

Enterococcus faecium; a Suitable Probiotic Candidate for Modulation of Immune Responses Against Pathogens

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Abstract

Probiotics are sets of nonpathogenic microorganisms without virulence which inhibit pathogen growth in animals. *Enterococcus faecium* has been introduced as a probiotic and its probiotic characteristics have been evaluated in several investigations. Evidence suggests that probiotics may modulate the immune system of the host to improve responses against pathogens. Thus, this review aimed to present recent studies regarding the effects of the *E. faecium* probiotic strains on the host immune responses. It seems that *E. faecium* AL41, CGMCC, NCIMB, SF68, Strain 26, JWS 833 and EF55 strains improve beneficially the immune responses of the host. However, most studies have been performed on animal models, therefore, clinical trials on humans are required to understand the beneficial mechanisms of *E. faecium* on the human immune system.

Keywords: Probiotics, *Enterococcus faecium*, Immune responses



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Introduction

Probiotics are nonpathogenic microorganisms which have several benefits on human health or physiology.¹ Probiotic is a Greek word 'pro bios' which means 'for life' as opposed to antibiotics which means 'against life.' Accordingly, it has been proposed that there are beneficial effects of probiotics on human health by a Russian researcher Ellie Metchnikoff. Probiotics have several positive and negative effects on the gut microflora and the microbial toxic activities, respectively.^{2,3} Both yeast and bacteria, especially lactic acid bacteria, are the main members of probiotics.¹ Probiotics modulate endogenous flora, pathogenic micro-organisms and also the immune responses of hosts in either a direct or indirect manner.⁴ Accordingly, it has been hypothesized that probiotics can be considered as potential agents to prevent or help to cure human infectious diseases, especially intestinal disturbances.⁵

Enterococcus faecium is a strain of gram positive bacteria Firmicutes phylum, ba-

cilli class, Lactobacillales order, Enterococcaceae family, *Enterococcus* genus, the *E. faecium* species, which exhibits some probiotic properties.⁶ Therefore, it has been proposed as a probiotic and several studies have evaluated the bacteria for treatment of several animal and human models.⁷⁻⁹ The following sections describe the bacteria in details. It has been hypothesized that probiotics, including *E. faecium*, fight the pathogenic microbes via several mechanisms.⁷ It appears that modulation of the immune responses against the pathogens is a plausible mechanism to fight against pathogens.¹⁰ Several probiotic strains including *E. faecium* SF68, AL41, EF55, JWS 833, Strain 26, NCIMB 10415 and CGMCC 2516 have been used to investigate the immunological modulation with focus on safety, anti-pathogenic and immunomodulatory properties which are listed in Table 1. Although, there are several research and review articles related to *E. faecium* and its benefits for human, the interaction of the bacterium with the immune system has

Table 1. The Major Effects of Enterococcus faecium Probiotic Strains on the Immune System of Hosts

Host	Effects on the Immune System	Strains	Ref.
Positive Results			
Mice	Increased phagocytic and digestive functions of macrophages	AL41	8
Chicken	Modulates the liver inflammatory molecules	CGMCC 2516	33
Ostrich	Mucosal immunity	AL41	12
Porcine	Increased intraepithelial lymphocyte numbers	NCIMB 10415	23
Holstein cows	Increased neutrophil infiltration and milk levels of MMP9	SF68	13
Chicken	Increased serum levels of IgM	-	25
Pig	Increased humoral immune responses against the SIV vaccination and increased number of activated T helper and cytotoxic T cell	NCIMB 10415	26
Pig	Regulation of the inflammatory molecules in the healthy animals. Increased expression of IL-8 in response to pathogens	NCIMB 10415	25
Holstein calves	Increased numbers of T helper and cytotoxic as well as $\gamma\delta$ T cells and their innate receptors such as TLR2	Strain 26	27
Chicken	Up-regulated of the mucosal IL-4, TNF- α and IgA	-	28
Fish	Increased myeloperoxidase and lysozyme activities	-	16
Rabbit	Stimulation of the mucosal immunity	AL41	9
Mice	Increased production of NO, IL-1 β and TNF- α	JWS 833	18
Chicken	Increased number of the peripheral blood lymphocytes and CD3, CD4, CD8, and IgM positive cells	EF55	29
Hen	Improvement of the intestine integrity	AL41	19
Chicken	Increased humoral immunity to vaccination with the Newcastle disease virus and infectious bursal disease	-	30
Fish	Increased expression levels of IL-1 α , TNF- α and TGF- β	-	17
Sow and piglet	Enhanced immune responses against Escherichia coli in CD8+ cytotoxic T cells independent manner	SF68	31
Dog	Increased production of specific IgA and IgG	SF68	32
Human	Increased neutrophil phagocytosis and humoral immunity	-	20
Dog	Increased expression of pro-inflammatory cytokines (IL-17A, IL-22, IFN- γ and TNF- α) in the duodenal biopsies and whole blood	-	21
Pig	Increased production of NO in response to swine influenza virus	NCIMB 10415	22
Negative Results			
Piglet	Reduction in the cell density counts of α/β CD8+ intraepithelial and percentage of α/β CD8+ circulating T cells	-	37
Dog	No differences in the IgA production	SF68	38
Dog	No alteration in the immune responses during the chronic enteropathy	NCIMB 10415	39

not been discussed previously. Additionally, *E. faecium* is now recognized as a probiotic, and hence, this review article collates recent information regarding the roles of *E. faecium* in modulation of the immune responses.

Enterococcus faecium and its Effects on the Immune Responses

Enterococcus faecium has been considered as a probiotic and several investigations have evaluated its effects on the immune responses in the human and animal models.¹¹ The probiotic may modulate innate and adaptive immunity to either increase responses to infectious disease to clear them or modulate immune responses in inflammatory conditions to decrease their complications. Accordingly, investigations on the immunomodulatory effects of *E. faecium* have been categorized into three sections including (1) The effects of *E. faecium* on the innate immunity, (2) The effects of *E. faecium* on the adaptive immunity, and (3) the effects of *E. faecium* on the modulation of the immune responses during pro-inflammatory diseases.

The Effects of Enterococcus faecium on the Innate Immunity

Based on the probiotic effects of *E. faecium*, it seems that the bacterium not only considered as a foreign particle for immune system, but also can modulate innate immune system. Accordingly, Dvoroznakova et al have reported the effects of *E. faecium* on the innate immunity (monocytes, polymorphonuclear leukocytes, phagocytosis and oxidative burst) of *Trichinella spiralis* infected mice⁸ and have demonstrated that the administration of *E. faecium* to mice prior to *T. spiralis* infection led to increased innate immunity against the parasite and elevated prolonged phagocytic activities of the host leukocytes. The administration of *E. faecium* also increases the ingestion capacity of the innate immune cells, including macrophages.⁸ The *E. faecium* also plays key roles in the induction of an appropriate mucosal immunity against the pathogenic bacteria such as *Salmonella typhimurium*.¹² Lauková et al also reported the positive effects of the *E. faecium* probiotic on the mucosal immunity of rabbits.⁹ Based on these results, Tiantong et al also showed the beneficial effects of

the *E. faecium* on the mucosal immunity and reported that the probiotic elevated the matrix metalloproteinase 9 (MMP9) and also improved the neutrophil infiltration to the infected glands of Holstein cows.¹³ In addition, Siepert et al showed that pre-treatment of the animals by the *E. faecium* NCIMB 10415 before *S. typhimurium* challenge led to significantly increased expression levels of IL-8 in the mesenteric lymph nodes.¹⁴ IL-8 is a pro-inflammatory cytokine which recruits neutrophils to the infected sites.¹⁵ Therefore, it may be concluded that *E. faecium* fights against pathogens via the induction of the immune responses at the site of infection.

The *E. faecium* also affects the immune responses in a strain of fishes (*Paralichthys olivaceus*). Accordingly, Lee et al revealed that the citrus by-products (CB) of fermentation with *E. faecium* enhanced the innate immunity of fish. Their results demonstrated that probiotic treatment led to increased myeloperoxidase and lysozyme activities in a dose-dependent manner.¹⁶ Another study on fish using rainbow trout, *Oncorhynchus mykiss*, as a model revealed that *E. faecium* enhanced leukocytes superoxide anion production and serum complement activities through the alternative pathway.¹⁷ The study also showed that expression levels of interleukin 1 alpha (IL-1 α), Tumor necrosis factor-alpha (TNF- α) and transforming growth factor beta (TGF- β) were increased significantly in the spleen and the kidney.¹⁷ Choi et al used *E. faecium* JWS 833 isolated from the duck intestine and reported that the heat-killed JWS833 enhanced production of nitric oxide (NO), IL-1 β and TNF- α by mouse peritoneal macrophages in response to *Listeria monocytogenes*.¹⁸ Using the *E. faecium* AL41 in hens also resulted in improvement of the intestine integrity, which is a part of the innate immunity against microbes.¹⁹ The probiotic also enhanced phagocytosis by neutrophils and production of antibodies in humans.²⁰ Schmitz et al studied the dog's duodenal biopsies and whole blood and demonstrated that *E. faecium* stimulated the expression of pro-inflammatory cytokines including IL-17A, IL-22, Interferon gamma (IFN- γ) and TNF- α in the duodenal biopsies and serum and subsequently enhanced the immune responses against pathogens.²¹ The probiotic form of *E. faecium* (NCIMB 10415) also increased the production of NO by the phagocyte cells of pigs.²² Based on the results, it appears that *E. faecium* modulates the innate immunity to fight against pathogens.

The Effects of *Enterococcus faecium* on the Adaptive Immunity

Enterococcus faecium also modulates the adaptive immunity. Rieger et al demonstrated that *E. faecium* treatment led to the improvement of the mucosal immunity against *S. typhimurium* by increasing the number of mucosal intraepithelial lymphocytes.²³ Intraepithelial lymphocytes are the first line of cellular immunity against microbes and play key roles in the mucosal immunity.²⁴

Ahmed et al demonstrated that the administration of *Citrus junos* probiotics, a by-product of fermentation with

E. faecium, to chicks showed that the product led to increased serum levels of immunoglobulin (Ig)M.²⁵ Wang et al also demonstrated the immunomodulatory effects of the *E. faecium* reporting that the *E. faecium* NCIMB 10415 in combination with zinc oxide stimulated the humoral immune responses against trivalent influenza vaccination and also led to an increased numbers of activated T helper and cytotoxic T cells in pigs.²⁶ Another study on Holstein calves using the *E. faecium* NCIMB 10415 showed that probiotic treatment led to increased numbers of the peripheral blood helper, cytotoxic and $\gamma\delta$ T cells.²⁷ Their study also demonstrated that the numbers of CD14(+), CD21(+) and TLR2(+) cells as well as the expression of IL-6, and IFN- γ were increased significantly in the probiotic treated animals on day 7.²⁸ Treatment of chickens with *E. faecium* prior to challenge with the *Escherichia coli* K88 also led to up-regulation of IL-4, TNF- α and IgA in the mucosal immunity which eventually assisted in the eradication of the *E. coli*.²⁹

Another study on chicks identified that the administration of *E. faecium* EF55 was associated with the elevated numbers of the peripheral blood lymphocytes and CD3, CD4, CD8, and IgM positive cells in response to *Salmonella enterica* serotype enteritidis phage type 4 (SE140 PT4).²⁹ Talebi et al reported that the administration of *E. faecium* to chicken improved the humoral immunity responses to vaccination with the Newcastle disease virus and infectious bursal disease.³⁰ Interestingly, the administration of *E. faecium* led to decreased numbers of intestinal CD8+ cytotoxic T cells and their results revealed that this was associated with suppression of the *E. coli* infection.³¹ It suggested that the probiotic enhances the immune responses against *E. coli* in a CD8+ cytotoxic T cells independent manner. *E. faecium* SF68 also increased fecal IgA and specific circulating IgG and IgA against the canine distemper virus (CDV) vaccine.³²

In sum, it may be hypothesized that *E. faecium* modulate the mucosal adaptive immunity to fight against pathogens.

The Effects of *Enterococcus faecium* on the Modulation of the Immune Responses During Pro-inflammatory Diseases

Although, *E. faecium* plays key roles in induction of appropriate immune responses against pathogens, there are some evidences which confirm the roles played by *E. faecium* to modulate immune responses during pro-inflammatory based diseases. Another study investigated the roles of *E. faecium* on the liver functions of broiler chickens which revealed that the probiotic decreased the inflammatory responses, damage associated with molecular patterns (PAMPs), and induced homeostasis.³³ Siepert et al also used the *E. faecium* NCIMB 10415 strain to treat the pigs prior to the infection with *S. typhimurium*.¹⁴ They reported that treatment of healthy pigs with the *E. faecium* NCIMB 10415 led to reduced expression of IL-8, IL-10 and CD86/B7-2 (a co-stimulatory molecule present on activated antigen presenting cells, and interaction of CTLA-4 [cytotoxic T-lymphocyte antigen-4 or

CD152] with CD86 inhibits human T-cell activation) (a co-stimulatory molecule) in the ileal Peyer’s patches and elevated serum levels of TGF-β.²³ TGF-β is an anti-proliferative cytokine with angiogenesis properties and its strong chemotactic capacity.^{34,35} It is noteworthy to know that this cytokine is essential for the maintenance of mucosal integrity.³⁶ Thus, it seems that *E. faecium* could play crucial roles in mucosal health programs. Thus, it seems that *E. faecium* may be considered as a therapeutic target for further investigations, especially on human subjects. Figure 1 summarizes the immunoregulatory effects of *E. faecium*.

In contrast with the aforementioned investigations, Mafamane et al showed that the administration of *E. faecium* was associated with a reduction in the cell density counts of CD8+α/β intraepithelial and percentage of circulating T cells, and did not influence the control or proliferation of *Salmonella* growth in the piglets.³⁷

In addition, short-term treatment of dogs suffering from giardiasis did not have any correlation with the associated response of IgA production against the worms.³⁸ Furthermore, Schmitz et al,³⁹ showed that *E. faecium* was unable to alter the immune responses during the chronic enteropathy in dogs.

Conclusion

The strong point of this review is that some strains of *E. faecium* can be considered as probiotics and can be used for the treatment against pathogens in the humans. Some *E. faecium* strains can be used in vivo in a safe manner and either induce the innate and adaptive immune

responses or inhibit the inflammatory related diseases. Thus, they may be useful for the immunomodulation of the human immune related diseases such as hypersensitivities, autoimmunity, transplantation and so on. However, some limited investigations revealed that the use of *E. faecium* was not associated with the improved immune responses and suggested negative results regarding the roles played by *E. faecium* against *Salmonella* growth in the piglets. Thus, it may be hypothesized that using short-term treatments, low doses of *E. faecium* or the use of the probiotic in chronic based disorders such as enteropathy, which is caused by a low grade chronic inflammation, are the main reasons for the negative results of *E. faecium* administration.

In sum, it seems that *Enterococcus faecium* AL41, CG-MCC, NCIMB, SF68, Strain 26, JWS 833 and EF55 strains may use common mechanisms of action that are seen in other probiotics.

Ethical Approval

Not applicable.

Competing Interests

There is no conflict of interest to declare.

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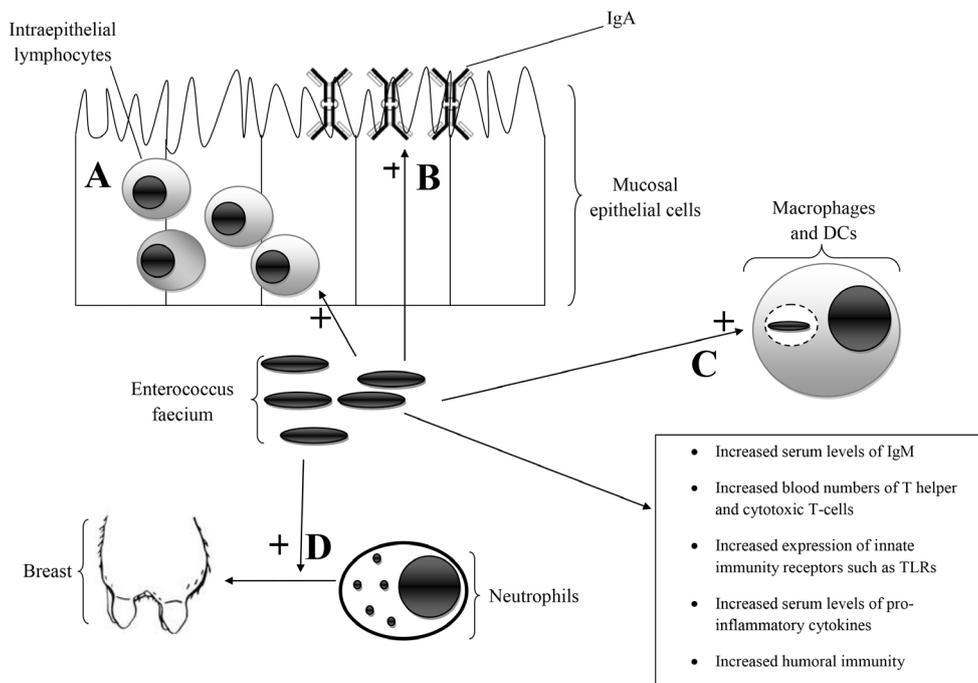


Figure 1. Immunoregulatory Effects of *Enterococcus faecium*. *E. faecium* enhances mucosal immunity by increasing numbers of intraepithelial T lymphocytes (A) and production of IgA (B). It also stimulates macrophages and dendritic cells (DCs) to produce NO and digest microbes (C). The probiotic also increases infiltration of neutrophils to the breast and milk as well as milk levels of pro-inflammatory cytokines (D).

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