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Health effects of occupational exposure in a dairy food industry, with a specific assessment of exposure to airborne lactic acid bacteria

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ABSTRACT

Objective: Lactic Acid Bacteria (LAB) are used in food industry as probiotic agents. The aim of this study is to assess the potential health effects of airborne exposure to a mix of preblend (LAB and carbohydrate) and milk powder in workers. **Methods:** A medical questionnaire, lung function tests and immunological tests were carried out on 50 workers. Occupational exposure to inhalable dust and airborne LAB was measured. **Results:** Workers not using respiratory masks reported more symptoms of irritation than workers using protection. Workers from areas with higher levels of airborne LAB reported the most health symptoms and the immune responses of workers to LAB was higher than compared to the immune responses of a control population. **Conclusions:** Measures to reduce exposure to airborne LAB and milk powder in food industries are recommended.

INTRODUCTION

Over the last 10-15 years, the addition of Lactic Acid Bacteria (LAB) strains to food, in particular in dairy food, is increasing since they are generally recognized to be beneficial to health [1], therefore, different species are used as food additives in order to have a positive effect on health.

For the U.S. Food and Drug Administration, *Bifidobacterium spp*. are clearly classified as Generally Recognized As Safe (GRAS), although this is not the case for all Lactobacilli. It should be kept in mind, however, that GRAS status is only for specified food use. The microbes themselves are not considered GRAS, but their traditional use in dairy foods is. Several *Lactobacillus* species (e.g., *L. paracasei*) are not specifically on this GRAS list. However, the accepted character of this list is that it is partial and a microbe's absence from the list does not imply that it is not safe for use[2]. These species are accepted as safe in Japan and

Europe[2]. Their occurrence as normal commensals of mammalian intestinal flora and their established safe use in a diversity of foods and food supplement products worldwide supports this conclusion. Thus, there is increasing general evidence that LAB have beneficial health effects when ingested and they are therefore more and more used in food industry. However, the health effects of airborne and dermal exposure to LAB strains by occupational contact in food processing plants had never been investigated.

It is well known that exposure to high levels of airborne bacteria in an occupational environment can be associated with a wide range of health effects, in particular respiratory impairment, allergy and organic dust toxic syndrome[3-7] . Moreover, exposure to high concentrations of specific bacteria could generate a specific immune response in the host. These immune responses are indicators of exposure and could also be indicators of an allergic reaction.

The aim of this study was to assess the frequency of general, respiratory, and self-reported health symptoms of workers exposed to dry LAB simultaneously to other powder (carbohydrate and milk powder). Moreover, workers' immune responses to different LAB strains were investigated and an assessment of airborne LAB exposure was made.

MATERIALS AND METHODS

Study site

The workers examined in this survey worked in a food processing plant. They handled LAB and mixed them with carbohydrate to make a so called preblend which is then mixed with milk powder. Evaluation took place at three different work areas on the site: i) laboratory (research area); ii) pilot plant (research and development area); and iii) factory (production site). Production of LAB in this dairy food industry started in 2002 and included strains of

Lactobacillus johnsonii, L. paracasei, L. rhamnosus, Bifidobacterium sp and *Streptococcus sp*. From 2003 onwards production was increased, and Personal Protective Equipment (PPE) was introduced for individuals exposed to airborne preblend.

Study population

All workers were currently exposed to preblends in one of the three different work areas described above. Fifty workers (43 men and 7 women) aged 22-60 years old participated in the study. No former employees were included in the study.

All but two of the individuals were full time workers. For analysis these two part time workers were not separated from the group of full time workers. All participants in the survey were matched to one of the three work areas (laboratory, pilot plant or factory) where exposure was measured: six individuals worked in the laboratory, 15 in the pilot plant and 29 in the preblend conditioning room.

No unexposed reference population was examined for health symptoms, however, the immunological analyses were obtained for an external unexposed group (n=32). All participants signed written informed consent before study started. In addition, all methods were approved by the Institute for work and Health Institutional Review Board.

Questionnaire

A questionnaire about general health symptoms, occupational exposure, and lifestyle factors was designed on the basis of a questionnaire, "Evaluating Organic Dust Exposure" [8], and was modified for the specific requirements of this survey. It included items about dust exposure before the present job, the number of years' exposure to preblend, the average percentage of working time (WT) exposed to preblend on during the last 12 months, and the average use of PPE (percentage of time wearing masks during critical periods of exposure)

during the last 12 months. All recorded symptoms were defined by their frequency, i.e: rare (less than once a month); sometimes (more than once a month) and consistently (nearly daily).
In addition all participants were asked about their previous dust exposure before starting work in this industry, the weekly duration of exposure and their use of PPE. Forty-five workers were able to estimate the percentage of work time during which they were exposed to preblend and to give adequate information about the length of time during which they used PPE. Participants were classed into three different groups: a low exposure group (14 workers exposed to LAB for 10% of their working time or less); an intermediate exposure group (5 workers exposed for more than 10% of their working time, yet who wore a mask for more than 90% of that exposure time); and a high exposure group (26 workers exposed for more than 10% of their working time, yet who wore a mask for less than or equal to 90% of the exposure time).

Medical examination, spirometry and blood sample collection

All participants were examined by the same occupational health physician, who collected data on weight, size, blood pressure, an auscultation of the heart and lungs, and a skin inspection. All participants performed a spirometry test using a MicroLab Spirometer (portable spirometer MicroLab CatNo ML3500 by Micro Medical Ltd, Kent, UK). The best out of three acceptable forced expirations was taken for forced expiratory volume in one second (FEV1) and for forced vital capacity (FVC). These were used as the outcomes of the lung function test. The spirometric parameters were expressed as a percentage of the predicted values according to height, age and gender of a Caucasian population[9].
Following their written consent, blood samples were collected from 44 of the 50 workers. They were collected on the last day of the survey, transported to the laboratory on the same day at +4°C, and stored in frozen conditions (-80°C) in several aliquots. To examine the

immunological status of each worker, the blood sample was tested for antibodies against the different LAB used in the production site, and to carbohydrate.

Airborne cultivable LAB and inhalable dust exposure assessment

Occupational exposure to airborne LAB and inhalable dust were measured at different places in the three different work areas. In total, 13 microbiological samples and 8 dust samples were collected. Airborne LABs were collected with an impactor (MAS-100 Eco, Merck, Darmstadt, Germany) at a flow rate of 100 l /min, onto Man Rogosa Shape agar plates (Oxoid, Basel Switzerland). Duplicate samples were taken at each sampling site. One of the duplicate plates was incubated at 30°C in an anaerobic atmosphere and the other plate in an aerobic atmosphere. All plates were checked daily for colony counts for 5 days. Results are expressed in Colony Forming Units (CFU) per cubic meter of air.

Inhalable dust was sampled on glass fiber filters at a flow rate of 2.0 l/min using pocket pumps (MSA Escort Elf, Mine Safety Appliance Company, Pittsburgh, USA or SKC pocket pump 210-1002, SKC Inc., USA) and IOM heads (SKC Inc.). Samplings were performed continuously for 1-4 hours at stationary points. The filters were pre and post-weighed on an analytical balance (Mettler Toledo AT201, Greifensee, Switzerland, 0.001 mg sensitivity)

Immunological tests

Every blood sample was tested for antibodies to the different LAB used and to carbohydrate, and then compared to a control group with an urban life-style.

The antigens were produced from the LAB strains and carbohydrate. Thus, seven different antigens were produced and the blood samples were tested using two different immunological methods (ELISA IgG and electrosyneresis on cellulose acetate) for antibodies against the 6 somatic antigens derived from each bacterium and one crude extract made with carbohydrate. Antigen extraction: Crude carbohydrate was covered with liquid of Coca (4g sodium chloride,

2.75g bicarbonate of sodium, 4g liquid phenol and completed to 1 l with sterilized distilled water) and put to soak for 7 days at room temperature under shaking at 300 rev/min. Then, a series of filtration steps were carried out using different filter papers, the last one having a porosity of 0.45µm (Millipore Corp., Bedford, MA, USA). The filtrate was lyophilized (Labconco, Kansas City, MB, USA) and the antigen was reconstituted with sterile distilled water at a concentration of 100mg/ml. Six somatic antigens were derived from six LAB strains. The antigens were produced as previously described [10]. Briefly, bacteria strains were cultured on Mueller Hinton medium (Bio-Rad, Marnes la Coquette, France) for 1 week, sonicated, extracted overnight in $NH_4\ CO_3$ at 4°C, centrifuged at 13,000 RPM, lyophilised and dosed for standardization at 100mg/ml of protein.

Serology techniques: Workers' sera were tested for each of the seven antigens using two parallel methods. Serum precipitins were analysed by electrosyneresis on cellulose acetate and ELISA with peroxydase-conjugated anti-human IgG. The results were interpreted separately by 2 persons; no differences were noted between their two interpretations. Serological techniques were previously described [11].

Electrosyneresis*:* It was performed with an electrosyneresis apparatus (Sebia®, Issy les Moulineaux, France). After 10 minutes in a bath of buffered tris-glycine solution (Sebia®, Issy les Moulineaux, France), cellulose acetate sheets (Sartorius®, Goettingen, Germany) were dried and placed in the electrophoresis vat filled with buffered tris-glycin solution (pH=8.8). Fifteen microliters of each serum were placed on 3 spots on the anode side and a 15µL line of antigen was placed on the cathode side. A 110V current was applied for 2 hours and 15 minutes. After washing, the cellulose acetate sheets were stained with Coomassie Brilliant Blue.

ELISA: The wells of flat-bottom microtiter plates (Immulon, Prolabo®, Fontenay Sous Bois, France) were coated with 200µl of 10µg/ml antigen solution in 50mmol/l K_2HPO_4

buffer, pH 8.5 at 4°C for 72 hours. Excess binding sites were blocked at 37°C for 1 hour with 250µl of 50mmol/l NaH_2PO_4, containing 0.5% bovine serum albumin and 60g/l of sorbitol. One hundred-microliter serum samples, diluted 1:50, were added to the wells in duplicate. The plates were incubated at 37°C for 1 hour and shaken constantly. Plates were washed four times with washing buffer (100mmol/l Tris-HCl, pH 7.5 containing 0.25% Tween). Next, 100µl of peroxidase conjugated goat anti-human IgG diluted to 1:4,000 (Sigma®, Saint Louis, Missouri) were added to all wells and the plates were incubated at 37°C for 1h and shaken constantly. The washing procedure was repeated and 100µl of 3.3'.5.5'-Tetramethylbenzidine (TMB) solution (TMB One-Step Substrate System, Dako®, Carpinteria, California) was added to the wells at room temperature for 10 minutes. To stop the TMB reaction, 100µL of an acid solution (a mixture of 1N HCL and 3N H_2SO_4) were added. Wells were read spectrophotometrically at 450nm (Titertek Multiskan®, Helsinki, Finland) and the results were expressed in optical density (od).

RESULTS

Airborne LAB and inhalable dust

Results for airborne LAB and inhalable dust are detailed in Table I. The level of airborne cultivable LAB was very low in the laboratory. In the pilot plant, low levels of aerobic LAB were measured, but intermediate levels of anaerobic strains. At the time of the study, the preblend conditioning room was the most exposed workplace with airborne bacteria level above Switzerland's occupational health recommendations (10'000 CFU/m^3; SUVA, 2009). We observed a positive correlation between inhalable dust levels and the level of airborne aerobic and anaerobic LAB (Spearmann $\rho = 0.829$).

Health assessment and heath symptoms

Data on participants are given in Table II. None of the participants had any relevant dust exposure before starting employment at the manufacturing site. The average career length of all the individuals in this industrial environment was 17 years (min = 1 year; max = 42 years).

In general, all participants were in good health conditions. There was no evidence of any relationship between medical consultation or regular prescribed medication and exposure to LAB and/or other powder. None of the individuals presented allergies related to exposure at work. There were no pathological results for the heart and lung auscultations. However, 46% (23/50) of participants reported past health symptoms or health incidents in relation to their exposure to preblend and milk powder. Some reported only one incident over the whole observation period (2002-2009); others were still reporting health problems. Most of the complaints concerned the eyes, nose or throat (31/36). Seventy-five percent of reported health complaints occurred rarely, 19% often, and 6% regularly. Tables III and IV show the prevalence of existing declared symptoms, as a function of their present workplace (Table III) and self declared exposure (Table IV). While most of the symptoms occur among subjects working in the factory, the difference is not significant, except for skin symptoms. On the other hand, it is noticeable that declared symptoms occur only in the group of subjects reporting a frequency of exposure greater than 10% and who did not consistently use the personal protective devices when exposed. This difference in symptoms prevalence between exposure groups is statistically significant ($p=0.002$ Fisher's exact test). Spirometric test values, on the other hand, are on average above the expected values and do not differ according to the exposure.

Immunological status

Strains of Lactobacillus induce the most specific immune response. In total, 52% (23/44) of the blood samples showed a positive result (defined as a result up to two standard

deviations from the control group's results) for one of the three lactobacilli (either in electrosyneresis or ELISA), most of them for *L. paracasei* and *L. rhamnosus.* None of the workers showed a reaction to carbohydrate.

We found no statistically significant association between positive blood results (positive immunological reaction to one of the three *Lactobacillus*, either in ES or ELISA) and the work area, self-reported exposure or self-reported symptoms (Chi-square test respectively: 0.076, p = 0.783; 0.613, p= 0.736; 0.08, p = 0.78).

Nevertheless the results indicate a specific immune reaction to the LABs *L. paracasei* and *L. rhamnosus* in exposed workers compared with an unexposed control group (Wilcoxon test, repectively: Z= -4.7, p<0.001; Z=-4.8, p<0.001). On average, workers exposed to LABs have an immune response to *L. paracasei* 3 times higher than that of the control group (Mean +/- SE = 684 +/- 27 vs 203 +/- 18; N= 44/31), and workers exposed to LABs have an immune response to *L. rhamnosus* 2 times higher than that of the control group (Mean +/- SE = 420 +/- 23 vs 193 +/- 18; N= 44/31).

DISCUSSION

The present study provides evidence that workers exposed to preblend (LAB mixed with carbohydrate) and milk powder, whether by inhalation or dermal contact and without adequate use of the PPE, are at an increased risk of developing irritating eye and nasal symptoms. We cannot compare the frequency of these health effects with a non exposed population, but the fact that about the half of the study population reported the occurrence of at least one eye or nose/throat symptom related to preblend handling during the period 2002-2009 means that symptoms are in relationship with that specific work and are not due to other environmental sources. As workers were exposed to LAB, carbohydrate and milk powder, we cannot know which of those three components is responsible for the observed health symptoms.

The fact that, workers from areas with higher levels of airborne LAB reported the most health symptoms and the fact that the immune responses of workers to LAB was higher than compared to the immune responses of a control population could suggest that LAB could have a role in the apparition of irritating symptoms. However, exposure to airborne milk powder is also known to induce nasal symptoms. Indeed, another study [12], in dairy food industries in Thailand had reported that workers exposed to milk powder and/or vitamin by inhalation, had an increased risk of nasal symptoms. This study reported also other health symptom such as wheezing, breathlessness and decrease of lung function tests. The difference in the gravity of the health symptoms between the Thai study and our study could be due to the fact that Thai workers do not used PPE. Comparison between health symptoms of workers exposed only to milk powder and workers exposed only to preblend would be necessary to clarify the specific health effect of each component.

Even if the exposed group of workers show an increased immunological reaction against some LAB strains compared to a control group, our data do not allow us to show a relationship between immunological results, reported symptoms and/or medical history. But the absence of a relationship between immunological reaction and symptoms could also be due to a lack of statistical power because of the low number of individuals included in the analysis. It cannot be excluded that the duration of the exposition (17 years on average) and microbial airborne concentrations were insufficient to induce any symptoms, but we cannot exclude the hypothesis that under certain environmental circumstances, symptoms result from an increase in precipitins: for instance in the factory where preblends are conditioned, with up to $2.6x10^6$ CFU/m^3 aerobic bacteria. However, our results suggest the self-reported symptoms seem to be more related to an irritating mechanism than to an allergic one. Currently, testing for specific antibodies against LABs seems unhelpful for the identification of individuals with health

problems or facing high exposure levels. This could provide an area for further research and studies.

Looking at the reported symptoms in relation to the total number of years worked in the company, we observed – even if there is no significant statistical association – that individuals having worked less than 8 years at the company, reported more symptoms than those who had worked there for more than 8 years. This could be related to the "healthy worker effect" (a selection effect by which ill workers are less likely to be employed) frequently observed in work situations, particularly in cases involving irritating and allergic problems [13] .

Among workers reporting at least one symptom related to contact with preblends during the last 12 months, we observed no complaints from staff in the laboratory and twice as many complaints in the staff of preblend conditioning room than in the pilot plant. These results are in accord with the airborne bacteria exposure measurements which showed that workers in the preblend conditioning room were exposed to up to five times more LAB than workers in the pilot plant, and that workers in the laboratory were exposed to very low amounts of airborne LAB since they handled very small quantities of powder.

The risk of developing irritating symptoms is rare (less than once a month), and the data do not allow the evaluation of whether the occurrence of symptoms is due to specific work processes (e.g. during extremely high exposure to the microbial substances) or whether they are due to other circumstances. We did not find any evidence for increased numbers of sick days related to exposure to preblends in the workers' medical histories. However, these workers should be regularly monitored for skin and mucous membrane problems and should receive sufficient training in the use of PPE in order to prevent health deteriorations.

The documented health problems in this survey were only reported retrospectively by the individuals themselves. It may be advisable to examine exposed individuals in a future prospective survey, evaluating and diagnosing health problems when they occur to get more

information about the relationship between health problems, exposure and work processes. To conclude, the present study indicates that workers exposed to preblend and/or milk powders by inhalation or dermal contact are at an increased risk of developing irritating symptoms of the eyes, throat, and nose. Moreover, the use of PPE at times of significant exposure is sufficient to protect against the risk of developing such irritating symptoms.

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Table I: Mean (range) of inhalable dust and airborne bacteria at different work areas of a food processing plant.

Work area	Mean of dust [mg/m^3]	Mean of aerobic LAB (CFU/m^3)	Mean of anaerobic LAB (CFU/m^3)
Laboratory: during weighing of LAB	0.772	150 N = 2 (100-200)	300 N = 2 (300)
Laboratory: during handling of LAB	< 0.015	350 N = 4 (300-400)	200 N = 4 (0-300)
Pilot plant: command airlock	-	60 N= 2 (40-80)	2'200 N=2 (1020-3380)
Pilot plant: inside the room where the dry LAB is distributed into big bags	0.791 N=2 (0.176-1.407)	200 N=2 (200)	4’200 N=2 (4100-4300)
Pilot plant: outside the room where the dry LAB is distributed into big bags	0.021	55 N=2 (50-60)	1’110 N=2 (1000-1220)
Factory: preblend conditioning room, during bag filling	4.714 N=2 (3.682-5.746)	21’100 N=2 (20’600-21160)	27’200 N=2 (26’700-27'700)
Factory: preblend conditioning room, during preblends mixing		> 2'628'000	> 2'628'000
Factory: airlock where big bags are loaded before being sent to others plants.	1.802	11’950 N=4 (8500-16’600)	16’525 N=4 (10’600-22’700)
Workers’ changing room: before entering in preblend conditioning room	-	6’840 N=2 (6’480-7’200))	8’525 N=2 (7’750-9’300)
Goods depot	-	420 N=2 (400-440)	230 N=2 (200-260)
Cafeteria (control)	-	150 N=2 (140-160)	30 N=2 (30)

Table II: Data on participants

	Laboratory N=6	Pilot Plant N=15	Factory N=29
Gender n (%)			
Male	1 (16.7%)	15 (100%)	27 (93.1%)
Female	5 (83.3%)	- (-)	2 (6.9%)
Age mean (sd)	38.0 (8.5)	46.7 (8.8)	44.0 (11.3)
Smoking habits n (%)			
Non smoker	5 (83.3%)	7 (46.7%)	15 (51.7%)
Former smoker	1 (16.7%)	6 (40.0%)	7 (24.1%)
Smoker	- (-)	2 (13.3%)	7 (24.1%)
Self declared exposure n (%)			
<10% exposure	5 (83.3%)	4 (28.6%)	5 (20.0%)
≥10% protection mask	-	2 (14.3%)	3 (12.0%)
≥10% no mask	1 (16.7%)	8 (57.1%)	17 (68.0%)
Missing information	-	1	4

Table III: Prevalence of existing declared symptoms, as a function of their present workplace

	Laboratory N=6	Pilot Plant N=15	Factory N=29	P
Respiratory parameters mean (sd)				
FEV1 (% predicted)	101.0 (4.1)	102.7 (12.1)	103.2 (14.4)	>0.5
FVC (% predicted)	108.7 (5.9)	104.1 (9.3)	105.9 (16.4)	>0.5
Present work-related symptoms n (%)				
Nose/Throat	0(-%)	2 (13.3%)	4 (13.8%)	>0.5
Skin	0(-%)	0(-%)	8 (27.8%)	0.03
Eyes	0(-%)	1 (6.7%)	4 (13.8%)	>0.5
Any symptom	0(-%)	3 (20.0%)	12 (41.4%)	0.09

Table IV: Prevalence of existing declared symptoms and self declared exposure.

Self declared exposure	**<10% exposure N=14**	**≥10% with protection mask N=5**	**≥10% no mask N=26**	**P**
Respiratory parameters mean (sd)				
FEV1 (% predicted)	106.6 (14.3)	107.6 (4.8)	101.6 (12.5)	>0.5
FVC (% predicted)	108.4 (16.3)	102.6 (5.4)	106.6 (13.0)	>0.5
Present work-related symptoms n (%)				
Nose/Throat	0 (-%)	0 (-%)	6 (23.1%)	0.10
Skin	0 (-%)	0 (-%)	7 (26.9%)	0.08
Eyes	0 (-%)	0 (-%)	3 (11.5%)	>0.5
Any symptom	0 (-%)	0 (-%)	12 (46.2%)	0.002