

Lack of evidence of IgE allergic sensitisation from working with lactic acid bacteria in the dairy foods industry

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This research communication aimed to evaluate the level of immunoglobulin E from lactic acid bacteria (LAB) that are used in dairy industries. Previous studies have demonstrated that workers report symptoms of irritation and are frequently IgG-sensitised to LAB. Workers ($n = 44$) from a probiotic production unit and the control lab were seen by a medical practitioner and responded to an occupational questionnaire. Specific IgE by the DELFIA® technique against 6 strains of LAB were measured on 44 exposed workers and 31 controls sera. Levels of specific IgE were low and no difference was observed between the two groups. This lack of IgE response could be explained by a healthy worker effect, an efficient implementation of personal protective equipment or by an absence of allergic mechanisms to account for the self-reported irritative symptoms. Despite the high concentrations of LAB, preventive measures are effective enough to guarantee no allergic effect and to prevent other adverse health effects. The implementation of preventive measures to avoid or reduce exposure to dust of LAB, and more generally to milk powder, is recommended in all dairy industry.

Occupational exposure to milk powder is recognised as a risk factor for impaired lung function (Sripaiboonkij et al. 2008). Dairy plant workers could also be exposed to lactic acid bacteria (LAB) that are used as additives in dairy industries because they are thought to be beneficial to health when they are ingested (Snydman, 2008) and are generally recognised as safe (GRAS) in food. However, workers of dairy food industries are exposed to airborne LAB, and when they do not wear respiratory masks they report more symptoms of irritation than workers using protection. Moreover, it has been shown that an occupationally exposed group is more sensitised (precipitins and IgG investigations) against LAB than a control group (Zeifelder et al. 2012), which could reflect a delayed immunological response considered as an exposure proof. However, specific allergic sensitisation (immediate immunological response) against these frequently used probiotic strains has not been tested yet and, to our knowledge, no allergic effect concerning the inhalation of LAB has been reported. The aim of this study is to evaluate the level of specific IgE anti-LAB using a new technique.

Materials and methods

Study population

The study site and worker population have previously been described in Zeifelder et al. (2012). Briefly, we investigated dairy industry strains of *Lactobacillus johnsonii*, *L. paracasei*, *L. rhamnosus*, *Bifidobacterium lactis*, *B. longum*, and *Streptococcus thermophilus*. Fifty workers were initially recruited, and 44 of them each gave a blood sample. Twenty-seven individuals worked in the factory (production site) and 17 in the laboratory or pilot plant (research and development areas). Thirty-one sera of non-exposed participants were also tested as a control group. All participants signed consent waivers before the study started. In addition, all methods were approved by Institute for Work and Health Institutional Review Board.

Experimental design

Dissociation-Enhanced Lanthanide Fluorescent immunoassay (DELFIA®) allows the measurement of specific IgE (Barrera et al. 2016). This technique has been used to dose specific IgE. It is recognised as sensitive and makes it possible to adapt the panel of antigens when they are not

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Table 1. Level of sensitisation in participants that were sensitised to at least one species

Participants #	<i>B. lactis</i>	<i>B. longum</i>	<i>L. johnsonii</i>	<i>L. paracasei</i>	<i>L. rhamnosus</i>	<i>S. thermophilus</i>
Sensitised exposed workers						
P02	—	+++++	—	—	—	—
P17	—	+	—	—	—	—
P26	—	+	—	—	—	—
P29	—	—	—	+	—	—
P30	—	+++	—	—	—	—
P33	—	+	—	—	—	—
P40	—	+	—	—	—	—
P42	—	+++++	+++	+++++	—	++++
Sensitised control participants						
T01	—	++	—	—	—	—
T04	—	+	—	—	—	—
T10	—	+	—	—	—	—
T14	—	—	—	++++	—	+++++
T15	—	+	+++++	—	—	—
T19	—	+	—	—	—	—
T21	—	+	—	—	—	+
T22	—	—	—	+++++	—	—
T29	—	+	—	++	+	++
T31	—	+	—	—	—	—

—: index value equivalent to the background.

+: index value >10% of the background.

++: index value >20% of the background.

+++: index value >30% of the background.

++++: index value >40% of the background.

+++++: index value >50% of the background.

commercially available. Preparation of antigen protein extract was obtained by culture of the 6 different strains on Mueller Hinton medium. Then bacteria were sonicated and lyophilised as described by Zeilfelder et al. (2012). The lyophilised powder was suspended in distilled water, and proteins were purified by the SDS-PAGE clean-up Kit (GE Healthcare, USA) according to the supplier's recommendations to obtain a purified protein extract of each strain. DELFIA® was performed as previously described by Barrera et al. (2016). Briefly, antigens were coated on a 96-well plate with 0.1 µg/ml of purified protein extract. Then the sera were incubated at a dilution of 1/100 in an assay buffer, before being revealed by further steps inducing the fluorometric reaction. The plate was read using the Victor 2 multilabel counter (Perkin Elmer®, Waltham, MA, USA). Each serum sample was tested six times. Unreliable count values were avoided, and the median of the replicates of each serum was calculated and then divided by the median of all sera count values. The resulting index was considered positive when at least greater than 10% of the background. Different levels of sensitisation were defined when index values were greater than 10, 20, 30, 40 and 50% of the background value. Variance analysis was performed with R software version 3.3.2.

Results

On the whole, levels of specific IgE were low and no difference was observed between the exposed workers group

and the control group (P -value >0.05). Eight exposed participants (18%) and 10 controls (32%) were considered as positive for at least one species, and none of the study population had a positive result for *B. lactis* (Table 1). Only one control showed a weakly positive level of sensitisation for *L. rhamnosus*. *B. longum* was the species that presented a positive result most often in both populations, and the level of sensitisation was higher in the exposed group. Only one exposed worker was highly sensitised to four antigens (Table 1). This multisensitised worker presented work-related nasal and eye irritation and was already working in the factory when the personal protective equipment was implemented. A second worker, weakly sensitised to only one LAB strain, also declared work-related symptoms. He worked in the pilot plant but had also been exposed before the introduction of protective measures. The remaining IgE sensitised exposed workers did not present any irritating symptoms. No correlation was made between specific IgE response and occupational characteristics (time of employment, type of exposure, exposure before or after introduction of protective measures).

Discussion

Immediate allergic response estimated by the level of specific IgE was low in our exposed population. Only eight workers out of 44 were sensitised to at least one of the six LAB strains tested, and one of them proved to be multisensitised. *B. lactis* and *L. rhamnosus* induced a weak or no

exacerbation of the specific IgE level. Few participants were sensitised to *L. johnsonii*, *L. paracasei* and *S. thermophiles*, but the associated specific IgE level could be high. However, the majority of the sensitised participants were sensitised to *B. longum* but in most cases with only a weak specific IgE level. To be exposed does not mean to become allergic to LAB and probably depends on individual factors, but we can also suggest factors that could explain the global lack of immediate allergic response :

- a healthy worker effect
- an efficient implementation of personal protective equipment
- an absence of allergic mechanisms to account for the self-reported irritative symptoms

It was reported that exposure to LAB (Zeilfelder et al. 2012), carbohydrates (Zeilfelder et al. 2012) and milk powder (Sripaiboonkij et al. 2008; Zeilfelder et al. 2012) increased the risk of developing irritating eye and nasal symptoms or adverse effect on lung function. Apart from all these considerations, our study proved the usefulness of the DELFIA® technique, that was sufficiently sensitive to detect low levels of specific IgE and have the advantages to test all antigenic extracts.

Conclusion

To conclude, no allergic reaction due to exposure to airborne LAB was demonstrated, only an irritative effect was observed. These results must be confirmed with a greater study population of dairy workers from several production sites to better understand environmental, occupational and individual factors that explain the occurrence of symptoms. Levels of specific IgE against milk compounds such as caseins, α -lactalbumin and β -lactoglobulin could be also measured as confounding factors. Moreover, it is well known that occupational exposure to dust in general and/or microorganisms is often deleterious to health (Roussel et al. 2012; van Kampen et al. 2012; Dorribo et al. 2015; Bittner et al. 2016; Paris et al. 2016). We recommend the implementation of technical and/or organisational preventive measures to avoid

or reduce this exposure (both exposure to milk powder and LAB).

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Conflict of interest

None declared.

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