Original Article

Probiotic Supplementations Preserve Protective Immunity Against SARS-CoV-2 Spike Protein After a Triple Combination of COVID-19 Vaccination

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Abstract

Introduction: Immunoglobulin G against SARS-CoV-2 spike protein declines over-time in vaccinated individuals. Trend to restore protective immunity is challenging.

Objectives: Probiotics have beneficial effects to human innate and adaptive immune responses. This study aimed to compare immune preservation of probiotic supplement versus placebo by measuring the mean and percent reduction of COVID-19 antibody level among those subjects receiving a triple dose combination of COVID-19 vaccine.

Methods: A randomized, double-blinded, placebo-controlled trial, conducted in Bangkok-Samui hospital between December 2021 to February 2022. There were 30 volunteers, divided randomly into 2 groups. The target group received probiotics supplement once daily for 6 weeks, while the control group received placebo. Blood testing for SARS-CoV-2 spike protein Immunoglobulin G level was collected at day baseline and week-6 visit. Data were analyzed by Statistical software with Statistical Package for the Social Sciences (SPSS) version 23.0, including descriptive and analytical statistics.

Results: The mean of SARS-CoV-2 spike protein IgG antibody level between probiotic and placebo group at the baseline was not difference ($P = .4178$). Subjects received probiotic supplement for 6 weeks found SARS-CoV-2 spike protein IgG antibody level reduction significantly ($P = .003$). The control group also found antibody level reduction significantly ($P < .001$). The Probiotic group found a significantly lower percent reduction of SARS-CoV-2 spike protein IgG antibody at week-6 visit from the baseline than the placebo group (35.7% vs. 48.3%, respectively, $P = .007$). There is no adverse event at the end of this study.

Conclusions: Taking probiotics supplement once daily for 6 weeks could preserve immunity level with less percent reduction than the placebo, with no adverse effect occurred.

Keywords: Probiotics, SARS-CoV-2 spike protein IgG antibody level, COVID-19, Supplement

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Introduction

A new emerging infectious disease, coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 virus, becomes the main health care problems as global pandemic disease. Within 2 years, SARS-CoV-2 virus infected more than 200 million people around the world and caused 4.7 million fatal deaths. The Thai government attempted to immunize their citizens by using COVID-19 vaccination; therefore, major protective immunity by immunoglobulin G (IgG) against SARS-CoV-2 spike protein level in completely vaccinated person (at least serial 3 doses of vaccination) rapidly dropped within the short period of time or a few months.[4,6]

Previous study reported viral vector COVID-19 vaccine could stimulate a peak level of neutralizing antibody within 28 days while mRNA coding SARS-CoV-2 spike antigens vaccine faster stimulated with the peak of 21 days after the first dose. The second dose of both viral vector and mRNA COVID-19 vaccine could boost protective immunity with the peak antibody response within 7 days after vaccination. The study reported that anti-SARS-CoV-2 IgG level could maintain specific immunity above the protective level after the second dose of vaccination in both viral vector and mRNA COVID-19 vaccines in longer than 49 days.[6] Even though, the vaccination could effectively boost the immunity against SARS-CoV-2 virus, but immunity level disappointingly persisted in short duration. This concern led to recommend the further booster dose.[4,5,6] It is a challenge on how to maintain a protective antibody level in an immunized individual as long as possible.

Although there were evidences suggested the booster dose, the shortage of COVID-19 vaccines during their 1st launch were inevitable in most of the countries around the world. Thai government try its best to prioritize healthcare providers in the first place, so it was impossible to provide vaccine to all citizens. At that time Thai ministry of public health try to solve the problem by recommended their citizens to maintain in a good health by taking some vitamins in combination (vitamin C, vitamin D, and zinc). As these vitamins was well known as an immune stimulator. Nevertheless, there are more supplements that could promote a healthy status to the host, especially probiotics.[1,7,8]

Recently, there are many studies claimed that probiotics gain the beneficial effects on health restoration and health promotion.[1,7,8,9] Especially an important role in innate and adaptive immune activation.[10] Probiotics helps digesting the indigestible food into short-chain fatty acids. These beneficial short-chain fatty acids such as acetate, butylate and propionate, which plays a role not only in metabolism regulation and gut homeostasis, but also in host innate immunity by promoting antimicrobial proteins, increasing immunoglobulins and stimulating phagocytic activities (Dendritic cell and NK cell activities). Moreover, these signals could activate host adaptive immunity by increasing T helper II, T lymphocytes, B lymphocytes, and antigen presenting cells.[17] Apart of that, the beneficial short-chain fatty acids also balance inflammation process (via T helper 17, IL-1, IL-6, and TNF alpha) and anti-inflammation processes (via T regulatory cells). Furthermore, a study in germ free mice found that short-chain fatty acids can increase plasma cell via Toll-like receptor 5 and IL-1ß activation.[9,10,12] So, probiotics play a crucial role in immunity level by their cell activation processes. As mentioned, host immune homeostasis could be maintained by probiotics. Especially in COVID-19 infection induced cytokine storm syndrome, probiotics could subside the systemic inflammation process within the body.[1,2,3,12]

Nowadays, there are so many beneficial probiotic strains found in supermarket. The recommendation of strain use depends on host status, age, sex, and purpose. Because of gut natural microorganism diversity, using probiotic supplement with multiple strains gain more benefits than using single strain alone.[15] According to some study records, probiotic strains such as *Bifidobacterium longum, Lactobacillus rhamnosus, and Lactobacillus acidophilus* maintain healthy status in infants.[11,14] One study earlier found that mixture of probiotic strains like *Lactiplantibacillus plantarum* KABP033 (CECT30292), *Lactiplantibacillus plantarum* KABP022 (CECT7484), *Lactiplantibacillus plantarum* KABP023 (CECT7485), and *Pediococcus acidilactici* KABP021 (CECT7483), could help COVID-19 patients to complete viral remission faster than the control group.[13,16] So, this study will focus on the use of *Lactobacillus* spp. and *Bifidobacterium* spp. strain only.

This research aimed to compare immune preservation of probiotic supplement versus placebo by measuring the mean and percent reduction of

COVID-19 antibody level among those subjects receiving a triple dose combination of COVID-19 vaccine.

Methods

Study Design: Randomized, double-blinded, placebo-controlled clinical study.

Population: This study enrolled 30 healthcare workers receiving full 3 consecutive doses of COVID-19 vaccination and currently worked at Bangkok hospital Samui, Surat Thani province on December 2021.

Inclusion Criteria:
1. Healthcare workers
2. 18-60 years of age
3. Full vaccination program with three consecutive doses of CoronaVac (Sinovac®), CoronaVac and Pfizer-BioNTech COVID-19 vaccine
4. The third booster COVID-19 vaccine within 60 days prior to an enrollment

Exclusion Criteria:
1. Known, serious or uncontrolled underlying diseases
2. Currently taking with some specific medications, herbal medicines, or food supplements within 6 months
3. Prior history or currently infected with COVID-19
4. Pregnant women

All subjects had randomly been assigned to receive either once daily, one sachet of 75,000 million colony forming unit (CFU) of lactic acid bacteria, probiotic supplementation mixed with a bottle of water for 6 weeks duration (intervention group) or once daily 10 milligrams maltodextrin powder (the placebo). Probiotic supplementation contains a combination of lactic acid bacteria strains including *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium longum, Bifidobacterium infantis, and Bifidobacterium bifidum*. SARS-CoV-2 spike protein IgG antibody level were tested at the baseline and week-6 visit (the endpoint).

Descriptive statistics were reported in the means and standard deviation in numeric data and with frequency and percentage in categorical data. Unpair student *t* test was used to compare SARS-CoV-2 spike protein IgG antibody level at the baseline and week-6 visit. Furthermore, unpair student *t* test was also used to compare the mean percent change from the baseline visit to the week-6 visit of SARS-CoV-2 Spike protein IgG level as the primary endpoint. Statistical software with Statistical Package for the Social Sciences (SPSS) version 23.0 was used in this present study. *P* value with less than .05 is set as statistical significance.

This study was reviewed and approved by Mae Fah Luang University Ethical committee prior to subject enrollment.

Results

There were 30 subjects enrolled and were randomized to receive either probiotic supplement (n = 15) or the placebo (n = 15). There were 5 subjects in each group, loss to follow-up at the final visit. In addition, there were 1 subject in probiotic group and 2 subjects in control group discontinue the study due to their personal discretion. (Figure 1)
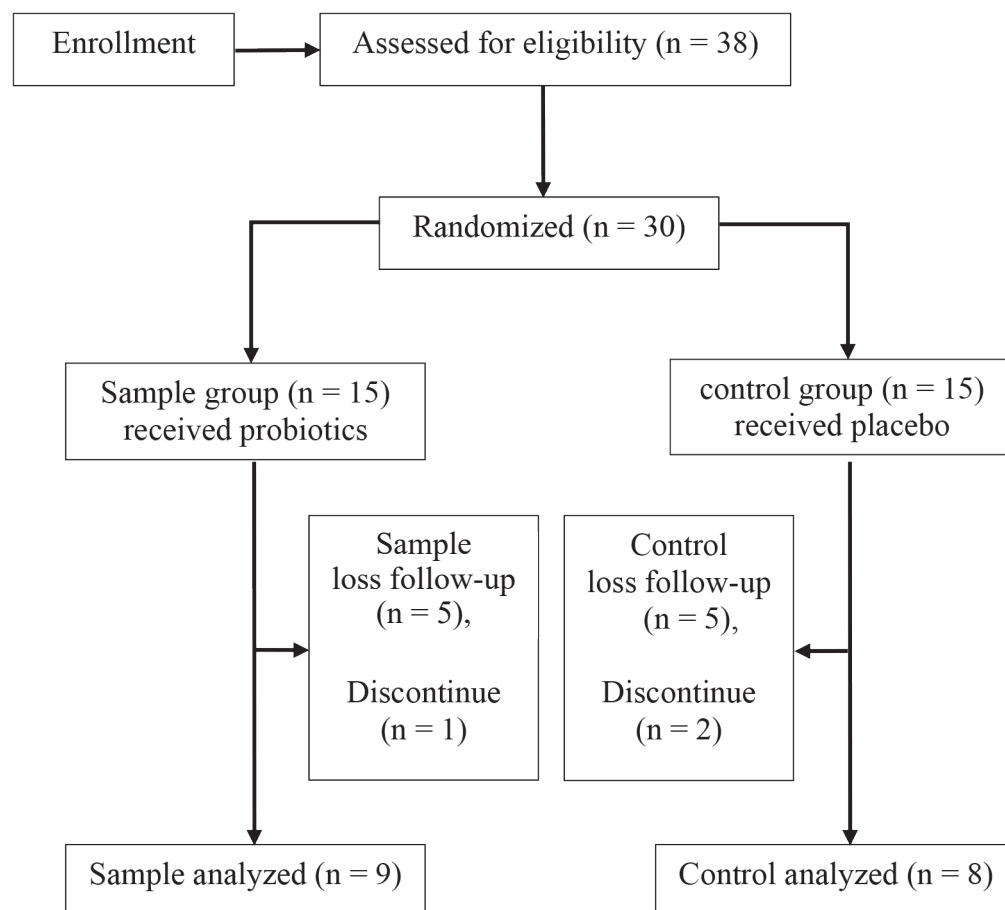
Figure 1 Study consort.

Demographic Data of the Subjects

There are 17 subjects completed the study, 9 subjects in probiotic group and 8 subjects in control group with well-balanced in gender and age factors ($P > .05$) (Table 1).

Table 1 Demographic data of the subjects (n = 17)

Demographics	Probiotic group (n = 9)	Placebo group (n = 8)	*P*-value
Gender			
• Male, no. (%)	1 (11.1)	1 (12.5)	.700
• Female, no. (%)	8 (88.9)	7 (87.5)	
Age (years)			
• Mean (SD)	34.2 (8.2)	35.7 (9.6)	.847
• Min.-Max.	24 - 50	24 - 55	

Abbreviation: SD = standard deviation, Min. = minimum, Max. = maximum

Immunoglobulin G Level Against SARS-CoV-2 Spike Protein

The mean of SARS-CoV-2 spike protein IgG antibody level between probiotic and placebo group at the baseline was not difference ($P = .4178$). Immunoglobulin G levels against SARS-CoV-2 spike protein at week-6 visit reduced significantly when compared with the baseline visit in both probiotic and placebo group ($P = .003$ and $< .001$, respectively) (Table 2 and Figure 2).
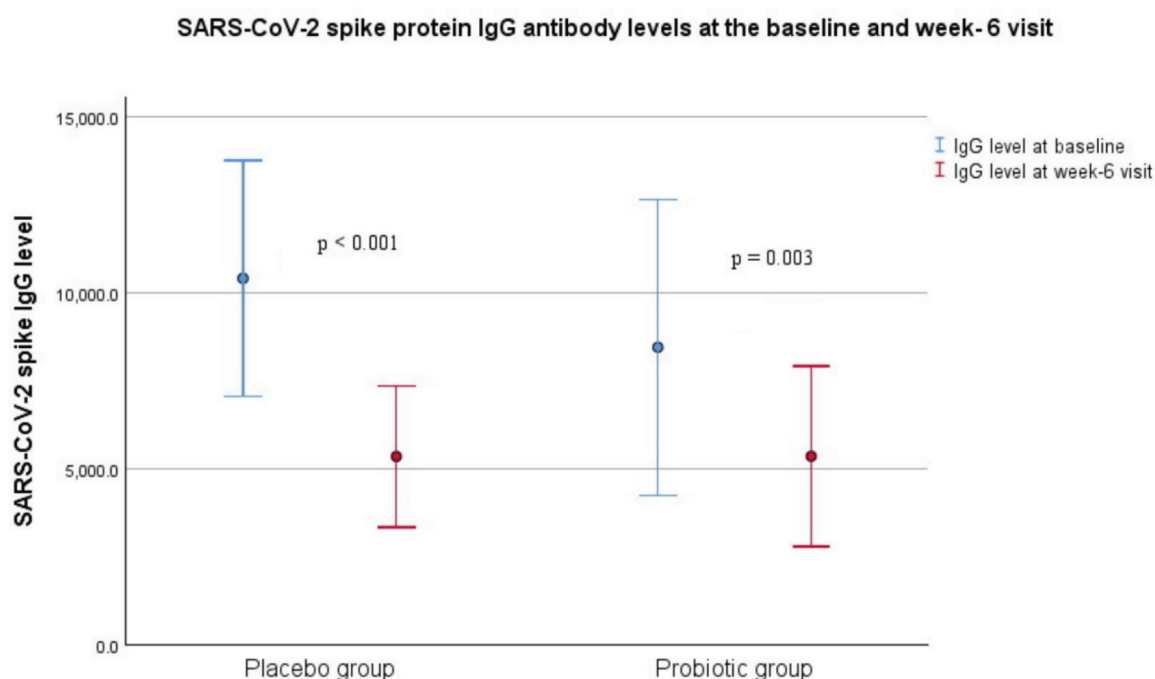
Figure 2 To compare SARS-CoV-2 spike protein IgG antibody levels at the baseline and week-6 visit.

Table 2 SARS-CoV-2 spike protein IgG antibody within group

SARS-CoV-2 spike protein IgG antibody level	n	Baseline visit Mean (SD)	Week-6 visit Mean (SD)	*P*-value*
• Probiotic group	9	8,456.7 (5462.4)	5,358.7 (3334.7)	.003
• Placebo group	8	10,415.1 (4004.9)	5,353.6 (2401.4)	< .001
P-value**		0.4178		

Abbreviation: SD = standard deviation

Comparison of Percent Reduction of SARS-CoV-2 Spike Protein IgG Antibody at Week-6 Visit Between Probiotic and Placebo Group

       Probiotic group has a significantly lower percent reduction of SARS-CoV-2 spike protein IgG antibody at week-6 visit from the baseline than the placebo group (35.7% vs. 48.3%, respectively, *P* = .007) (Table 3, Figure 3).

Table 3 Comparison of percent reduction of SARS-CoV-2 spike protein IgG antibody at week-6 visit compared with the baseline between probiotic and placebo group

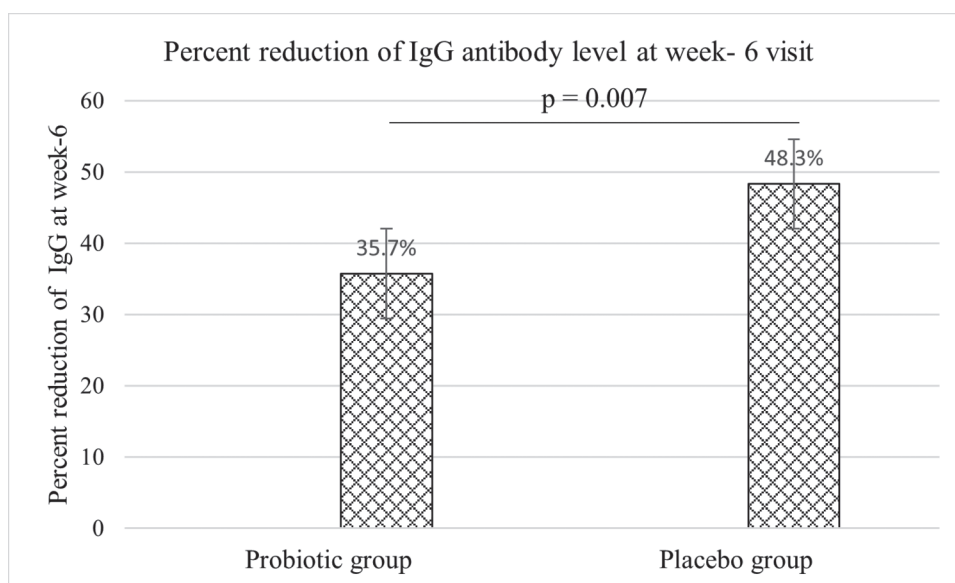| Study outcome	Probiotic group (%, 95% CI)	Placebo group (%, 95% CI)	*P*-value*
Percent reduction of SARS-CoV-2 spike protein IgG antibody at week-6 visit	35.7 (31.9 - 39.5)	48.3 (40.5 - 56.2)	.007

Abbreviation: CI = confident interval

Figure 3 To demonstrate percent reduction of SARS-CoV-2 spike protein IgG at week-6 visit.

There was no adverse event reported during the study. The number of drop-out rate is quite high due to fear of testing substances and ongoing 4th COVID-19 booster dose in Koh Samui, Thailand (40% for probiotic group and 46% for placebo group) (Figure 4).
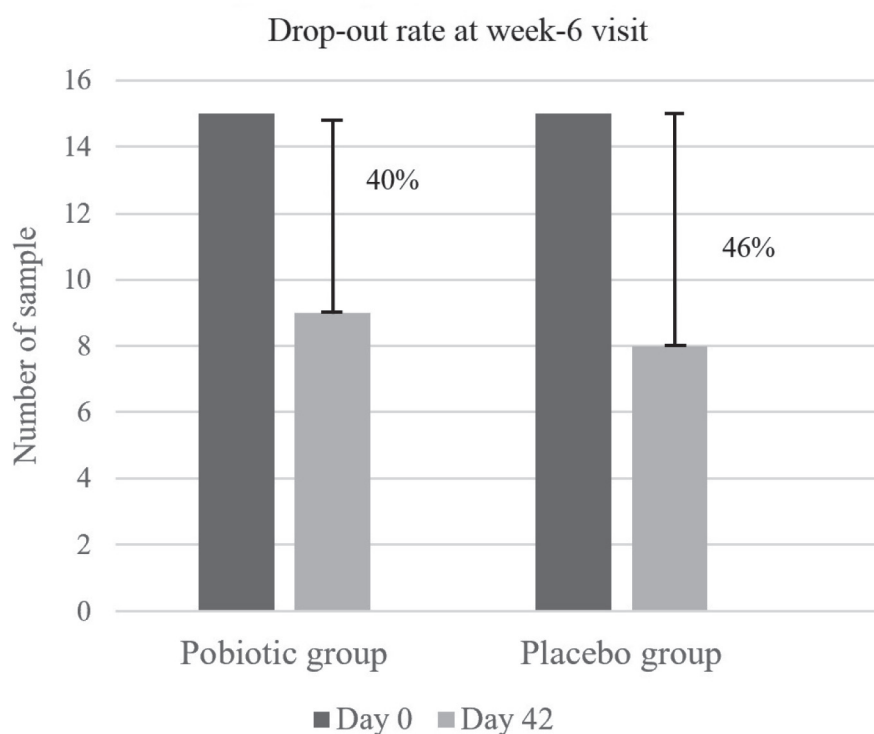

Figure 4 Number of drop-out rate at week-6 visit.

Discussion

Coronavirus disease 2019 is a new emerging disease and there are many aspects that are still not clearly understood. Recent studies have shown that COVID-19 patients had some degree of gut dysbiosis which might explain why most of patients had a diarrhea symptom and inflammation marker rising. When gut dysbiosis symptom aggravated, some studies found that it correlated with severely ill disease. As a result, probiotics might play an important role in gut homeostasis in COVID-19 patients.

Currently, there are many evidences supporting the benefits of probiotics to human health. It can help in regaining normal gut function and alleviate the symptoms of some diseases. According to this study, we found that taking probiotic once daily for 6 weeks could prolong SARS-CoV-2 spike protein IgG antibody level in the volunteers.

Nevertheless, this pilot study was established along with Thai government policy to prioritize its citizens to be vaccinated. According to the policy, medical healthcare workers were the top priority, so the demographic data of the volunteers were mostly in middle-aged and healthy. Further study is needed to evaluate the outcomes in people from other age groups and health conditions. This study has some limitations. There are many different strains of probiotics supplement that could promote host immune homeostasis. However, this study selected the 75,000 million CFU of lactic acid bacteria combination with *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium longum, Bifidobacterium infantis,* and *Bifidobacterium bifidum* strains only. So, probiotic strains apart from this study should be study for further information.

Acknowledgements

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