Multi-strain Symbiotic Preparations as a Novel Adjuvant Approach to Allergic Rhinitis

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Abstract

Objective. To investigate the effects of Lactobacillus acidophilus NCFM / Bifidobacterium lactis BL-04 / fructo-oligosaccharide and Lactobacillus plantarum LP01/ Lactobacillus paracasei LPC00 / fructo-oligosaccharide preparations in the routine clinical management of seasonal allergic rhinitis (SAR) and perennial allergic rhinitis (PAR), respectively.

Materials and Methods. Seventy-six and fifty-one outpatients attending 36 Allergy Clinics in Italy with clinically documented allergic rhinitis consumed a symbiotic combination of Lactobacillus acidophilus NCFM / Bifidobacterium lactis BL-04/ fructo-oligosaccharide and Lactobacillus plantarum LP01/ Lactobacillus paracasei LPC00 / fructo-oligosaccharide over a period of 4 months. Data on Allergic Rhinitis and Impact on Asthma (ARIA) classification, nasal symptoms severity by Visual Analogical Scale (VAS), and concomitant use of corticosteroids and antihistamines drugs were collected after 2 and 4 months of treatment (T1 and T2 respectively).

Results. After the treatment with two multi-strain symbiotic preparations a significant reduction between baseline evaluation (T0) and T1/T2 on total nasal symptoms and ARIA classification of rhinitis were observed. A significant decrease on VAS index at all time points of the treatment vs pre-treatment was detected for Lactobacillus plantarum / Lactobacillus paracasei symbiotic combination; significant decrease was observed for Lactobacillus acidophilus / Bifidobacterium lactis symbiotic combination between T0 and T1, while a further reduction at T2 did not reach statistical significance. In addition a decrease in consumption of orally-administered corticosteroids and antihistamines drugs was found.

Conclusion. These data on the effect of Lactobacillus acidophilus NCFM / Bifidobacterium lactis BL-04 / fructo-oligosaccharide and Lactobacillus plantarum LP01/ Lactobacillus paracasei LPC00 / fructo-oligosaccharide preparations support their potential positive effect in the routine clinical management of subjects with SAR and PAR, respectively. Future studies are warranted to confirm this potential beneficial effect.

Keywords: Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus plantarum, Lactobacillus paracasei, Allergic rhinitis, Clinical practice, Symbiotic preparations

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1. Introduction

Allergic rhinitis is a chronic disorder which significantly impacts quality of life. Its prevalence ranges from 10% to 25% in the general population, with a substantial increase over the last decades (Bousquet et al., 2001; Janson et al., 2001; Asher et al., 2006). Several treatment strategies for allergic rhinitis are available, and their use is well established (Bousquet et al., 2001; Bousquet et al., 2008; Brozek et al., 2010; Demoly et al., 2003). Recently, novel approaches aimed at reducing and preventing allergic symptoms have been proposed. Data from pre-clinical and clinical research have highlighted new trends based on the use of probiotics in the management of allergic rhinitis (Kalliomaki et al., 2010; Laitinen et al., 2005; O’Flaherty 1982) as well as in other allergic diseases.

Probiotics are defined as live microorganisms capable to provide a health benefit to the host and are frequently co-administered (symbiotic preparation) with non-digestible dietary compounds (prebiotics) in order to enhance the growth/activity of specific probiotics (Guarner et al., 1998; Gibson and Roberfroid, 1995; Van Zanten et al., 2012). Probiotics such as Lactobacillus and Bifidobacterium species are commonly used for prevention/treatment of immune-mediated diseases due to their immunomodulatory effect on immune response (Madsen et al., 1999; Madsen et al., 2001; Pessi et al., 2000).

The effects of specific probiotic strains in the management (Isolauri et al., 2000) and in the prevention of atopic eczema (Kalliomäki et al., 2007; Kalliomäki et al., 2003; Kalliomäki et al., 2001; Manzotti et al., 2014) have been formally addressed in studies demonstrating their positive effects on inflammation. To date, data on the effect of probiotics on allergic rhinitis are scarce (Isolauri et al., 2000).

The aim of the present study is to evaluate the effect of specific multi-strain symbiotic preparations in subjects with clinically confirmed seasonal (SAR) and perennial allergic rhinitis (PAR).

2. Materials and Methods

2.1 Participants, Study Protocol, Characteristics and Outcomes

Two different observational studies were performed to assess the effect of the administration of multistrain symbiotics. In the first one, Lactobacillus acidophilus NCFM / Bifidobacterium lactis BL-04 / fructo-oligosaccharide were used in the management of SAR, while in the second one Lactobacillus plantarum LP01 / Lactobacillus paracasei LPC00 / fructo-oligosaccharide were used in the routine management of PAR.

The populations involved in the two studies comprised of 76 and 51 outpatients affected by SAR and PAR, respectively, attending 36 participating outpatient Allergy Clinics from April 2013 to May 2014 with clinically documented allergic rhinitis. Patients using prebiotics and/or probiotics at enrollment were excluded from the study.

Industrial combinations of 2 different products were used in each individual study: i) Lactobacillus acidophilus NCFM (1.2510⁹ UFC/sachet) / Bifidobacterium lactis BL-04 (3.75 10⁹ UFC/sachet) /
fructo-oligosaccharide (1 g) preparation (Pollagen®, Allergy Therapeutics Italia, Milan, Italy) in the study involving patients with SAR ii) *Lactobacillus plantarum* LP01 (≥ 10⁹ UFC/sachet) / *Lactobacillus paracasei* LPC00 (≥ 10⁹ UFC/sachet) / fructo-oligosaccharide (2.5 g) preparation (AtiProb®, Allergy Therapeutics Italia, Milan, Italy) in the study performed to assess their effect on PAR.

Each outpatient used 1 oral powder sachet per day (≥ 10⁹ of bacteria for each strain). This dosage is in agreement with the guidelines of the Italian Ministry of Health on probiotics (Italian Ministry of Health, 2013). In addition, the AtiProb combination was microencapsulated in order to offer significant protection against gastric juices, while the Pollagen combination is composed of acid-resistant bacteria strains.

The study consisted of three consecutive time points: baseline visit (T0), evaluation after 2 months (T1) and evaluation after 4 months (T2) of symbiotic consumption. The primary outcome of the study was the change of total nasal symptoms evaluated by VAS, the shift in ARIA classification of rhinitis (Bousquet et al., 2001; Bousquet, et al., 2008; Brozek et al., 2010; Demoly et al., 2003), and the change of corticosteroids and antihistamines assumption before and after the treatment.

### 2.2 Data Recording and Questionnaire

All included subjects were evaluated using a questionnaire based on the following categories:

- Demographic characteristics
- VAS for total nasal symptoms
- ARIA classification of allergic rhinitis
- Concomitant treatment with corticosteroids and antihistamines medications

### 2.3 Statistical Analysis

Descriptive statistics were performed using Excel Microsoft Office. Comparisons at different time points (T1/T2) vs baseline (T0) were analyzed using the Student’s unpaired t-test. The McNemar test was used to compare the effect on ARIA severity score. A two-tailed P value ≤0.05 was considered statistically significant. Statistical analyses were conducted using PSP (psppire.exe 0.8.3-g5f5de6; A program for the analysis of sampled data; Free Software Foundation; [http://www.gnu.org/software/pspp/](http://www.gnu.org/software/pspp/); GNU GENERAL PUBLIC LICENSE Version 3, 29 June 2007).

### 3. Results

#### 3.1 *Lactobacillus acidophilus NCFM* / *Bifidobacterium lactis BL-04* / fructo-oligosaccharide preparation effect in SAR

Seventy-six participants (54% males and 46% females) were evaluated at T0/T1. The dropout rate at T2 was 30% (23 patients withdrew from the study). At baseline the mean age of subjects was 32 years and the distribution of allergic sensitization was heterogeneous among different patients with no clear prevalence of a specific airborne allergen (data not shown). Forty-five percent of the
evaluated patients were not concomitantly treated with allergen-specific immunotherapy, 17% were treated with subcutaneous immunotherapy (SCIT) and 21% with sublingual immunotherapy (SLIT) (Figure 1).

![Diagram showing immunotherapy treatments]

**Fig. 1.** Immunotherapy (SIT) treatments of the study subjects at baseline.

Abbreviations: SIT = Specific immunotherapy; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy; ND = not determined.

The mean VAS score for total nasal symptoms was significantly reduced after 2 months of *Lactobacillus acidophilus NCFM /*Bifidobacterium lactis BL-04*/fructo-oligosaccharide supplementation (5.16±0.29, p=0.02); after 4 months a further reduction trend could be observed, but this change was not significantly different from T0 (4.94±0.37, p=0.06) (Figure 2).

![Graph showing VAS scores for total nasal symptoms]

**Fig. 2.** Mean VAS score for total nasal symptoms at T1 and T2 vs T0 in subjects affected by SAR. (* p<0.05). Abbreviations: NS = not statistically significant vs T0.
Concomitantly, a shift in ARIA classification of rhinitis was observed, with more patients being affected by intermittent (rather than persistent) and mild (rather than moderate-severe) rhinitis at T1 and T2 (p=0.0003 and p=0.0011, respectively, compared to T0) (Figure 3).

**Fig. 3.** Patient classification of rhinitis (number of subjects in each group) according to ARIA, in subjects affected by SAR (T1 vs T0, p=0.0003; T2 vs T0, p=0.0011).

Use of antihistamine drugs (mean ± se) in the last 2 months significantly decreased from 23.39±2.96 at T0 to 15.25±2.31 at T1 and finally to 10.15±1.73 at T2 (p<0.0001) (Figure 4). The concomitant use of orally administered corticosteroid drugs (prednisone mg/die or other drug of equivalent potency, mean ± se) in the last 2 months significantly decreased from 5.82±2.36 at T0 to 3.53±1.45 at T1 and finally to 1.72±0.96 at T2 (p=0.03 at T1 and p=0.02 at T2 vs T0) (Figure 5). The concomitant use of intranasal corticosteroids in the last 2 months (number of packages, mean ± se) significantly decreased at T1 (0.53±0.09, p<0.0001) vs T0 (1.01±0.12) but significantly increased at T2 (3.81±0.45 p<0.0001) (Figure 6).
Fig. 4. Use of antihistamine drugs at T1 and T2 compared to T0, in the last 2 months in subjects affected by SAR (**p<0.0001).

Fig. 5. Use of corticosteroid drugs (oral formulation) at T1 and T2 compared to T0, in the last 2 months in subjects affected by SAR (**p=0.03 at T1 and p=0.02 at T2 vs T0).
**Fig. 6.** Use of intranasal corticosteroid drugs (number of packages used) at T1 and T2 compared to T0, in the last 2 months in subjects affected by SAR (***p<0.0001).

### 3.2 Lactobacillus plantarum LP01/ Lactobacillus paracasei LPC00/ fructo-oligosaccharide preparation effect on PAR.

Fifty-one subjects (57% males and 43% females) were evaluated at T0/T1. The dropout rate at T2 was 37%. At baseline the mean age of subjects was 31 years and the distribution of allergic sensitization was heterogeneous among the different patients with no clear prevalence of a specific airborne allergen (data not shown). Twenty-seven percent of the evaluated patients were concomitantly treated with allergen-specific immunotherapy, 10% were treated with SCIT and 17% with SLIT (Figure 7).

**Fig. 7.** Immunotherapy (SIT) treatments of the study subjects at baseline. Abbreviations: SIT = Specific immunotherapy; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy; ND = not determined.
With regard to total nasal symptoms VAS, the mean value was significantly decreased after 2 months of treatment (4.90±0.31, p=0.04) and after 4 months (3.88±0.49, p=0.01) vs baseline (5.84±0.31) (Figure 8).

![VAS Score for Total Nasal Symptoms](image.png)

**Fig. 8.** Mean VAS score for total nasal symptoms at T1 and T2 vs T0, in subjects affected by PAR (* p<0.05 vs T0).

A statistically significant shift in ARIA classification of rhinitis was observed, with more patients being affected by intermittent (rather than persistent) and mild (rather than moderate-severe) rhinitis at T1 (p=0.02); a further trend toward reduction was observed at T2 vs T0, but this change was not significantly different from T0 (p=0.17) (Figure 9).
**Fig. 9.** Patients classification of rhinitis (number of subjects in each group) according to ARIA, in subjects affected by PAR (T1 vs T0, p=0.02; T2 vs T0, p=0.17).

**Fig.10.** Use of antihistamine drugs in the last 2 months, at T1 and T2 compared to T0, in subjects affected by PAR (***p<0.0001).
The concomitant use of antihistamine drugs (mean ± se) in the last 2 months significantly decreased from 37.53±5.47 at T0 to 16.82±3.51 at T1 and finally to 5.73±1.68 at T2 (p<0.0001) (Figure 10).

The concomitant use of orally administered corticosteroids drugs (Prednisone mg/die or other drug of equivalent potency) in the last 2 and 4 months showed a decreased trend that did not reach statistical significance because of the reduced sample size of the study population due to drop-outs (data not shown).

The concomitant use of intranasal corticosteroids in the last 2 months (number of packages, mean ± se) significantly decreased at T1(0.51±0.11, p<0.0001) vs T0 (1.27±0.21) and significantly increased at T2 (2.59±0.50 p<0.0001) (Figure 11).

![Intranasal Corticosteroids](image)

**Fig. 11.** Use of intranasal corticosteroid drugs (number of packages used) at T1 and T2 compared to T0, in the last 2 months in subjects affected by PAR (***p<0.0001).

### 4. Discussion

Direct evidence of specific *Lactobacillus plantarum* LP01, *Lactobacillus paracasei* LPC00, *Lactobacillus acidophilus* NCFM, *Bifidobacterium lactis* BL-04 strains activity in the control of allergic rhinitis symptoms is well reported in literature with a number of studies using these stains separately. Here we reported the effects of two different multi-strain symbiotic preparations on patients affected by SAR and PAR after 2 and 4 months of treatment.
Of the total of 76 subjects affected by SAR who were evaluated at T0/T1, 53 were checked at T2; 23 patients didn't show for the follow-up evaluation. No significant adverse events were reported by any participant. The dropout rate of 30% is acceptable given that this is an observational study.

Of the total of 51 subjects affected by PAR who were evaluated at T0/T1, 32 were checked at T2; nineteen patients didn't show for the follow-up evaluation, with a dropout rate of 37%. No significant adverse events were reported by any participant. The reasons for the higher rate of dropout, compared to the subjects affected by SAR, are not known.

The results obtained from these two different observational studies showed a significant reduction between T0 and T1/T2 on total nasal symptoms and a shift in ARIA classification of disease during the four-month observation period.

A significant decrease on VAS index at all time points of treatment vs pre-treatment was detected for the symbiotic multi-strain composed of *Lactobacillus plantarum* LP01/*Lactobacillus paracasei LPC00*/ fructo-oligosaccharide (ATIprob®; Allergy Therapeutics, Milan, Italy); for the other symbiotic multi-strain (Pollagen®; Allergy Therapeutics, Milan, Italy), composed of *Lactobacillus acidophilus NCFM*/Bifidobacterium lactis BL-04*/fructo-oligosaccharide*, a significant decrease was observed only between T1 and T0, with a further reduction trend at T2 which approached statistical significance (without reaching it possibly because of the reduced size of the study population due to dropouts).

These data support a potential positive effect of *Lactobacillus acidophilus NCFM*/Bifidobacterium lactis BL-04*/fructo-oligosaccharide* preparation and *Lactobacillus plantarum* LP01/*Lactobacillus paracasei LPC00*/fructo-oligosaccharide preparation in subjects with SAR and PAR, respectively, in the context of routine clinical practice, with a significant reduction in severity after 2 and 4 months of treatment compared to baseline. This suggests the importance of the strain-specific effects of *Lactobacillus acidophilus NCFM*, *Bifidobacterium lactis BL-04*, *Lactobacillus plantarum* LP01, *Lactobacillus paracasei LPC00* highlighting the concept of the strain-specific effects of probiotic preparations.

The observed decrease in the utilization of co-administered oral antihistamine drugs and oral corticosteroids, during the study period with this symbiotic multi-strain combination, may represent additional evidence of the beneficial effect of the two studied multi-strain symbiotic preparations in the management of SAR and PAR. These studies also show an increase in the concomitant use of intranasal corticosteroids at T2, probably due to an improvement in nasal symptoms which prompts the patient to consume less oral drugs and use more intranasal drugs.

Importantly, the symbiotic multi-strain formulation used in the present study contained ≥10⁹ bacteria for each strain (at the end of the 2 year shelf-life). Moreover, the utilization of microencapsulated products capable of offering considerable protection against gastric juice and the addition of prebiotic FOS could be important factors ensuring a better colonization of the intestinal tract with an increased number of viable bacteria, and therefore positively influencing the effect of these probiotic strains.
Our data support the hypothesis that an adequate symbiotic multi-strain supplementation can be suggested as a novel adjuvant approach in the management of SAR and PAR, and that *Lactobacillus acidophilus NCFM*, *Bifidobacterium lactis BL-04*, *Lactobacillus plantarum LP01*, *Lactobacillus paracasei LPC00* are relevant candidates for this purpose. Further studies and randomized trials with larger patient populations are needed to confirm this positive effect with particular focus on strain-specific outcomes, usefulness of concomitant prebiotic and probiotic supplementation, dose optimization, microencapsulation, and other relevant manufacturing specifications.

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**Conflict of Interests**

Filippo Fassio received consultancy fees from Allergy Therapeutics Italia.

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