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ORIGINAL CONTRIBUTION

Randomised, double-blind and placebo-controlled study using new probiotic lactobacilli for strengthening the body immune defence against viral infections

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Abstract
Background The aim of this study was to investigate whether consumption of *Lactobacillus plantarum* HEAL 9 (DSM 15312) and *Lactobacillus paracasei* 8700:2 (DSM 13434) could affect naturally acquired common cold infections in healthy subjects.
Methods A randomised, parallel, double-blind placebo-controlled study was performed to investigate whether intake of this probiotic mixture could reduce the risk of common cold episodes, number of days with common cold symptoms, frequency and severity of symptoms, and cellular immune response in common cold infections. A total of 272 subjects were supplemented daily with either 10^9 cfu (colony forming units) of probiotics ($N = 135$) or control ($N = 137$) for a 12-week period.
Results The incidence of acquiring one or more common cold episode was reduced from 67% in the control group to 55% in the probiotic group ($p < 0.05$). Also, the number of days with common cold symptoms were significantly ($p < 0.05$) reduced from 8.6 days in the control group to 6.2 days, in the probiotic group, during the 12-week period. The total symptom score was reduced during the study period from a mean of 44.4 for the control group to 33.6 for the probiotic group. The reduction in pharyngeal symptoms was significant ($p < 0.05$). In addition, the proliferation of B lymphocytes was significantly counteracted in the probiotic group ($p < 0.05$) in comparison with the control group.
Conclusion In conclusion, intake of the probiotic strains *Lactobacillus plantarum* HEAL 9 (DSM 15312) and *Lactobacillus paracasei* 8700:2 (DSM 13434) reduces the risk of acquiring common cold infections.

Keywords Probiotic · Immune defence · Common cold · *Lactobacillus plantarum* · *Lactobacillus paracasei* · DSM 15312 · DSM 13434

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Introduction

The occurrence of common cold is very frequent in the community, and on average, children have 6–8 and adults 2–4 colds per year [1]. By tradition, foods or dietary supplements with high levels of vitamin C or Echinacea have been consumed to try to reduce the incidence or severity of common cold. It is known that the symptoms associated with common cold are a result of the inflammatory response by the host towards the infection. Therefore, compounds with the right type of anti-inflammatory activity are supposed to be effective antivirals. Examples of compounds with anti-inflammatory activity are probiotics. The current definition by The World Health Organization of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [2]. However, the effect on the immune system is highly strain specific, and evaluation of effects on the immune system must be done using the strains per se.

Different probiotic strains have been shown to affect the immune system and also the incidence of common colds. In young children, intake of probiotics reduced missed school days [3] and absence from day care [4] because of illness. In the study by Hatakka et al. [4] conducted on 571 children aged 1–6, intake of *Lactobacillus* GG during a 7-month period resulted in a relative reduction in the number of children suffering from respiratory infections

with complications and lower respiratory tract infections in comparison with a control group without probiotic supplementation. In the study by Leyer et al. [3] conducted on 326 children between 3–5 years, a 7-month intake of a probiotic mixture containing *Lactobacillus acidophilus* NCFM (ATCC 700396) and *Bifidobacterium animalis* lactic Bi-07 reduced duration as well as incidence of fever, rhinnorhea and cough in comparison with placebo.

In healthy adults, intake of a probiotic mixture (*Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *Bifidobacterium bifidum* MF 20/5 (5×10^7 cfu/tablet)) reduced the severity of symptoms as well as the duration of common cold [5, 6]. A significantly higher enhancement of T cytotoxic plus T suppressor cells (CD8+) and a higher enhancement of T helper cells (CD4+) were also observed in the probiotic-treated group. In a similar study by Winkler et al. [7], using the same probiotic mixture but administered in a higher dose (5×10^8 cfu/tablet), the incidence of respiratory tract infections was lower in the probiotic group compared to the placebo group ($p = 0.07$). An increase particularly in T lymphocytes including CD4+ and CD8+ cells was also observed in the probiotic group during the first 14 days of supplementation compared to placebo.

The strains used in this study have earlier been shown to have an effect on the immune system. Intake of *Lactobacillus paracasei* 8700:2 efficiently induced cell-mediated immune functions in healthy volunteers (Rask et al. unpublished data). Further, an induction of TGF-beta and IL-10 was reported after intake of *Lactobacillus plantarum* HEAL 9, *Lactobacillus plantarum* HEAL 19 and *Lactobacillus paracasei* 8700:2 in a model for experimental autoimmune encephalomyelitis [8]. The ability of these bacteria to get established in the human intestine has been documented in earlier studies [9–11].

Materials and methods

Design of the study

The study was randomised, double-blind and placebo-controlled with two parallel arms and was carried out at two different sites in Sweden between January 2007 and May 2007. One site was situated in Lund (site 1) in the south of Sweden and the other in Uppsala (site 2), situated approximately 650 km north of Lund.

Sample size

The calculated sample size was based on the assumption that the risk to get an episode would be at least 20% less in the group receiving probiotics (probiotic group) in comparison with a control. To detect such a difference with a two-tailed test significance level of 0.05 and a power of 90%, 140 subjects were needed in each group.

Subjects

A total of 318 healthy volunteers were recruited by advertising in local press. The subjects were healthy and aged 18–65. Exclusion criteria were known intolerance or allergy to any ingredient included in the formulations, medically treated allergy, current treatment for severe gastrointestinal disorders, pregnancy or lactation, vaccination against influenza within the last 12 months or smoking. Prior inclusion, all subjects gave their informed consent to participate in the study.

Forty-six subjects withdrew from the study, and finally 272 subjects (151 from Lund and 121 from Uppsala) were included in the statistical analysis (Fig. 1; Table 1). Demographic characteristics for the subjects are described in Table 2. There were no significant differences in gender distribution, age and BMI between the probiotic and the control group or between the two study sites.

The subjects consumed either *Lactobacillus plantarum* HEAL 9 and *Lactobacillus paracasei* 8700:2 (1×10^9 cfu/day) or a control product. There was a 2-week run-in period before the subjects got the study product for 12 weeks. The subjects were not allowed to ingest other products containing probiotic bacteria during the study period including the 2 weeks of run-in period.

Faecal samples were handed in after the run-in period (0-sample), after 2 weeks of consumption of the study products (I-sample) and at the end of the study (II-sample) to control compliance and faecal recovery of the probiotic

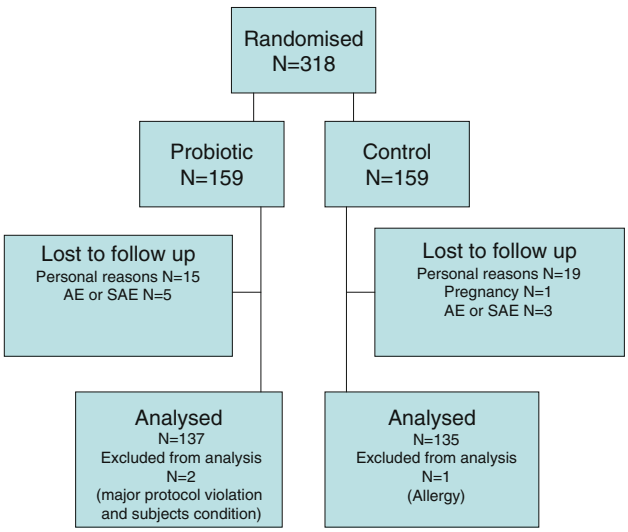

Fig. 1 Diagram showing the flow of participants through the stages of the study

Table 1 Distribution of subjects per site, sex and treatment

Site	Included	Withdrawn	Treatment	Sex		Total
				Women	Men	
Site 1	178	27	Probiotic	47	30	77
			Control	45	29	74
			Total	92	59	151
Site 2	140	19	Probiotic	43	17	60
			Control	45	16	61
			Total	88	33	121
Total	318	46	Probiotic	90	47	137
			Control	90	45	135
			Total	180	92	272

Table 2 Demographics characteristics per treatment group

Characteristic	Control	Probiotic	P^a
N	135	137	
Gender			
Male *N* (%)	45 (33.3)	47 (34.3)	–
Female *N* (%)	90 (66.7)	90 (65.7)	
Ethnicity			
Caucasian *N* (%)	135 (100)	136 (99.3)	–
Other *N* (%)		1 (0.7)	
Age (year)	43.7	46.5	0.066
BMI (kg/m^2)	24.2	24.8	0.091
Weight (kg)	72.4	73.2	0.376
Height (cm)	172.4	171.7	0.702

[a] Significance for changes in the variable considered during each treatment when compared to control group

bacteria. In addition, at site 2, blood samples were collected after the run-in period and after 2 weeks of product intake for analysis of cellular immune responses.

Evaluation of symptoms

Daily records of health status as well as symptoms of common cold, if acquired, were made by the participants throughout the study period in a diary. The included symptoms for common cold were nasal (runny nose, stuffed nose, yellow secretion, bloody secretion, sneezing), pharyngeal (scratchy throat, sore throat, hoarseness), bronchial (cough, secretion, yellow secretion), and headache, myalgia, conjunctivitis, ear ache, sinusitis, fatigue, loss of appetite, nausea and fever. The severity of the symptoms was graded: no symptoms as 0, mild symptoms as 1, moderate symptoms as 2 and severe symptoms as 3. Sneezing, fatigue, loss of appetite and fever were graded as yes or no. The subject's intake of the product, adherence to the dietary regulations and sample collection were also noted in a diary. The diary was inspected by the investigator upon each visit (day 14, day 84), and all diaries were collected immediately upon completion of the study.

The start of a common cold episode was defined as the occurrence of a total symptom score of 2 or more [12] and defined as over when sickness score was less than 1 for nasal, pharyngeal or bronchial symptoms. A total score was calculated by adding up the daily individual symptom scores. With the symptoms graded yes or no, yes was calculated as 1 in case of sneezing, fatigue, loss of appetite and as 3 with fever.

Permission to carry out the study was approved by the Ethical Review Board Uppsala. The study was carried out following the principles in the World Medical Association Declaration of Helsinki and GCP.

Test preparations

Study products were handed out in sachets, and the volunteers were instructed to ingest the powder in association with a meal once a day. They were also instructed not to add the powder to hot drinks or food.

The control product contained 1.0 g maltodextrine (Glucidex®6**,** Roquette, France), and the probiotic product contained 1.0 g of maltodextrine and lyophilised bacteria (*Lactobacillus plantarum* HEAL 9 (DSM 15312) [11] and *Lactobacillus paracasei* 8700:2 (DSM 13434) [13] (1×10^9 cfu/day). The two test products were identical in appearance and taste.

Storage stability of the probiotic product was performed throughout the study. The study material was analysed for viable count after storage in room temperature (23 ± 2 °C) and in fridge (8 ± 2 °C) by Probi AB:s laboratory (accredited by SWEDAC*, according to ISO/IEC 17025:2005). The probiotic product contained $> 1 \times 10^9$ cfu/g for more than 120 days after storage in both room temperature and fridge.

Faecal analysis of lactobacilli

Faecal samples were handed in after run-in (day 0), after 14 days intake of the study products and at the end of study. The samples were analysed with regard to the lactobacilli flora using a quantitative real-time PCR methodology.

Stool samples were refrigerated and stored in plastic containers at −80 °C until analysis. DNA was extracted with QIAamp DNA Stool Mini kit (Qiagen) according to the manufacture's instructions, after prior double-wash in PBS. The purity and quality of the extracted DNA was checked by SmartSpec Plus spectrophotometer (Bio Rad). To detect the concentration of *L. plantarum* and *L. paracasei* in the stool samples, quantitative real-time PCR assays (by using a RealPlex^2, Eppendorf) were performed

as previously described [14] after optimisation. Primers and probes used for the real-time Q-PCR analysis were from Applied Biosystems and are shown in Table 3. The assays were performed with a 25 µl PCR amplification mixture containing 12.5 µl Platinum Quantitative PCR SuperMix-UDG (Invitrogen), optimised concentrations of the primers and probes, and 5 µl DNA extracted from stool samples. The temperature profile for the amplification consisted of 2 min at 50 °C, 2 min at 95 °C, followed by 40 cycles of 3 s at 95 °C and 30 s at 57 °C for *Lactobacillus plantarum*/30 s at 64 °C for *Lactobacillus paracasei*. DNA extracted from pure cultures of *Lactobacillus plantarum* HEAL 9 and *Lactobacillus paracasei* 8700:2 was used as reference control in the assays.

Flow cytometry

Cellular immune response was assessed by measuring proliferation of certain cell types using flow cytometry for the group of 120 participants from site 2. They had been randomly divided into two groups: the probiotic group (60 participants) and the control group (61 participants). Blood samples were taken after run-in (day 0) and 14 days after intake. The cell types measured were NK cells (CD45+, CD16+, CD56+), T lymphocytes (CD45+, CD3+), T-helper cells (CD45+, CD3+, CD4+), T-suppressor and T-cytotoxic cells (CD45+, CD3+, CD8+) and B lymphocytes (CD45+, CD19+).

Statistical evaluation

Descriptive statistics (number of observations, minimum and maximum values, standard deviation and standard error) were used and evaluated using analysis of variance and χ^2-test. The amount of lactobacilli in faeces was compared before and after intake using the Wilcoxon signed rank test. The level of significance was set at $p = 0.05$. SPSS and SAS software were used.

Results

Faecal recovery of probiotic bacteria

A significant increase in *L. plantarum* and *L. paracasei* was measured in faecal samples in the probiotic group (Fig. 2),

Table 3 Primers and probes used for the Q-PCR analysis

Primer/probe	Direction	Dye	Sequence (5′ → 3′)	Conc (nM)	Tm (°C)
L. plantarum	Forward primer	–	CGG TGT TCT CGG TTT CAT TAT G	900	58
	Reverse primer	–	CCT ACA CAC TCG TCG AA A CTT TGT	900	58
	–	6-Fam	CTT GTT CTT TGA AAA CTA G-MBG	300	68
L. paracasei	Forward primer	–	ACA TCA GTG TAT TGC TTG TCA GTG AAT AC	900	60
	Reverse primer	–	CCT GCG GGT ACT GAG ATG TTT C	900	60
	–	6-Fam	TGC CGC CGG CCA G-MBG	300	70

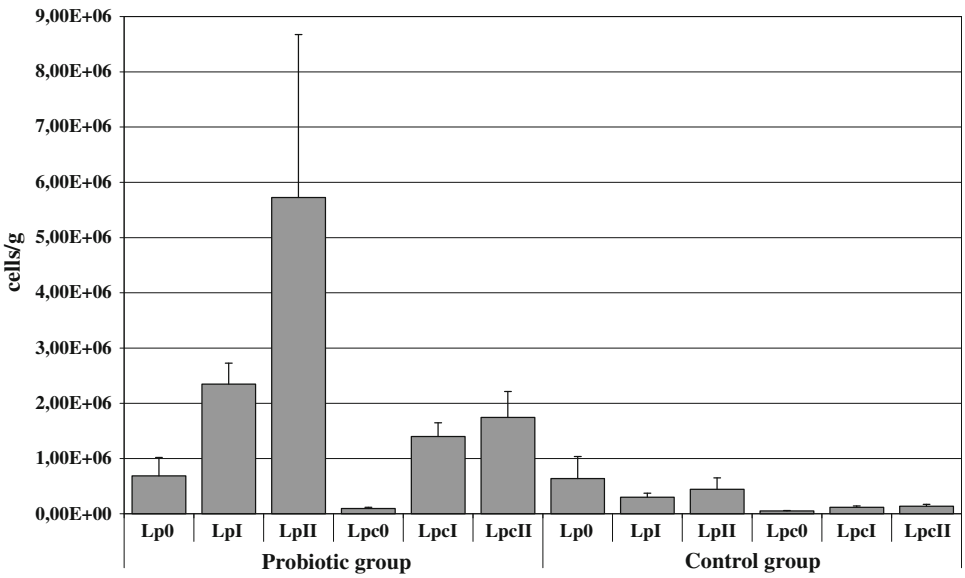

Fig. 2 Effect on faecal concentrations (cells/g) of *Lactobacillus plantarum* (Lp) and *Lactobacillus paracasei* (Lpc) after 2 weeks of run-in (0-sample), 2 weeks of intake (I-sample) and 12 weeks of intake (II-sample) of probiotic and control product (mean (SEM))

indicating a good compliance. *L. plantarum* increased from 6.9×10^5 cells/g to 2.4×10^6 cells/g ($p < 0.001$) and 5.7×10^6 cells/g ($p < 0.001$) in the samples collected after 2 and 12 weeks of product intake. Similarly, *L. paracasei* increased from 9.7×10^4 cells/g to 1.4×10^6 cells/g ($p < 0.001$) and 1.8×10^6 cells/g ($p < 0.001$) in the samples collected after 2 and 12 weeks, respectively. A significant decrease in *L. plantarum* was observed in the control group from 6.4×10^5 cells/g to 3.0×10^5 cells/g after 2 weeks of product intake ($p = 0.02$) and an increase in *L. paracasei* from 5.1×10^4 cells/g to 1.2×10^5 cfu/g after 2 weeks ($p = 0.01$) and 12 weeks ($p = 0.03$) of intake.

The levels of *L. plantarum* were 6.9×10^5 and 6.4×10^5 cells/g in the probiotic and control group, respectively, and similar ($p = 0.26$) after run-in (day 0) in both groups, while the levels of *L. paracasei* were 9.7×10^4 cells/g in the probiotic group and significantly lower and 5.1×10^4 ($p = 0.02$) in the control group (Fig. 2).

Adverse events

During the course of the study, 37 adverse events (AEs) (possibly/probably related to the study product) were reported totally, 17 of these were in the probiotic group and 20 in the control group ($N = 318$). The AEs in the active group were bowel pain (3), flatulence (6), loose stools (2), nausea (2), borborygmi (1), diarrhoea (2), sore gums (1), and in the placebo group bowel pain (1), flatulence (3), nausea (2), bloating (3), constipation (3), diarrhoea (4), heartburn (1), cold sores (1), halitosis (1) and aching tongue (1).

Incidence

A total of 170 cold episodes occurred in the control group in comparison with only 121 episodes in the probiotic group. For those participants who did have a common cold episode, either in the probiotic or the control group, the mean episode duration was 7 days.

A significantly ($p = 0.043$) lower incidence to get one or more common cold episodes was observed in the probiotic group in comparison with the control group (Table 4). Only 55% of the subjects in the probiotic group got one or more episodes in comparison with 67% of the subjects in the control group. The difference was even more pronounced ($p = 0.024$) when observing the incidence of getting 2 or more episodes during the study period (21% for the probiotic group and 33% for the control group).

The occurrence of the first episode was postponed in the probiotic group in comparison with the control group, and occurred after, in mean, 30.5 and 21.9 days, respectively ($p = 0.038$, Mann–Whitney *U*-test). A similar trend was also observed for the following episodes.

Table 4 Number of subjects with common cold episodes during the 12-week study period

	Control group		Probiotic group		P^a
	N	%	*N*	%	
≥1 episode	91	67	76	55	0.043
≥2 episodes	45	33	29	21	0.024
≥3 episodes	20	15	10	7	0.048

[a] Significance for changes in the variable considered during each treatment when compared to control group

Days sick

Intake of the active product resulted in significantly ($p < 0.05$) less days sick during the whole 12-week study period, and in mean 6.2 days in the probiotic group and 8.6 days in the control group.

Symptom scores

The total symptom score was lower in the probiotic group and was 33.6 compared to 44.4 in the control group ($p = 0.168$). However, only the score for pharyngeal symptoms was significantly reduced ($p = 0.027$) and was 5.2 in comparison with 8.6 in the control group (Table 5). If acquiring a second episode, the total symptom score during that episode was significantly lower in the probiotic group when compared to the control group ($p = 0.031$).

Cellular immune response following the ingestion of the study product

The changes in the cellular immune response after ingestion of either the probiotic product or the control product were measured after 2 weeks of product intake. This was in accordance with the blood sample analyses reported in a

Table 5 Symptom score during the study period (mean (SEM))

Symptom scores	Probiotic mean (SEM)	Control mean (SEM)	P^a
Nasal	10.30 (1.82)	13.20 (1.80)	0.257
Pharyngeal	5.20 (0.74)	8.60 (1.30)	0.027
Bronchial	7.00 (1.46)	9.40 (1.63)	0.282
Total respiratory tract	22.60 (3.34)	31.20 (4.06)	0.104
Total	33.60 (5.01)	44.40 (5.97)	0.168

[a] Significance for changes in the variable considered during each treatment when compared to control group

Table 6 Effect of probiotic bacteria (10^9 cfu/day) on the cellular immune response of the participants in site 2

	Δ (day 14–day 0) cells/μl blood				P^a
	Probiotic group ($N = 48$)		Control ($N = 49$)		
	Mean	SEM	Mean	SEM	
NK (CD16 CD56)	40.9	27.5	24.1	28.9	0.67
T lymphocytes (CD3)	73.9	84.5	237.8	81.4	0.16
T-helper cells (CD3 CD4)	−8.1	56.9	99.7	59.4	0.19
Ts and Tc cells (CD3 CD8)	73.6	41.9	124.5	47.2	0.42
B lymphocytes (CD19)	16.6	18.3	73.9	22.0	0.04

Blood samples were taken on day 0 and 14 after the first intake of study product. Results are expressed as the difference Δ (day 14–day 0) cells/μl blood (mean (SEM)). *NK* natural killer cells, *Tc* T cytotoxic cells, *Ts* T suppressor cells. Participants with abnormally high leucocyte counts in the first blood sample were excluded from the analysis of the results (11 participants excluded in the probiotic group and 12 in the control group)

[a] Significance for changes in the variable considered during each treatment when compared to control group

similar probiotic study by de Vrese et al. [5]. The aim of this analysis was mainly to document how probiotics can affect the baseline status of different cell populations in the blood.

A significantly increased number ($p = 0.04$) of peripheral B lymphocytes was measured in the control when compared to the probiotic group. In the probiotic group, in general, the different cell populations studied did not increase in numbers as much as in the control, and a reduction was observed for T helper cells (CD4+), however, not significant (Table 6).

In the statistical analysis, participants with abnormally high leucocyte counts in the first blood sample were excluded (11 participants in the probiotic group and 12 in the control group).

Discussion

The aim of this study was mainly to determine the effect of *Lactobacillus plantarum* HEAL 9 and *Lactobacillus paracasei* 8700:2 on reducing the risk of common cold episodes, number of days with common cold symptoms, frequency and severity of symptoms, and cellular immune response in common cold infections.

Common cold is mainly caused by human rhinoviruses and is a relatively mild infection. However, it is responsible for most absences from work and school every year. It has been estimated that the total annual cost of non-influenza-related viral respiratory tract infections (VRTI) approached 40 billion $ a year [15]. Thus, treatments that shorten the duration, reduce the incidence of infection and/or lessen the severity of symptoms are of high interest both for the individual as well as for the whole society.

Previous studies have proven the ability of *Lactobacillus plantarum* HEAL 9 and *Lactobacillus paracasei* 8700:2 to colonise the gastrointestinal tract after oral administration [9–11]. In this study, Q-PCR analysis was applied to quantify the levels of *Lactobacillus plantarum* and *Lactobacillus paracasei* in faecal flora and to check for compliance.

In the control group, a significant decrease in *L. plantarum* was observed after 2 weeks of intake of the study product. This indicates that the run-in period was too short. The control product was pure maltodextrine, and the intake was only 1 g per day, which could not have affected the microflora as maltodextrine is completely digested before entering the colon.

Forty-six subjects out of 318 withdrew from the study. This relatively high number of volunteers who did withdraw could be explained by the long study duration of 14 weeks (2 weeks of run-in + 12 weeks of intervention).

In this study, oral intake of a probiotic mixture contributed to the body's defence against common cold infections. This was reflected in a significantly ($p < 0.05$) lower incidence to get one or more common cold episodes and in significantly ($p < 0.05$) less days sick with common cold symptoms after intake of the probiotic product during the study. The effect was most pronounced when observing the second common cold episode.

The effect of probiotic bacteria on common cold has previously been demonstrated. In a study by Tubelius et al. [16] on 262 employees at TetraPak in Sweden, intake of the probiotic *L. reuteri* (1×10^8 cfu/day) significantly reduced ($p < 0.01$) the sick leave as well as the frequency of sick days caused by gastrointestinal and respiratory tract infections.

Further, a probiotic mixture containing *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3 and *Bifidobacterium bifidum* MF 20/5 (5×10^7 or 5×10^8 cfu/tablet) with an added vitamin/mineral mixture reduced the severity of symptoms ($p = 0.056$) as well as the duration

($p = 0.045$) of common cold infection [5] in comparison with a placebo containing vitamins and minerals. The same probiotic mixture (5×10^8 cfu/tablet) reduced the incidence of respiratory tract infections in comparison with a placebo without added vitamins and minerals ($p = 0.07$) [7].

In the study by de Vrese et al. [5, 6], 158 common cold infections were observed in the group receiving 5×10^7 cfu/day of the probiotic mixture ($N = 238$) and 153 in the control group ($N = 241$). On the other hand, the higher dose, 5×10^8 cfu/day, of the probiotic mixture used in the study by Winkler et al. [7] reduced the incidence of respiratory tract infections with 13.6% ($p = 0.07$) from 140 ($N = 238$) episodes in the control group to 121 ($N = 239$) in the group receiving probiotics. In the present study, the total number of episodes was reduced with 29% from 170 in the control group to only 121 in the probiotic group. The incidence of common cold episodes was rather high in the present study, 0.9 and 1.3 in the probiotic and control group, respectively. However, the study was run over a 3-month period during the winter–spring period when there is an increased risk for cold infections. Therefore, a mean of one cold episode per participant is within the limits of an expected average of 2–4 cold episodes per year [1].

It has also been demonstrated using synbiotic formulations [17] that a regular, long-term intake of synbiotics containing *L. plantarum* may improve health by reducing the incidence and severity of respiratory diseases during the cold season. Prebiotics are known to affect the immune system beneficially [18] and may have had an additional impact on the immune system.

In this study, we detected a significantly increased number of B lymphocytes in the control group when compared to the probiotic group, after 2 weeks of ingestion of the study product. It is difficult to explain these cellular changes in the apparently healthy population of study participants. Since the study was conducted during the common cold season, one could hypothesise the existence of underlying minor viral infections that could trigger the proliferation of B lymphocytes, despite the fact that the study participants were feeling healthy. If this is the case, then intake of the probiotic product might be linked to an inhibited/reduced B-cell proliferation and indirectly linked to a reduced inflammation. It is in general considered that the common cold symptoms do not result from common cold viruses themselves but from the inflammatory response of the host towards the viruses [19]. Therefore, a mild down-regulation of such an inflammatory response could result in less severe common cold symptoms. However, a more thorough analysis of inflammatory mediators and surface markers for cellular status would be required to explain and support such a hypothesis. Nevertheless, down-regulation of cell-mediated immune functions has previously been reported for different lactobacilli strains such as *Lactobacillus rhamnosus* and *Lactobacillus fermentum* (Rask et al., unpublished data). Moreover, a reduction in CD4+ T cells and induction of IL-10 and TGF-beta were reported for *Lactobacillus plantarum* HEAL 9, *Lactobacillus plantarum* HEAL 19 and *Lactobacillus paracasei* 8700:2 in a model for experimental autoimmune encephalomyelitis [8]. In contrast to our results, significantly higher numbers of cytotoxic plus T suppressor cells (CD8+) and T helper cells (CD4+) were observed in the probiotic-treated group in the study by de Vrese [5, 6]. In a similar way, an induction of CD4+ and CD8+ cells was reported by Winkler [7]. However, it is important to clarify that in the above-mentioned studies, the probiotic bacteria tested differed from those applied in the present study, and as mentioned before, the probiotic activities are strain specific.

Conclusion

In this study, oral intake of the strains *Lactobacillus plantarum* HEAL 9 (DSM 15312) and *Lactobacillus paracasei* 8700:2 (DSM 13434) contributes to the body's defence against common cold infections. This was reflected in a significantly ($p < 0.05$) lower incidence of one or more common cold episodes. In addition, there were significantly ($p < 0.05$) less days sick with common cold symptoms after intake of the probiotic product during the 12 weeks of study period. Furthermore, the total symptom score and in particular the pharyngeal symptom score ($p < 0.05$) was lower in the probiotic group in comparison with the control group.

Conflict of interest The authors are employees at Probi AB, and the study was funded by Probi AB and VINNOVA (The Swedish Governmental Agency for Innovation Systems).

References

1. Heikkinen T, Järvinen A (2003) The common cold. Lancet 361(9351):51–59
2. FAO/WHO Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf
3. Leyer GJ, Li S, Mubasher ME, Reifer C, Ouwehand AC (2009) Probiotic effects on cold and influenza-like symptom incidence and duration in children. Pediatrics 124(2):72–79
4. Hatakka K, Savilahti E, Pönkä A, Meurman JH, Poussa T, Näse L, Saxelin M, Korpela R (2001) Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 322(7298):1327
5. de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J (2005)

Effect of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, B. bifidum MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. Clin Nutr 24(4):481–491
6. de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J (2006) Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. Vaccine 24(44–46):6670–6674
7. Winkler P, de Vrese M, Ch Laue, Schrezenmeir J (2005) Effect of a dietary supplement containing probiotic bacteria plus vitamins and minerals on common cold infections and cellular immune parameters. Int J Clin Pharmacol Ther 43(7):318–326
8. Lavasani S, Dzhambazov B, Nouri M, Fåk F, Buske S, Molin G, Thorlacius H, Alenfall J, Jeppsson B, Weström B (2010) A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. PLoS One 5(2):1–11
9. Antonsson M, Molin G, Probi AB (2003) New strains of *Lactobacillus paracasei*, patent EP1313837. Available at http://v3.espacenet.com/publicationDetails/originalDocument?CC=EP&NR=1313837B1&KC=B1&FT=D&date=20051214&DB=EPODOC&locale=se_SE
10. Molin G, Ahrné S, Jeppsson B, Vasquez A, Berggren A, Probi AB (2006) WO patent application 2006038869. Available at http://v3.espacenet.com/publicationDetails/biblio?CC=WO&NR=2006038869A1&KC=A1&FT=D&date=20060413&DB=EPODOC&locale=se_SE
11. Vásquez A, Ahrné S, Jeppson B, Molin G (2005) Oral administration of *Lactobacillus* and *Bifidobacterium* strains of intestinal and vaginal origin to healthy human females: Re-isolation from faeces and vagina. Microbial Ecology in Health and Disease 17:15–20
12. Macknin ML, Piedmonte M, Calendine C, Janosky J, Wald E (1998) Zinc gluconate lozenges for treating the common cold in children: a randomized controlled trial. JAMA 279(24):1962–1967
13. Ahrné S, Nobaek S, Jeppsson B, Adlerberth I, Wold A, Molin G (1998) The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. J Appl Microbiol 85:88–94
14. Haarman M, Knol J (2006) Quantitative real-time PCR analysis of fecal *Lactobacillus* species in infants receiving a prebiotic infant formula. Appl Environ Microbiol 72(4):2359–2365
15. Fendrick AM, Monto AS, Nightengale B, Sarnes M (2003) The economic burden of non-influenza-related viral respiratory tract infection in the United States. Arch Intern Med 163(4):487–494
16. Tubelius P, Stan V, Zachrisson A (2005) Increasing work-place healthiness with the probiotic *Lactobacillus reuteri*: a randomised, double-blind placebo-controlled study. Environ Health 4:25
17. Pregliasco F, Anselmi G, Fonte L, Giussani F, Schieppati S, Soletti L (2008) A new chance of preventing winter diseases by the administration of synbiotic formulations. J Clin Gastroenterol 42(Suppl 3):S224–S233
18. Macfarlane GT, Steed H, Macfarlane S (2008) Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J Appl Microbiol 104(2):305–344
19. Kirchberger S, Otto Majdic, Stöckl J (2007) Modulation of the immune system by human rhinoviruses. Int Arch Allergy Immunol 142:1–10