

# Potential immunomodulator

*by* Andriana Tjitria

---

**Submission date:** 11-Jan-2019 10:17PM (UTC+0800)

**Submission ID:** 1063150982

**File name:** 39545-Article\_Text-141534-1-10-20190103.pdf (703.49K)

**Word count:** 2287

**Character count:** 12920

**Original article:**

**Potential Immuno-modulatory activity of Probiotics containing *Lactobacillus acidophilus* and *Lactobacillus casei* to increase the ratio of IFN  $\gamma$ /IL-4 in patients with Allergic Rhinitis**

Andriana Tjitria Widi Wardani<sup>1</sup>, Wiratno<sup>2</sup>, Dina Fatmawati<sup>3</sup>

**Abstract:**

**Aims:** This study aimed to determine the effect of probiotic combination on ratio of IFN- $\gamma$ : IL-4 levels in patients with allergic rhinitis. **Materials and methods:** Single blind, randomized controlled trial. This study included 40 patients with severe allergic rhinitis according to WHO criteria. This study used 2 treatment groups with probiotics and placebo control group. IFN  $\gamma$  and IL-4 levels were evaluated using ELISA method. Data were analyzed using paired T test with 95% confidence intervals. **Results and Discussion:** In the treatment group, the levels of IFN- $\gamma$  increased significantly ( $p < 0.05$ ). In contrast, IL-4 levels decreased significantly ( $p < 0.05$ ). There was a significant difference between the IFN- $\gamma$  ratio: IL-4 treatment and control group ( $p < 0.05$ ). **Conclusion:** The administration of combination of probiotics can improve the levels of IFN- $\gamma$ /IL-4 in patients with moderate to severe allergic rhinitis.

**Keywords:** allergic rhinitis; Interferon gamma; interleukin-4; Probiotics

3

Bangladesh Journal of Medical Science Vol. 18 No. 01 January' 19. Page : 42-45

DOI: <https://doi.org/10.3329/bjms.v18i1.39545>

**Introduction**

Allergic rhinitis is a global health problem that can affect the quality of life and is often associated with multiple co-morbidity. The characteristic of patients with allergic rhinitis include changes in the body normal immune response such as increased serum levels of immunoglobulin E (IgE), local eosinophil infiltration and Th<sub>2</sub> cells and various other response caused by Th<sub>1</sub> and Th<sub>2</sub> imbalance toward Th<sub>2</sub> polarization.<sup>1</sup> Allergic rhinitis therapy using probiotics has been currently developed as it is more effective and specific in modulating the immune response. Specific probiotic strains demonstrated efficacy in prevention and treatment of allergies such as atopic eczema, reduce allergic inflammation both locally and systemically.<sup>2</sup> There have been few studies on efficacy of probiotics in allergic rhinitis and conflicting results due to the ability of the immune system modulation depending on the species of probiotic used and the target population.<sup>3</sup> Thus, the studies on the potential of probiotics containing *L. casei* and *L. acidophilus* in patients with against

allergic rhinitis are needed.

*Lactobacillus casei* and *Lactobacillus acidophilus* are potential probiotics to be developed as a new alternative immunomodulatory-based therapy of allergic rhinitis.<sup>4</sup> The administration of *L. acidophilus* has been shown to activate Th<sub>1</sub> and increase levels of IFN- $\gamma$  specifically but did not affect the production of IL-4. Induction of immune responses mediated by Th<sub>1</sub> has been also observed after administration of *L. casei* of which include increased production of IFN- $\gamma$ , IL-12 and IL-4 and decreased the levels of IgE.<sup>5</sup> The previous study has shown that the administration of fermented milk containing *L. casei* Shirota strain can reduce levels of IgE in allergic rhinitis patients.<sup>6</sup> As an immunomodulatory probiotics containing *L. casei* and *L. acidophilus* are more specific so that it has the potential to be developed as a new alternative method of treatment of allergic rhinitis. This study aimed to determine the effect of probiotic containing *L. casei* and *L. acidophilus* on levels of IFN  $\gamma$ /IL-4 in patients with allergic rhinitis.

10

1. Andriana Tjitria Widi Wardani, Department of otorhinolaryngology-Head & Neck, Faculty of Medicine, Universitas Islam Sultan Agung.
2. Wiratno, Department of Otorhinolaryngology-Head & Neck, Faculty of Medicine, Universitas Islam Sultan Agung.
3. Dina Fatmawati, Department of Biology, Faculty of Medicine, Universitas Islam Sultan Agung.

**Correspondence to:** Dina Fatmawati, Jl. Raya Kaligawe KM 4, Semarang, Indonesia.  
Email: [dienafatma@unissula.ac.id](mailto:dienafatma@unissula.ac.id)

**Materials and Methods**

**Study design**

This single blind, consecutive controlled clinical trial study was performed in 30 patients with moderate to severe allergic rhinitis. All the eligible subjects in this study were randomly divided into 2 groups: treatment group and control group. All groups underwent a 4 week treatment. Prick test was performed to determine allergen distribution triggering allergic rhinitis in the study subject.

**Ethics, consent and Permission**

The study protocol was approved by bioethic commission of Universitas Islam Sultan Agung, Semarang Indonesia (reg:149/VII/2013/Komisi Bioetik). All participants were fully informed about all aspects of the research project and consented to complete and return the questionnaire. Moreover, they could ask for their names and addresses to be deleted from the enrollment list, by simply signing an appropriate box on the inform consent form and returning it without costs.

**Participant**

This study was conducted in otorhinolaryngology, head and neck center and biology laboratory. All participants were recruited from via daily outpatient services and advertisement. The criteria of allergic rhinitis were determined by using a questionnaire according to ARIA criteria. All the patients aged 18-21 years with moderate to severe allergic rhinitis were eligible to participate. Patients with allergic asthma, autoimmune diseases, moderate to severe atopic dermatitis, any severe chronic inflammatory disease, specific immunotherapy during last 2 years, continuous using antihistamines and corticosteroids medication in last 2 weeks, pregnancy and breast feeding were excluded.

**Intervention**

Patients in treatment group received probiotics containing *L. casei* and *L. acidophilus* in liquid milk based preparation much as 65 mL/day for 4 weeks orally while control group were given placebo in the form liquid milk skim based preparation as much as 65 mL/day for 4 weeks.

**Immune cytokines analysis**

IFN- $\gamma$  and IL-4 levels in serum were measured by ELISA kit (DRG cat. EIA-4434 and DRG cat. EIA-4435 respectively) according to the manufacturer's instructions and analyzed using a Luminex 100 instrument. Samples were diluted 1:2 in deionized water.

**Statistical Analysis**

Data of study results presented in tabulation of mean

and deviation standard were subjected to normality test to determine their distribution. The increased mean levels of IFN- $\gamma$ /IL-4 were determined after administration of probiotics containing *Lactobacillus acidophilus* and *Lactobacillus casei* using a parametric paired t-test with 95% confidence intervals.

**Results**

The result of prick test showed that all respondents had more than 1 type of allergy with the highest percentage of 73.3% allergy to dust mite, 60% subjects was allergic to cockroach, 26.67% subjects were allergic to house dust, 30% were allergic to the mixed fungi, 10% was allergic to human dander, and 20% of allergy respondents was allergic to dog dander, 3.3% of were allergic to horse dander (figure 1).

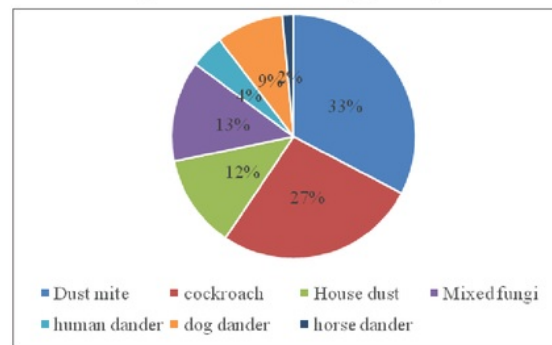


Figure 1. Distribution of allergen in allergy rhinitis.

Table 1 showed no difference in that the characteristics of the respondents of the placebo and probiotics groups.

**Table 1: Characteristics of respondents**

Characteristics	Groups		sig.
	Placebo	Probiotics	
<b>Gender</b>			
a. Male (%)	40	40	0,67
b. Female*%)	60	60	
Mean of IFN- $\gamma$ baseline (pg/mL)	143.88 $\pm$ 5.32	146.73 $\pm$ 5.51	1.000
Mean of IL-4 Level baseline (pg/mL)	29.62 $\pm$ 5.76	29.18 $\pm$ 6.50	1.000
Mean ratio of IFN- $\gamma$ /IL-4 Level	5.02 $\pm$ 0.94	5.28 $\pm$ 1.20	1.000

To elucidate the possible mechanism of probiotics in allergic rhinitis, IFN- $\gamma$  levels were measured using ELISA. The level of IFN- $\gamma$  was significantly higher in treatment group compared to that of control group (table 2).



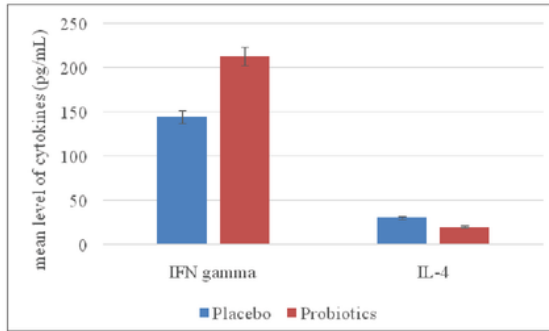


Figure 2. mean level of cytokines after 4 weeks treatment

Figure 1 showed that the administration of milk as a placebo did not increase IFN  $\gamma$  in patients with severe allergic rhinitis. In the probiotics group, IFN  $\gamma$  was by 1.5 times higher after the probiotic treatment ( $p = 0.000$ ). Increased levels of IFN  $\gamma$  may be due to the effect of the administration of probiotics in patients with allergic rhinitis. This is indicated a significant difference between placebo group and probiotic after treatment ( $p < 0.05$ ).<sup>12</sup>

Interleukin-4 level in treatment group was lower compared to that of control group ( $p = 0.000$ ). Levels of IL-4 allergic rhinitis patients before and after treatment is presented in table 3. The decreased in levels of IL-4 may be due to the effect of the administration of probiotics in patients with allergic rhinitis for the treatment group. This is supported by the test results of IL-4 levels before<sup>16</sup> and after treatment in the placebo group showing no significant difference ( $p > 0.05$ ) and significant difference between the placebo group and the probiotic after the treatment ( $p < 0.05$ ).

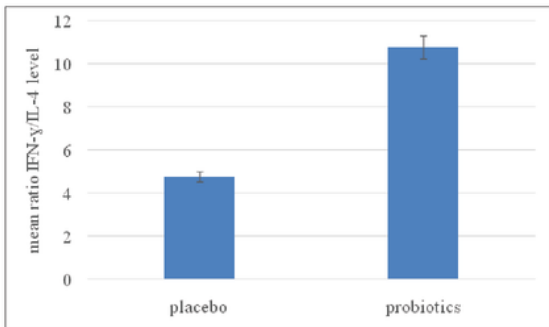


Figure 3. mean ratio of IFN  $\gamma$ /IL-4 level after 4 weeks treatment

In order to evaluate the<sup>19</sup> immunodulatory activity, ratio of mean levels of IFN  $\gamma$  / IL-4 was evaluated. Mean ratio of IFN  $\gamma$  / IL-4 significantly increased in probiotics group compared to the control group (figure 2). The increase was 2 times higher after probiotic treatment ( $p = 0.000$ ).

### Discussion

Increased IFN- $\gamma$ /IL-4 ratio in the probiotic group might have been due to increased IFN- $\gamma$  and decreased levels of IL-4. This decrease can be caused by increased levels of IL-12 and IL-10. The study by Kaji *et al.* in 2010 showed that *Lactobacillus casei* has the ability to increase the ratio of IL-12 / IL-10 while *Bidifobacterium* has the ability to increase IL-10. Increased IL-10 plays a role in increasing the ratio.<sup>7</sup>

This finding supports a study by Mohamadzadeh in 2005 showing that results that probiotics can activate human dendritic cells tend to Th1 polarization indicated by increased IFN  $\gamma$ . The study used three different types of *lactobacilli* including *Lactobacillus gasserii*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*. All of the three *Lactobacillus* increased IFN  $\gamma$ , but inversely correlated to the IL-2, IL-4 and IL-6.<sup>8</sup> In the probiotic group there was a significant decrease in IL-4 levels after probiotic treatment ( $p = 0.000$ ).

Previous study showed that IL-4 levels after treatment were lower than before treatment. This was likely due to the stimulation of probiotic bacteria as a strong activator for the innate immune system because their specific molecules on the cell wall of peptidoglycan and lipoteichoic acid which will interact with TLR2 and TLR4 resulting in activation of T cells in the immune system, by polarization toward Th1 and Treg cells<sup>9,10</sup>

Probiotics have a role in the prevention of allergic diseases besides having the ability to inhibit pathogens, probiotics can trigger S-IgA immune mucosal response that systemically trigger T regulator inhibiting Th<sub>2</sub>, or overactivity of Th<sub>1</sub> redundant.<sup>11</sup>Wahn *et al.* in 2004 stated that current therapeutic development is directed at improving the homeostasis of allergic biological systems intended to immunomodulating the immune response by balancing the Th1 and Th2 immune responses. The advancement of science and technology allows the paradigm shift from preventing allergies in the form of prevention of risk factors toward active induction of immunologic tolerance.<sup>12</sup>

### Conclusions and recommendations

The administration of combination of probiotics can improve the levels of IFN- $\gamma$ /IL-4 in patients with moderate to severe allergic rhinitis. Further studies are needed to determine the effect of *Lactobacillus*

*acidophilus* and *Lactobacillus casei* treatment on symptom recurrence and the quality of life in allergy rhinitis patients.

#### Acknowledgment

This research was funded by Indonesian Minister of Research Technology and Higher Education and medical faculty of Universitas Islam Sultan Agung.

**Conflict of interest:** The authors declared no conflict of interest

#### Authors' contributions:

Data gathering and idea owner of this study: Dina Fatmawati

Study design: Andriana Tjitria Widi Wardani, Wiratno

Data gathering: Wiratno, Dina Fatmawati

Writing and submitting manuscript: Dina Fatmawati,

Editing and approval of final draft: Dina Fatmawati,

Wiratno, Andriana Tjitria Widi Wardani

#### References:

1. Bousquet J, Cauwenberge PV, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108, (5 Suppl):S147-334. PMID: 11707753. <https://doi.org/10.1067/mai.2001.118891>
2. Well JM., 2011. Immunomodulatory Mechanisms of Lactobacilli. *Proceeding 10th Symposium on Lactic Acid Bacterium. Wells Microbial Cell Factories*,
3. Michail S. The Role of Probiotics in Allergic Diseases. *Allergy, Asthma & Clinical Immunology*. 2009;5 (5).
4. Gill HS., Rutherford, KJ., Prasad J., Gopal PK., 2000. Enhancement of Natural and acquired immunity by *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*. *British Journal of Nutrition* (2000);83;167-176 <https://doi.org/10.1017/S0007114500000210>
5. Baken K A, Ezendam J., Gremmer E R., et al., Evaluation of Immunomodulation by *Lactobacillus casei* Shirota: Immune Function, Autoimmunity and Gene Expression. *Int J of Food Microbiology*. 2006; 112: 8-18 <https://doi.org/10.1016/j.ijfoodmicro.2006.06.009>
6. Wardani, AT., Rian. Pengaruh Pemberian Yogurt Terhadap Kadar IgE. *Laporan Penelitian*. 2011.
7. Kaji R, Shibata JK., Nagaoka M., Nanno M., Shida., Bacterial Teichoic Acids Reverse Predominant IL-12 Production Induced by Certain *Lactobacillus* Strains into Predominant IL-10 Production via TLR 2-Dependent ERK Activation in Macrophages. *J Immunol*; 2010;184:3505-3513 <https://doi.org/10.4049/jimmunol.0901569>
8. Mohamadzadeh, M., 2005, Lactobacilli Active Human Dendritic Cells That Skew T Cell Toward T Helper 1 Polarization, Department Of Microbiology, Louisiana State University, New Orleans
9. Iwasaki A, Medzhitov R., Toll-like receptor control of the adaptive immune responses. *Nature Immunol* 2004;5(10): 987-995. in: <http://www.nature.com/nature/journal/v5/n10/full/ni1112.html>. Dikutip tanggal 3 Mei 2012.
10. Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, Ogawa H., Differential responses of mast cell Toll-Like receptor 2 and 4 in allergy and innate immunity. *J Clin. Invest* 2002;109:1351-9. Dalam : <http://www.jci.org/articles/view/14704>. Dikutip tanggal 3 Mei 2012.
11. Endaryanto, A., Harsono, A., Prospek Prebiotik Dalam Pencegahan Alergi Melalui Induksi Toleransi Aktif Imunologis, *Bag. Ilmu Kesehatan Anak FK UNAIR*;2010,
12. Wahn U, Nickel R, Illi S, Lau S, Gruber C, Hamelmann E., Strategies for early prevention of allergic disorders. *Clin Exp Allergy*, 2004;4:194-199. Dalam : <http://onlinelibrary.wiley.com/doi/10.1111/j.1472-9725.2004.00054.x/abstract>. Dikutip tanggal 25 Februari 2012

# Potential immunomodulator

---

## ORIGINALITY REPORT

---

18%

SIMILARITY INDEX

11%

INTERNET SOURCES

13%

PUBLICATIONS

0%

STUDENT PAPERS

---

## PRIMARY SOURCES

---

1

[krishikosh.egranth.ac.in](http://krishikosh.egranth.ac.in)

Internet Source

2%

---

2

[ar.scribd.com](http://ar.scribd.com)

Internet Source

2%

---

3

Titik Sumarawati, - Israhnanto, Dina Fatmawati. "Anticancer Mechanism of Melia azedarach, Doxorubicin and Cyclophamide Combination against Breast Cancer in Mice", Bangladesh Journal of Medical Science, 2017

Publication

2%

---

4

Cazzoletti, Lucia, Marcello Ferrari, Mario Olivieri, Giuseppe Verlato, Leonardo Antonicelli, Roberto Bono, Lucio Casali, Isa Cerveri, Pierpaolo Marchetti, Pietro Pirina, Andrea Rossi, Simona Villani, and Roberto de Marco. "The gender, age and risk factor distribution differs in self-reported allergic and non-allergic rhinitis: a cross-sectional population-based study", Allergy Asthma & Clinical Immunology, 2015.

Publication

1%

---

5	<a href="http://respiratory-research.com">respiratory-research.com</a> Internet Source	1%
6	"XXIIst CINP Congress: Brussels, Belgium, 9-13 July 2000", The International Journal of Neuropsychopharmacology, 2007 Publication	1%
7	<a href="http://www.nrcresearchpress.com">www.nrcresearchpress.com</a> Internet Source	1%
8	"Poster Communications", Acta Physiologica, 2015. Publication	1%
9	<a href="http://openaccess.city.ac.uk">openaccess.city.ac.uk</a> Internet Source	1%
10	"Poster Sessions", Tropical Medicine & International Health, 2017 Publication	1%
11	<a href="http://dergipark.ulakbim.gov.tr">dergipark.ulakbim.gov.tr</a> Internet Source	1%
12	<a href="http://www.spandidos-publications.com">www.spandidos-publications.com</a> Internet Source	1%
13	Muhammad Thorieq Ali, Muhammad Fadli Ashari, Santika Puji Rahayu Wijaya, Endang Lestari, Rina Wijayanti. "Evaluation of Wound Healing Effect of Eel Mucus Ointment (Belutidine) in Mice by Incision Model",	1%

# JOURNAL OF NATURAL REMEDIES, 2018

Publication

---

14	<a href="http://onlinelibrary.wiley.com">onlinelibrary.wiley.com</a> Internet Source	1%
15	<a href="http://d-scholarship.pitt.edu">d-scholarship.pitt.edu</a> Internet Source	<1%
16	<a href="http://www.jstage.jst.go.jp">www.jstage.jst.go.jp</a> Internet Source	<1%
17	<a href="http://epdf.tips">epdf.tips</a> Internet Source	<1%
18	Novak, Jan, Thibault Griseri, Lucie Beaudoin, and Agnès Lehuen. "Regulation of Type 1 Diabetes by NKT Cells", International Reviews of Immunology, 2007. Publication	<1%
19	Dietert, Rodney R., and Michael S. Piepenbrink. "Lead and Immune Function", Critical Reviews in Toxicology, 2006. Publication	<1%
20	<a href="http://link.springer.com">link.springer.com</a> Internet Source	<1%
21	<a href="http://www.doria.fi">www.doria.fi</a> Internet Source	<1%
22	<a href="http://journals.plos.org">journals.plos.org</a> Internet Source	<1%

---



23

Quanxin Gao, Qian Gao, Minghua Min, Chenjie Zhang, Shiming Peng, Zhaohong Shi. "Ability of Lactobacillus plantarum lipoteichoic acid to inhibit Vibrio anguillarum-induced inflammation and apoptosis in silvery pomfret (*Pampus argenteus*) intestinal epithelial cells", *Fish & Shellfish Immunology*, 2016

Publication

---

<1%

24

Gerald W. Volcheck. "Clinical Allergy", Springer Nature America, Inc, 2009

Publication

---

<1%

---

Exclude quotes      On

Exclude matches      < 7 words

Exclude bibliography      On