acid, and bacteriocin to protect the human vagina [16]. In our previous study, we discovered that *L. plantarum* Pro 1 protects against diabetes-related complications [18].

Table 3: Sperm parameters in mice treated with probiotic.

	Sperm count ±S.E.	Number of abnormalities	Mean % ±S.E.	Amorphous head	Without hock head	Small head	Triangle head	Coiled tail
Control	35^{a} $X10^{6}$±0.5	97	1.94^{a}±0.6	25	11	22	11	28
Pro2	37^{a} $X10^{6}$±0.7	108	2.16^{a}±0.5	31	10	20	10	37
Pro1	45^{b} $X10^{6}$±0.8	43	0.86^{b}±0.7	12	6	10	5	10
Mix	46^{b} $X10^{6}$±0.9	34	0.68^{c}±0.8	10	5	8	3	8

Total number of screened sperm is 5000 for 5 mice per group

In the current study, we evaluated this strain and it's mixed with *L. rhamnosus* Pro1 as an enhancer for fertility in male mice. Rats were given probiotics exhibited similar testis weight as in the control group [19].

In mice, treatment with *L. plantarum* TW1-1 reversed testis weight reduction [20]. The testis weight of mice treated with *L. plantarum* pro1 and *L. rhamnosus* pro2 was not statistically different from the control group in the current research. Histopathological examination of testicular tissue of mice given a combination of *L. plantarum* pro1 and *L. rhamnosus* pro2 showed that seminiferous tubules had normal form accompanied with the normal distribution of spermatogenic cells and interstitial cells. Testicular sections from rats administered a probiotic containing arsenic (PRO) exhibited a normal appearance of the seminiferous tubules, as well as a normal distribution of spermatogenic cells and interstitial cells, similar to that of the untreated control testicles [19]. Even though some germ cells already were exfoliated into the lumen after *L. plantarum* TW1-1 injection to DEHP-exposed rats, the uneven arrangement of germ cells was avoided, indicating that *L. plantarum* TW1-1 partially reduced DEHP-induced testicular injury [20]. Furthermore, spermatogenic cells in PRE animals' testes lost their usual architecture as a result of vacuolation and increased Leydig cell size. The structure of spermatogenic cells in SYN rats' tests revealed no significant modifications [19]. In DEHP-exposed animals, oral administration of *L. plantarum* TW1-1 dramatically raised serum testosterone levels, improved semen quality, and reduced gonad development abnormalities [20]. *Lactobacillus reuteri* administration raises testosterone levels in aged mice [21], and *Lactobacillus rhamnosus* improves fish backbone calcification and reproductive [22,23]. The current findings demonstrated that blood levels of free testosterone (FT), luteinizing hormone (LH), and Follicle stimulating hormone (FSH) increased considerably in both groups when *L. plantarum* pro1 and *L. rhamnosus* were administered alone or in combination. When comparing testes to other tissues, semi-quantitative PCR and qPCR demonstrated considerable overexpression of the SYCP3 gene at 6 and 30 months [24]. The increased incidence of SYCP3 in the human testis was investigated by Aarabi et al. [25], who claimed that the absence of SYCP3 expression in testis had a negative impact on fertility and spermatogenesis. Furthermore, the elevated mRNA expression level of SYCP3 corresponds to Syrjänen et al. [26,27]. Wang et al. [28] found a considerably greater expression level of bovine SYCP3 mRNA in cow testes, which is consistent with our findings. This suggests that bovine SYCP3 plays a key role in yak spermatogenesis meiosis. Furthermore, SYCP3 defective male mice failed to generate synaptonemal complexes, axial/lateral elements, and chromosomes in mutant spermatocytes could not synapse, according to Yuan et al. [29]. Sertoli cells and germ cells release VEGFA isoforms that are required for the preservation of undifferentiated spermatogonia, sperm counts, and normal male fertility [30]. Aarabi et al. [25] looked at the greater prevalence of SYCP3 in human testis and concluded that the lack of SYCP3 expression in the testis was deleterious. Wt1, a Wilms tumor gene, is expressed only in Sertoli cells (SCs), which aid in spermatogenesis. Wt1 knockout led to widespread germ cell death, with only Sertoli cells present in the majority of seminiferous tubules, evoking non-obstructive azoospermia patients in humans. Blood-testicular barrier disease (BTB) is caused by Wt1-deficient testicles [31]. The genes Sycp3, Vegfa, and Wt1 were significantly elevated in the LP and mix groups when compared to the control group, while there were no significant changes in the LR group when compared to the control group. The increased expression of these genes was associated with increases in the quality of sperm. The combination of *L. plantarum* pro1 and *L. rhamnosus* pro2 increases sperm motility and improves sperm morphology, and decreases sperm abnormalities. Valcarce et al. [32] used a combination of *Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347 to increase sperm motility, concentration, and shape, as well as reduce DNA fragmentation and ROS levels in asthenozoospermic males. In both animal models and humans, previous research had well confirmed the

causal link between endotoxemia/epididymal inflammation and poor sperm motility/spermatogenic dysfunction [33,34]. According to Bejarano et al [35], men who were given melatonin had greater seminal antioxidant activities and improved sperm quality. Another study looked at how taking an antioxidant supplement affected sperm characteristics [36]. In the female factor, some studies have indicated positive benefits of probiotic strains in reproductive biology, concentrating solely on their qualities as a bacterial vaginosis treatment [37,38]. The probiotic behavior and activities of *L. plantarum* KU15149 have been proven, involving antioxidant and anti-inflammatory properties [39].

Conclusion

This research looked at the role of probiotics in boosting male fertility. Sexual hormones such as FT, LH, and FSH, as well as sperm motility, sperm count, and sperm quality, were all increased when *L. plantarum* pro1 and *L. rhamnosus* pro2 or *L. plantarum* were combined. These improvements were followed by normalized seminiferous tubule shape and increased expression of the Sycp3, Vegfa, and Wtl genes.

Conflicts of interest

There are no conflicts to declare.

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