

Allergen-free Probiotics

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Abstract: Food sensitivities are constantly increasing in “westernized” countries and may pose serious health risks to sensitized individuals. Severe allergy episodes have also been reported after the intake of probiotic products containing milk protein residues, especially in children. The need for safe and effective probiotic strains and food supplements, which contain them, is now emerging clearly. The present work describes the way of achieving this aim by the avoidance of any kind of raw materials at risk, both in probiotic strain industrial manufacturing and finished product formulation. Allergen-free probiotics represent, without any doubt, an innovative and safe tool for human health.

Key Words: food allergies and intolerances, microbial stimulation, probiotic strains, milk proteins, pediatric formulations

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FOOD SENSITIVITIES

Food allergies and food intolerances are both types of “food sensitivities.”

Food allergies are anomalous immunologic reactions (IgE mediated) to generally safe foods. The food component, which triggers this reaction (allergen), is typically a protein in the molecular weight range of 5 to 200 kd. Many allergens may be found in foods and adverse reactions generally occur shortly after product ingestion. Most reactions are short-lived and relatively harmless, but severe allergic reactions leading to anaphylactic shock and death are not uncommon. Main symptoms associated with food allergies are: glottis edema, urticaria (hives), vomiting, diarrhea, rash, asthma, allergic rhinitis, and headache.

Even though their symptoms are similar to food allergies, food intolerances do not involve IgE production and adverse reactions may occur even hours after consumption. Food intolerance can develop toward a wide range of foods. Intolerances can be triggered by enzymatic deficiencies or biochemical reactions due to substances naturally present in the food or specifically used as additives. For example, lactose intolerance is due to a deficiency of the enzyme lactase, needed to break the

disaccharide down into the single sugars, glucose and galactose. Typical associated symptoms are gas production, intermittent diarrhea, constipation, irritable bowel syndrome, and skin rashes.

Both food allergies and intolerances are constantly increasing in developed countries. It is estimated that 2% to 4% of adults and 6% to 8% of children up to 3 years of age suffer from these food sensitivities. Two out of 100 babies under 12 months are allergic to cow’s milk. Approximately, 35% of children with moderate-to-severe atopic dermatitis also have food allergy.

Moreover, among allergic people between 20% and 30% may have an adverse reaction to food, which is not revealed by skin or blood tests.

Currently, the only way to treat food allergies is to avoid the foods, which trigger these reactions.

EUROPEAN LEGISLATION AND LABELING

The European Community has defined a list of 12 classes of potential allergens (cereals containing gluten, crustaceans, eggs, fish, peanuts, nuts, soybeans, milk, celery, mustard, sesame, and sulfur dioxide at levels above 10 mg/kg or 10 mg/L expressed as SO₂), which are included in Annex IIIa of Directive 2003/89/EC,¹ whose aim is to achieve a high level of health protection for consumers and guarantee the right to information through clear and complete product labeling. Together with Directives 2004/77/EC and 2005/63/EC, these regulations have been transformed into Law Decree no. 114 of 2006. A further update is given in Directive 2006/142/EC, which explains additional allergen labeling requirements in respect of molluscs and lupins and products thereof.

The new Legislation removed the “25% rule” in the previous Directive and introduced the criterion according to which all potential allergens, as defined in Annex IIIa, must be clearly indicated on the label even if present as components of compound ingredients and independently from their final concentration in the product. The rule has to be applied to potential allergenic substances either directly used in the manufacture of food products or those present as residues in the finished product, even in an altered or modified form.

FOOD HYPERSENSITIVITIES AND GASTRO-INTESTINAL MICROBIOTA... A POSSIBLE MECHANISM OF ACTION

A significant proportion of the population is either affected by or concerned about food allergy.

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Scientific knowledge about food allergens and how they stimulate the immune system has increased dramatically over the past decade.

Evidence has emerged supporting the hypothesis that a reduction in microbial antigenic stimulation brought about by widespread vaccination, improvements in standards of hygiene, and extensive use of antibiotics especially in “westernized” countries has contributed to the dysregulation of T-helper 2 cell (T_H2) type responsiveness that typifies allergy and asthma (Hygiene hypothesis).^{2,3}

The intestinal microflora may play a particular role in this respect, as it is the major external driving force in the maturation of the immune system after birth, and animal experiments have shown it to be a prerequisite for normal development of oral tolerance.⁴

The recent increase in allergic disorders has been attributed to a relative lack of microbial stimulation of the infantile gut immune system.⁵ The intestinal microflora can be considered as a postnatally acquired organ that is composed of a large diversity of bacterial cells that perform different functions for the host. Innate immune cells, such as macrophages and dendritic cells, express pattern recognition receptors that recognize pathogen-associated molecular patterns that are associated with microorganisms; activation of these receptors induces a T_H1 type response. Toll-like receptor 2 (TLR2) and TLR4 are members of a family of pattern recognition receptors that recognize various conserved microbial components or pathogen-associated molecular patterns.⁶

It was therefore suggested that a lack of microbial antigen-induced immune deviation from the T_H2 cytokine profile, which predominates at birth, to a T_H1 type profile might explain the development of enhanced T_H2 cell responses to allergens.⁷

COULD A PROBIOTIC SUPPLEMENT BE OF SOME HELP IN THE TREATMENT OF ALLERGIC DISEASES?

Recent epidemiologic studies and experimental research suggest that the microbial environment and exposure to microbial products in infancy, modifies immune responses and enhances the development of tolerance to ubiquitous allergens.

The composition of the microflora differs between healthy and allergic infants and in countries with a high and low prevalence of allergies.⁴

Many efficacy trials have been conducted and have demonstrated the ability of probiotic strains to modulate immune responses.

The results of a randomized double-blind placebo-controlled study provided the first clinical demonstration of specific probiotic strains modifying the changes related to allergic inflammation. The data further indicate that probiotics may counteract inflammatory responses beyond the intestinal milieu.⁸

A recent study showed that administration of 2 *Lactobacillus* strains for 6 weeks moderately reduced the extent and severity of preexistent atopic eczema.⁹

Furthermore, Bongaerts and Severijnen¹⁰ have suggested that adequate probiotics may (1) prevent the increased characteristic intestinal permeability of children with atopic eczema and food allergy, (2) may thus prevent the uptake of allergens, and (3) finally, may prevent the expression of the atopic constitution.

Specific components of the intestinal microflora, including *Lactobacilli* and *Bifidobacteria*, have been associated with beneficial effects on the host, such as promotion of gut maturation and integrity, antagonism against pathogens, and immune modulation.¹¹

The ability of probiotic bacteria to shift the equilibrium of the mucosal immune system toward T_H1 response provides a strategy for treatment of atopic disorders.¹²

The use of live microorganisms that might be beneficial to health has a long tradition and its safety is well documented. Prospective intervention studies, in which the gut flora was modified from birth have yielded encouraging results and may suggest a new mode of primary prevention of allergy in the future.

PROBIOTICS: ALLERGY PREVENTION TOOL...OR ALLERGY TRIGGERING CAUSE?

Despite some encouraging trial results, other clinical studies with probiotic supplements have identified unfavorable reactions, especially in children.

The reason for these negative results has been investigated in some cases. Lee et al¹³ have analyzed and confirmed that about 70% of probiotic supplements, commonly used today in France, contain milk and/or its derivatives, with particular reference to β -lactoglobulin. They also reported a case of an infant with cow's milk allergy who experienced anaphylactic shock a few minutes after ingesting a probiotic supplement.

The administration of probiotics, which are not “allergen-free” poses a serious risk, especially to children. In fact, the allergologic profile of a child is generally not well known and unexpected adverse effects may occur after the intake of even traces of one or more allergenic molecules.

NEED FOR SAFE PROBIOTIC PRODUCTS

The most recent findings further reinforce the need for safe and effective probiotics according to the European Directive.

The nonuse of all potential allergens or raw materials derived from risky substances by means of hydrolysis, heating, and/or mechanical treatments has to be guaranteed.

ALLERGEN-FREE PROBIOTIC STRAINS IN BIOBULK

The first step toward achieving this objective is the identification of the primary allergenic or sensitizing raw materials that are typically used as carbon sources, nitrogen sources, cryoprotectants, and/or excipients in the production of probiotic strains.

Some common examples are:

- milk proteins (milk and whey powder, peptones, and protein hydrolyzed products) commonly used as sources of nitrogen for bacterial growth. The sensitizing substances are caseins, β -lactoglobulin, α -lactalbumin, which are resistant to heat, homogenization, and digestive processes. Typical reactions occur after ingestion, contact, and inhalation after dispersion in the air;
- lactose and by-products that are used as sources of carbon or as cryoprotectants. Reactions are those typical of food intolerance, such as gas production, intermittent diarrhea, constipation;
- soy proteins (peptones and hydrolyzed proteins) commonly used as additional nitrogen sources. Sensitizing substances are stable during digestion and adverse reactions take place especially after ingestion;
- gluten-containing cereal derivatives such as maltodextrin and glucose, often used as carbon sources and excipients. Gliadins, glutelins, globulins are sensitizing substances and reactions occur mainly after ingestion.

The second step is the substitution of these risk materials with alternative substances, particularly of vegetable origin, which do not belong to the list of potential allergen types and are capable of guaranteeing suitable, or even higher, process performance. Infact, the use of vegetable raw materials, usually rich in bioactive peptides, provides an excellent nutritional source for probiotic bacteria and guarantees both their healthy growth and a more robust stability, even after freeze-drying.

The last step is the optimization of all manufacturing phases as regards both the achievement of the highest industrial yield and especially an extremely vital biomass that is indispensable in minimizing mortality and stress of the successive freezing phase. The main common industrial steps in the production of a probiotic microorganism are fermentation, concentration, cell washing to remove catabolites which are toxic for cells and affect the shelf life

of the freeze-dried culture, cryoprotection, and freeze-drying (Fig. 1).

The overall allergen-free manufacturing process is able to guarantee safe consumption even for individuals with food allergies and/or food intolerances and also improve stability of freeze-dried probiotic in biobulk at room temperature due to the physiologic integrity of bacterial cells grown in media rich in bioactive peptides.

ALLERGEN-FREE PROBIOTIC SUPPLEMENTS

A food supplement containing one or more probiotic strains should be prepared using only other active components, such as prebiotic fibers, amino acids, vitamins, minerals, and technologic excipients, which are allergen-free and certified as such by their supplier.

Pediatric Formulations

Pediatric supplements should be optimized with particular attention to the following points:

- use of strains that are typical of the microbiota of children;
- formulation totally allergen free;
- the avoidance of fructose or saccharose for individuals suffering from hereditary fructose intolerance;
- use of only natural sweeteners, flavorings, and, if present, colorants;
- organoleptic characteristics specifically targeted to appeal to children's tastes (sweetness, fruit color, and flavor, etc.).

Many kind of allergen-free product typologies such as sachets, unit dose vials, capsules, and tablets (swallowable, chewable, and modified release) may be manufactured.

QUALITY SYSTEM AND CERTIFICATION

The Quality System must guarantee the nonuse of allergenic raw materials and their derivatives throughout the manufacturing process of the probiotic strain and the finished product. The application of an effective Hazard Analysis and Critical Control Point control plan must be

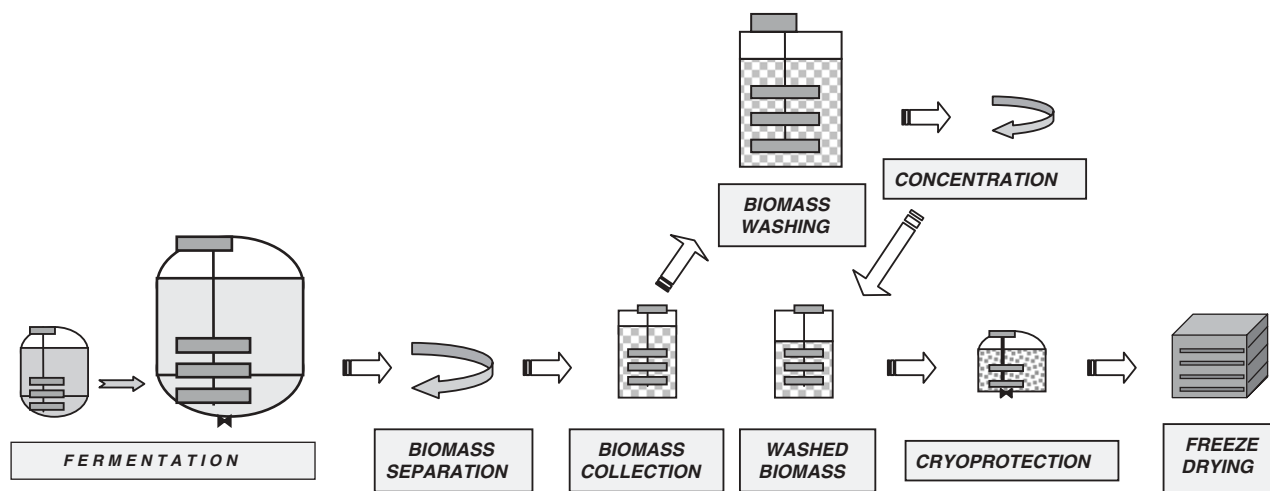


FIGURE 1. Overview of the industrial phases during manufacturing of probiotic strains.

TABLE 1. Range of the Most Recent LOAEL Established by the FDA (2007)

Food	Range of LOAEL (mg Protein)
Crustacea	0.25-10
Egg	0.13-1
Fish	1-100
Milk	0.36-3.6
Tree nuts	0.02-7.5
Peanut	0.25-10
Sesame	0.25-10
Soy	88-522
Gluten	20-100 ppm

Reported in Explanatory Notes of VITAL Allergen Action Level Grid.

Australia New Zealand Food Standards Code Standard, 2007.

FDA indicates Food and Drug Administration; LOAEL, Lowest Observed Adverse Effect Level.

able to continuously monitor possible cross-contamination by means of ultrasensitive and reliable analytical methods for the detection of even traces of allergens in raw materials, process intermediates, and finished products.

Quality Control Certification requires the use of the most sensitive analytical methods available validated by Accredited International Institutions and with detection limits well below the critical dose and Lowest Observed Adverse Effect Level identified in Double-Blind Placebo-Controlled Food Challenges (Table 1).

A further point is the application of tests based on complementary analytical strategies to guarantee the utmost reliability of the analytical findings concerning the presence of trace allergens by means of protein and DNA analysis. The most common examples are enzyme-linked immunosorbent assay, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Immunoblot for the former and polymerase chain reaction or polymerase chain reaction-enzyme-linked immunosorbent assay for the latter.

CONCLUSIONS

Food allergies and intolerances are in constant increase worldwide, especially among children.

It is important to guarantee probiotics with proven health benefits, which are stable at room temperature throughout their shelf life and are free from allergens.

New European rules have been defined not only concerning allergen declaration, but also health claims. This represents a very important opportunity for safe and really effective functional foods, particularly for probiotic products, providing that a “significant quantity” of viable cells at the end of the shelf life is supplied, and safety for all consumers is guaranteed.

Technology, research, and a proper quality system are indispensable for the production of safe and effective probiotic strains and the food supplements, which contain them.

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