Clinical Efficacy of Probiotic 
*Bifidobacterium longum* for the 
Treatment of Symptoms of Japanese 
Cedar Pollen Allergy in Subjects 
Evaluated in an Environmental 
Exposure Unit

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**ABSTRACT**

**Background:** Japanese cedar pollinosis (JCPsis) affects nearly one in six Japanese. Oral administration of *Bifidobacterium longum* BB536 has been shown to be effective in relieving JCPsis symptoms during the pollen season.

**Methods:** This double- two-way crossover study was designed to evaluate the efficacy of BB536 on reducing symptoms in JCPsis patients exposed to Japanese cedar pollen (JCP) in an environmental exposure unit (EEU) outside of the normal JCP season. After a 1-week run-in period, subjects (n = 24) were randomly allocated to receive BB536 powder (approximately 5 × 10¹⁰) or placebo twice a day for 4 weeks. After a 2-week washout period, subjects were crossed over to another 4 weeks of intake. At the end of each intake period, subjects received controlled JCP exposure for 4 hours in the EEU. Symptoms were self-rated 30 minutes before and every 30 minutes during the exposures. From the first day of exposure through the next 5 successive days, participants self-rated their delayed symptoms and medication uses. Blood samples were taken before the exposures. The mean JCP levels for exposures were 6500 to 7000 grains/m³ air.

**Results:** In comparison with placebo, BB536 intake significantly reduced the ocular symptom scores during JCP exposures. Evaluating delayed symptoms after exposures indicated that scores for disruption of normal activities were significantly lower in the BB536 group compared with the placebo group. Prevalence of medication use was markedly reduced by BB536 intake.

**Conclusions:** These results suggest the potential beneficial effect of BB536 in relieving symptoms of JCP allergy

**KEY WORDS**

Allergy, *Bifidobacterium*, environmental exposure unit (EEU), Japanese cedar pollinosis, probiotic

**INTRODUCTION**

Allergic rhinitis is a common disorder affecting a large percentage of the population, with estimates of the prevalence ranging from 9 to as high as 42%.¹,² This disorder can be classified as seasonal or perennial depending on whether symptoms are manifested at defined yearly intervals or throughout the year, respectively. Japanese cedar pollinosis (JCPsis) is an immunoglobulin E (IgE)-mediated type I allergy.
caused by exposure to Japanese cedar (Cryptomeria japonica) pollen (JCP) that is normally released from early February until the end of April. Over recent decades there has been an increasing prevalence of this allergy and this public health issue now affects >16% of the Japanese population. Drugs such as antihistamines and local steroids are commonly used to relieve symptoms, but some of these therapies can cause adverse effects such as sleepiness and thirst.

As of yet, there is no complete recovery measurement known for this disorder, although presently, allergen-specific immunotherapy is considered to be the most promising.

Numerous studies have reported that the intake of probiotics such as lactobacilli and bifidobacteria is associated with the prevention of several allergic diseases, but for other strains conflicting results have been reported. We previously reported that the intake of Bifidobacterium longum BB536 was potentially effective in relieving JCPsis symptoms and modulating the Th1/Th2 balance. In 2004, we performed a human trial to investigate the effects of yogurt supplemented with BB536 in the treatment of JCPsis during the pollen season. We found that some of the subjective symptoms were alleviated following BB536 intake, while decreased interferon (IFN)-γ levels were particularly suppressed at the early stage of pollen season. The effect of BB536 in the treatment of JCPsis was confirmed during the 2005 pollen season by using lyophilized powders of BB536. We found that BB536 intake alleviated subjective symptoms leading to an increase in the number of JCPsis subjects who did not need to use their prescribed pollinosis medicines during the pollen season. BB536 was found to significantly suppress the increase of plasma thymus- and activation-regulated chemokine (TARC), a Th2 marker, during the pollen season. These results suggested that BB536 was effective in relieving JCPsis symptoms, with the mechanism most likely due to the modulation of the immune response.

The present study was a double-blind two-way crossover study designed to be performed out of the JCP season and to assess the efficacy of BB536 intake on the reduction of symptoms after JCP exposure in an environmental exposure unit (EEU) in JCPsis patients. The EEU concept has been developed to overcome the problems associated with field-based studies. The EEU used in this particular study is located in Wakayama, Japan. The design of the unit allows for control of the temperature and humidity and enables simultaneous allergy challenge in up to 28 subjects under controlled and reproducible conditions for several hours at a time. In studies of the efficacy of anti-histamine drugs against JCP challenge, the Wakayama EEU has been validated to provide reproducible and consistent results. EEU located in Canada, Austria and other countries have also been demonstrated to be useful in the evaluation of the onset, efficacy and duration of medicines used for treating different kinds of seasonal allergies. However, to the best of our knowledge, there have been no prior reports on the application of an EEU in the evaluation of the efficacy of probiotics in the treatment of allergy diseases.

**METHODS**

**PARTICIPANTS**

For this intervention, a total of 24 adult volunteers (10 men, 14 women) with a clinical history of JCPsis were recruited from the area around Wakayama Prefecture. Participants were screened on the basis of a 2-year clinical history of JCPsis, the presence of serum JCP-specific IgE and the presence of relatively severe symptoms during the last cedar pollen season as evaluated in accordance with the Practical Guidelines for the Management of Allergic Rhinitis in Japan (PGMARJ) 2005. None of the subjects had been taking any rhinitis medication prior to the start of this intervention.

All participants provided written informed consent. All study protocols were approved and controlled by the Local Ethics Committee of the NPO (non-profit organization) Japan Health Promotion Supporting Network, Wakayama, Japan, and the Local Ethics Committee of Morinaga Milk Industry Co., Ltd., Tokyo, Japan.

**SAMPLE INTAKE AND PARTICIPANT MONITORING**

The study was designed as a double-blind two-way crossover study. After a 1-week run-in period, subjects (n = 24) were randomized into 2 groups (group A and group B) and allocated to receive either a freeze-dried powder of BB536 (approximately 5 × 10^10) or a placebo powder (only internal matrix, which consisted mainly of dextrin) twice a day for 4 weeks. After a 2-week washout period, volunteers were crossed over to another 4 weeks of intake (Fig. 1). BB536 and placebo powders looked and tasted identical. To improve the survivability of probiotics in delivery to the intestinal tract, participants were instructed to suspend the powder in 100 ml of ultrahigh temperature pasteurized milk prior to ingestion of the samples. At the end of each interventional period, each subject received controlled JCP exposure (4 hours) in the EEU. The mean pollen levels were 6500 to 7000 grains/m³ air, which were approximately 2 times higher than the heaviest dispersion documented during the 2005 pollen season. Participants were instructed to complete a questionnaire on compliance, medication and health conditions throughout the intervention. For the JCP exposure, all participants arrived at the site in the morning, and prior to pollen exposure, physical examinations and blood draws were performed. Symptoms were self-rated 30 minutes before exposure and every 30 min-
Fig. 1 Study protocol.

utes during the exposure. Nasal blockage, eye itching, watery eyes, throat symptoms (such as a scratchy or sore throat) and disruption of normal activities (for example, the impact of the symptoms on reading a book or watching TV in the EEU) were evaluated using a 10-cm visual analogue scale (VAS; maximum score, 10). Counts of the episodes of sneezing and nose blowing (rhinorrhea) were recorded. Scores for eye itching and watery eyes were summed for the measure of ocular symptoms for each subject. Since the outcomes of episode counts of sneezing and nose blowing were well balanced with those observed for the VAS scores of nasal blockage, they were directly summed to provide the measure of nasal symptoms for each subject.

After exposure, each participant underwent a second physical examination and nasal lavage with approximately 100 ml of phosphate-buffered saline used to remove pollen from the nasal cavity. For delayed symptoms, eye drops (histamine release inhibitors), local nasal sprays (focal administration of steroids) and oral medications (second generation antihistamines) were prescribed. Participants were instructed to record delayed subjective symptoms and medications that were used starting from the first day of exposure and continuing for 5 successive days after exposures. Participants were allowed to try self-care measures such as the use of prescribed eye drops, local nasal sprays or oral medication when necessary. All participants were requested to record use of these items on a daily basis. Nasal blockage, eye itching, watery eyes, throat symptoms, and disruption of normal activities (for example, the impact of the symptoms on work, study, housework, sleep, or outdoor activities) were evaluated daily using a 10-cm visual analogue scale (VAS; maximum score, 10). Counts of episodes of sneezing and nose blowing (rhinorrhea) were recorded daily. Daily scores for eye itching and watery eyes were summed for the measure of ocular symptoms for each subject. The daily counts of episodes of sneezing and nose blowing after exposures were transformed to ordinal scores from 0 to 4 as follows; 0, none; 1, 1-5 episodes; 2, 6-10 episodes; 3, 11-20 episodes; and 4, ≥21 episodes in accordance with the PG-MARJ 2005. The outcomes of ordinal scores of sneezing and nose blowing were well balanced with those observed for daily VAS scores of nasal blockage, and therefore they were summed as a measure of nasal symptoms for each subject.

BLOOD SAMPLING
Physical examinations and blood samplings were performed during the screening, and after each of the sample intake periods. Blood samples were drawn from an antecubital vein after overnight fasting. Serum total IgE antibodies and JPC-specific IgE antibodies were analyzed using the UniCap system (Diag nostic, Sweden).

STATISTICAL ANALYSIS
The basic data are presented as geomeans with 95% Confidence intervals (CI). To compare scores over the entire phase, area-under-the curves (AUCs) during and after exposures were then calculated based
Table 1  Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group A (placebo → BB536)</th>
<th>Group B (BB536 → placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.0 ± 8.0</td>
<td>37.6 ± 7.5</td>
</tr>
<tr>
<td>(range, years)</td>
<td>(27 – 56)</td>
<td>(25 – 50)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>n = 4</td>
<td>n = 5</td>
</tr>
<tr>
<td>Female</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>Total IgE†</td>
<td>IU/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>113.5 (67.6 – 417.3)</td>
<td>148.1 (95.7 – 447.0)</td>
</tr>
<tr>
<td>JCP-specific IgE‡</td>
<td>UA/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.9 (10.2 – 26.3)</td>
<td>21.2 (18.0 – 48.5)</td>
</tr>
</tbody>
</table>

†Means ± SD  ‡Geometric means (95% confidence intervals)

on subjective symptom scores for each subject. Goodness of fit test for the normality of the data revealed that the symptom scores and AUCs did not follow a normal distribution. Therefore, logarithmic transformation of the data was used to alleviate the deviation from the normality. Since this study is two-way crossover design, firstly, the presence of a treatment-by-period interaction was checked by analysis of variance methods including treatment, period and treatment-by-period effect. Because the interaction was not identified in this study for each variable, the Wilcoxon signed rank test was performed to compare BB536 efficacy with placebo. Medication scores were analyzed similarly as symptom scores. Furthermore, prevalence of medication use between the BB536 and the placebo groups were compared using a chi-square test or Fisher’s exact test. All statistical analyses were performed using SAS statistical software version 9.1.3 (SAS Institute, NC, USA). Values of $p < 0.05$ were considered statistically significant.

Fig. 2  Mean scores for subjective symptoms before and during pollen exposure in the environmental exposure unit for the BB536 group (closed squares) and placebo group (open circles). (A) Nasal symptoms; (B) Ocular symptoms; (C) Throat symptoms; (D) Disruption of normal activities. Data are expressed as geomeans. †, $p < 0.1$; *, $p < 0.05$, difference between groups.
Table 2  AUC analysis of symptom scores

<table>
<thead>
<tr>
<th>Symptoms during exposure</th>
<th>Placebo group</th>
<th>BB536 group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular symptoms</td>
<td>6.85 (4.20–11.18)</td>
<td>4.40 (2.70–7.18)</td>
<td>0.033</td>
</tr>
<tr>
<td>Throat symptoms</td>
<td>2.08 (1.43–3.03)</td>
<td>2.89 (1.85–4.51)</td>
<td></td>
</tr>
<tr>
<td>Disruption of daily routines</td>
<td>2.23 (1.45–3.45)</td>
<td>2.30 (1.44–3.67)</td>
<td></td>
</tr>
<tr>
<td>Symptoms post exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular symptoms</td>
<td>2.81 (1.96–4.03)</td>
<td>2.48 (1.64–3.76)</td>
<td></td>
</tr>
<tr>
<td>Throat symptoms</td>
<td>2.07 (1.48–2.89)</td>
<td>1.79 (1.31–2.44)</td>
<td></td>
</tr>
<tr>
<td>Disruption of daily routines</td>
<td>2.03 (1.45–2.85)</td>
<td>1.52 (1.15–2.00)</td>
<td>0.011</td>
</tr>
<tr>
<td>Oral medicines</td>
<td>1.39 (1.07–3.07)</td>
<td>1.17 (1.03–1.40)</td>
<td>0.093</td>
</tr>
<tr>
<td>Nasal sprays</td>
<td>1.44 (0.82–3.42)</td>
<td>1.31 (0.97–2.07)</td>
<td></td>
</tr>
<tr>
<td>Eye drops</td>
<td>1.48 (0.99–3.20)</td>
<td>1.20 (0.99–1.98)</td>
<td>0.047</td>
</tr>
<tr>
<td>Total medications†</td>
<td>2.27 (1.34–3.83)</td>
<td>1.79 (1.20–2.65)</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Values are expressed as geomeans (95% CIs).
†Sum of medication scores for oral medicines, nasal sprays and eye drops.

RESULTS

BASELINE CHARACTERISTICS OF PARTICIPANTS AND CONTINUED PARTICIPATION
Among the 24 subjects, one dropped out due to failure to undergo the second pollen exposure. Two subjects (one in group A and one in group B) had colds during the second exposure test and had extremely high baseline symptom scores prior to the exposures. Therefore, these two subjects were excluded from the analysis of symptoms scores during and after exposures. Baseline characteristics of the other 21 subjects are shown in Table 1. The characteristics were similar between the placebo and BB536 groups (Table 1).

SYMPTOM SCORES DURING POLLEN EXPOSURE
Figure 2 shows mean scores for subjective symptoms in each group. The baseline scores recorded 30 minutes before exposures were similar between the placebo and the BB536 groups for scores of nasal symptoms, ocular symptoms and disruption of normal activities; however, slightly higher baseline scores were found for throat symptoms in the placebo group compared with the BB536 group (p = 0.114). Nasal symptom scores increased rapidly, but other symptom scores (ocular symptoms, throat symptoms and disruption of normal activities) increased gradually upon exposure to pollens. No significant difference was observed for the scores of nasal symptoms and disruption of normal activities; however, significantly lower scores were found for ocular symptoms starting from 30 minutes after exposure to the pollens. There was also no difference in the scores for each of the nasal symptoms of sneezing, nose blowing and nasal blockage between the two groups, but both of the scores of eye itching and watery eyes were reduced in the BB536 group (data not shown). Owing to the difference of the baseline, a significant difference was found on the throat symptom scores that were rated from 30 to 60 minutes. AUC analysis indicated a significant difference for the ocular symptom scores (p = 0.033), but there was no difference found for the other symptoms (Table 2).

DELAYED SYMPTOM AND MEDICATION SCORES AFTER POLLEN EXPOSURE
Remarkable delayed symptoms were observed beginning with the first day of exposures and continued through the next day (Fig. 3). No marked differences were apparent in the nasal and ocular symptom scores between the placebo and BB536 groups. There was no difference in the scores for each of the nasal or ocular symptoms between groups (data not shown). Throat symptom scores tended to be lower on the day after the exposures, and scores for disruption of normal activities tended to be lower on the two days following exposure in the BB536 group compared with the placebo. AUC analysis indicated a significant difference (p = 0.011) for the scores of disruption of normal activities between the two groups, but there was no difference for the other symptoms (Table 2).

Medication use for relieving symptoms was observed in some subjects, particularly on both the first
Fig. 3 Mean scores for subjective symptoms and medications after pollen exposure in the environmental exposure unit for the BB536 group (closed squares) and placebo group (open circles). (A) Nasal symptoms; (B) Ocular symptoms; (C) Throat symptoms; (D) Disruption of normal activities. (E) Oral medicines; (F) Nasal sprays; (G) Eye drops; (H) Total medications. Data are expressed as geomeans. Total medications are the sum of scores for oral medicines, nasal sprays and eye drops. †, p < 0.1; *, p < 0.05, difference between groups.
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Table 3  Prevalence of medication used post exposures

<table>
<thead>
<tr>
<th>Medication</th>
<th>Placebo</th>
<th>BB536</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>Oral medicines</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<td>2</td>
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<td>2</td>
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</tr>
<tr>
<td>Nasal sprays</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Eye drops</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>3</td>
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<td>2</td>
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<td>Total study group</td>
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* p < 0.05, difference in total counts vs placebo groups, Fisher’s exact test.

Day and the day following exposures (Fig. 3). No significant differences were apparent in the numbers of subjects taking these medications; however, significantly lower prevalence was found for the total counts of the number of days for oral medication and eye drop usage in the BB536 group when compared to the placebo group (Table 3). AUC analysis indicated significantly lower scores for eye drops (p = 0.047) and the total medications used (p = 0.041) along with a tendency towards a decrease in oral medication use (p = 0.093) (Table 2).

**BLOOD PARAMETERS**

No marked changes were found for the total and JCP-specific IgE levels between the first and the second blood samplings as well as between the BB536 and placebo groups.

**DISCUSSION**

This double-blind, placebo-controlled, crossover study indicated that as compared to placebo, BB536 intake was associated with a significant improvement of ocular symptoms during pollen exposures, and an improvement of both the scores for the disruption of normal activities and the need for medication use after JCP exposure in patients with JCP allergy. These results confirm the effect of BB536 intake in relieving subjective symptoms.

The efficacy of probiotics in preventing allergic development and treating already established allergic diseases remains controversial. Many reasons could account for the different outcomes in the various clinical trials and include variation of the dose and intake period, differences in the type and the severity of the symptoms involved, and most importantly, differences in the species and the strains of the probiotics used. In order to overcome the problems associated with field-based studies, such as differences in pollen amounts and symptom severities that are found within or between seasons, we assessed the efficacy of BB536 intake on the output of symptoms under controlled exposures to JCP in an EEU during the non-pollen season in a two-way crossover design. Our results suggest that this method is useful for comparative study of different agents in allergy disease treatment.

Allergic disorders, such as allergic asthma, rhinitis and atopic dermatitis, are characterized by an immediate hypersensitivity reaction followed by a delayed inflammatory reaction (late-phase response). Type I allergy is characterized by a Th2-skewed immune response upon exposure to allergens, which causes the synthesis of allergen-specific IgE. The immediate reactions, which included responses such as sneezing, rhinorrhea, eye symptoms, etc, which are observed with early contact with the sensitizing allergen, are consecutive with the IgE-dependent activation of the mast cells that subsequently release stored mediators that are preformed in the granules, such as histamine or tumor necrosis factor-α, or are newly synthesized, such as arachidonic acid metabolites prostaglandin. The late inflammatory reactions, which included responses such as nasal blocking, etc, that occurs seven hours after contact with the allergen and which contributes to cell damage are characterized by tissue infiltration by eosinophils and T lymphocytes (mainly CD4+ T helper 2 cells). The infiltration is tightly controlled by chemokines such as TARC and MDC (macrophage-derived chemokine) that selectively attract Th2 memory T cells.

The reason why BB536 demonstrates benefits only on ocular symptoms and not on other symptoms during exposures is unclear, however, several explanations can be proposed. First, differences existed for exposure patterns between EEU and the pollen season. In the EEU, it was designed to be a concentrated, heavy exposure for a few hours. Within such a short time, immunological changes with possible relation to symptom development would not be expected. Under natural conditions, however, the exposure is successive for several months. There is also a preseason with a mild pollen scattering before the onset of pollen season. Such an induction phase as well as the successive exposure would affect the immunological system. These differences might have influenced the outcomes of BB536 intake. Second, there
were differences in responses for each symptom in the EEU. Nasal symptoms responded abruptly upon exposure in the EEU, especially with regard to sneezing and rhinorrhea, which increased to maximum scores within 30 to 60 minutes. However, eye symptoms showed gradual responses upon exposure. Furthermore, our results and a previous report indicated that EEU exposure tended to cause rapid response of nasal blocking, and delayed symptoms occurred several hours after exposure to allergens under natural exposure. The unusual responses of nasal symptoms might have limited the effects of BB536 intake. Thirdly, another limitation of BB536 efficiency may be related to the EEU exposure. The effect of probiotics in the treatment of allergy diseases has been suggested to occur via a modulation of the immune responses. BB536 was found to significantly suppress the increase of plasma TARC in a trial carried out in the 2005 JCP season. In contrast to other treatments such as histamine release inhibitors or antihistamines, the effects of probiotics are expected to be mild and have a lag in the effect on expression. For these reasons, in the present study BB536 was administrated for 4 weeks before pollen exposure; however, the short time (4 hours) combined with the heavy amounts of pollen used might have surpassed the efficiency limit of BB536 in alleviating all symptoms during pollen exposures.

Interestingly, we found that some of the delayed symptoms and the medication use after exposures were reduced by BB536 intake. Similarly, we found that BB536 intake alleviated the subjective symptoms and increased the number of JCPs's subjects that did not have to take their prescribed pollinosis medicines during the heavy 2005 pollen season. Eye drops, nasal sprays or oral pollinosis medications are usually prescribed for practical precaution, self-care, or medical treatment. Since possible adverse effects can be a problem with such treatments, it would be desirable if the use of these medications could be decreased, and therefore, probiotic use is a worthwhile goal to pursue.

In conclusion, the present study confirms the beneficial impact of BB536 intake on JCP allergies that have been reported in studies undertaken during previous pollen seasons. Additionally, other benefits noted include the relief of subjective symptoms and a concurrent reduction in medication use as compared to the placebo response to allergen exposure.

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REFERENCES

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