Fermented Dairy Products

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Dairy products were shown to represent a source of bacteria able to establish, at least temporarily, in the human gut. It was found that traditional Pasta Filata fresh cheeses obtained with natural bacterial cultures contributed bacterial components to the intestinal microbiota since identical sequences of selected genetic markers, *rpo*D and *clp*P, discriminant at intra-species level, were found both in the cheese and in healthy children who consumed the cheese for one week. Studies carried out in vivo by administering bacteria isolated from traditional dairy products and belonging to the species involved in fermentation and ripening of these foods proved able to exert varied health promoting effects in animal models and in clinical trials. Therefore, fermented dairy products can be considered a source of naturally occurring probiotics well adapted to the food matrix that deserves further investigations regarding the beneficial effects of the microbiota and their enhancement.

dairy microorganisms SLAB NSLAB probiotic effects fermented dairy products

1. Introduction

Probiotic foods are currently defined as foods that supply “probiotics” proven to exert health-promoting effects in human trials. These effects must derive at least in part from the microorganisms present and must be distinct from those of the food matrix [1]. For probiotic foods, the recommendation of providing an adequate number of microorganisms per serving, i.e., at least 10^9 CFU, is re-affirmed, in accordance with the recommended intake for probiotics of the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) [2]. Indeed, the term “probiotic” can be defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO), later emended by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [3].

Probiotic benefits include regulation of intestinal transit, normalization of perturbed intestinal microbiota, competitive exclusion of pathogens, hypolipidemic, anti-allergic and antioxidant effects, immunomodulation, and production of bioactive compounds. These effects are strain-specific so they differ for microorganisms belonging to the same species [3].

In vitro assessment of acid and bile salt tolerance prior to undertaking in vivo trials is required to indicate if a potential probiotic is able to survive in the host’s gastrointestinal tract (GIT). In vivo trials in animal models are necessary to predict beneficial effects in human beings and explain those effects by the observation of changes in tissue, cell structure, and biomarker expression in different organs.

Bacteria naturally present and involved in fermentation and ripening of dairy products comprise strains able to behave as probiotics with efficacy proven in animal models and in clinical trials [4]. For some of these, the ability to produce bacteriocins, such as different nisin forms, lacticin 3147, pediocins, and enterocins, enhanced the beneficial effects against infections and immunomodulation in animal models [5].

2. Fermented Dairy Products

Dairy products were shown to represent a source of bacteria able to establish, at least temporarily, in the human gut. It was found that traditional Pasta Filata fresh cheeses obtained with natural bacterial cultures contributed bacterial components to the intestinal microbiota since identical sequences of selected genetic markers, *rpo*D and *clp*P, discriminant at intra-species level, were found both in the cheese and in healthy children who consumed the cheese for one week. Some of those fecal isolates were obtained after two weeks of cheese administration suspension, showing a good colonization ability [6].

In an in vivo pilot study involving healthy individuals *Bifidobacterium mongoliense* BMONG18, detected along the whole Parmesan cheese production chain, was detected in the feces of all individuals during cheese consumption. After one week of suspension, *B. mongoliense* BMONG18 decreased revealing low persistence capacity in the human gut. Despite the in vivo function of *B. mongoliense* BMONG18 not being elucidated, the study demonstrated that cheese consumption contributes intestinal microbiota components [7].

Illikoud et al. [4] inferred from clinical studies regarding the effects on health of fermented dairy products that their deprivation from human diet caused a lower efficiency of the innate immune response and that daily consumption of yogurt containing a conventional starter constituted by *S. thermophilus* and *L. delbrueckii*, counteracted this effect [4]. Six of those studies showed that yogurt, cheese, or sour cream consumption decreased biomarkers of inflammation in different categories of subjects, including obese women with non-alcoholic fatty liver disease and metabolic syndrome, premenopausal women, obese people, normal weight, and overweight volunteers. They also cited a systematic review of the literature considering 10 trials with contrasting conclusions suggesting that the effectiveness of different fermented dairy products can vary, possibly for the different functional activities of the microorganisms present [4]. More recent studies are summarized below and separated according to the observed effects.

2.1. Amelioration of Intestinal Health

Antimicrobial peptides of the regenerating family member 3 (REG3) family, that maintain the intestinal barrier, are reduced in the small intestines by aging, but the long-term ingestion of yogurt fermented with *L. delbrueckii* subsp. *bulgaricus* 2038 and *S. thermophilus* 1131 by aged mice increased their expression. The REG3 family is induced in the intestinal epithelial cells by interleukin IL-22, predominantly produced by type 3 innate lymphoid cell (ILC3) that is, in turn, induced by IL-23, produced by dendritic cells (DCs) and macrophages after stimulation of Toll-like receptors (TLRs) by bacterial cell components. In the study by Kobayashi et al. [8], oral administration of these

strains to specific pathogen-free (SPF) male BALB/c mice led to *Reg3g* induction in the small intestine cells and production of IL-22 and IL-23 with a higher effect exerted by *S. thermophilus* 1131.

2.2. Amelioration of Atopic Dermatitis

Numerous studies have reported beneficial effects of probiotics on atopic dermatitis, e.g., for the widely used probiotic *L. rhamnosus* GG [9]. Based on this evidence, a study aimed to evaluate the effect of cream cheese containing *Lactococcus chungangensis* CAU 28 (CAU 28) (1.4 g/kg/mouse daily) and the dry cells (10^{10} CFU/mouse, daily) of the same strain compared to an untreated positive control and treatment with bepotastine besilate (BB) on the amelioration of atopic dermatitis in female BALB/c mice sensitized with ovalbumin (OVA) when administered for 8 weeks [10].

In that study, it was observed that cytokines produced by regulatory T (Treg) cells, such as IL-10 and IL-1β and Th2 cytokines IL-4 and IL-5 were significantly lower and levels of the Th1 cytokines, IL-12, IFN-γ, and TNF-α were significantly higher in the CAU 28 and CAU 28 cream cheese groups, indicating an enhancement of the Treg-mediated suppression of Th2 immune response [10].

The activation of CD 86 T cell protein expression was significantly lower in the CAU 28 cream cheese group and the expression of CD 274 suppressor of adaptive immune response was significantly higher in the CAU 28 and CAU 28 cream cheese groups. Serum IgE levels and eosinophil, neutrophil, lymphocyte, and monocyte percentages significantly decreased in the latter groups. Mast cell accumulation at the dorsal skin and ileal lesions was suppressed in mice treated with CAU 28 and CAU 28 cream cheese and reduced eosinophil infiltration was observed in the dorsal skin lesions of mice treated with CAU 28, CAU 28 cream cheese, and BB [10].

High-throughput sequencing of 16S rRNA gene from feces showed a positive correlation between CD 274 and *Bacteroidales*, *Deferribacteraceae*, *Prevotellaceae*, *Oscillospiraceae*, *Rikenellaceae*, and *Veillonellaceae*, while CD 86 levels were correlated with several bacterial families, including *Verrucomicrobiales* and negatively correlated with *Desulfovibrionaceae*. *Bacteroides* and *Akkermansia* were present at significantly higher abundance in the CAU 28 cream cheese-treated group. The levels of short chain fatty acids (SCFA), associated with the maintenance of gut health and positively correlated with *Lactobacillus*, *Bacteroides*, *Ruminococcus*, and *Akkermansia* were found to be higher in the feces of mice treated with CAU 28 cream cheese [10]. SCFAs, mainly acetic, propionic, butyric, and valeric acids, are produced from complex dietary carbohydrates by components of the colon microbiota. These represent an energy supply and exert regulatory and immunomodulatory functions in the intestinal epithelial cells (IEC). Butyrate, produced from acetic acid, lactate, and amino acids, is the preferred energy source for IEC and its metabolism maintains hypoxia-favoring anaerobic commensal bacteria. Butyrate also promotes the differentiation and inhibits the proliferation of intestinal cells in physiological conditions, thus also repressing cancerous cells [11].

2.3. Allergy Amelioration

Milk fermentation was found to alleviate indicators of the immune reaction in CMPA. This immune hypersensitivity condition is a complex disorder in which the main symptoms are atopic dermatitis, eczema, asthma, vomiting, and recurrent diarrhea. In CMPA, B cells induced by antigen activated $CD4^+$ T helper (Th) cells produce IgE that bind to a high-affinity Fc receptor on the surface of mast cells or basophils that binds to allergen epitopes and triggers the release of inflammatory mediators such as histamine and mast cell protease-1 (MCPT-1). It was shown that fermentation with combined LAB could reduce the antigenicity of cow milk protein by destroying the linear epitopes in vitro [12]. Therefore, *L. helveticus* KLDS 1.8701, selected for antioxidant capacity and isolated from traditional fermented dairy products in Sinkiang, China and endowed with genes coding two cell envelope proteases (CEP), and *L. plantarum* KLDS 1.0386, isolated from an Inner Mongolia traditional fermented dairy product, endowed with 21 peptidase genes and transport systems, were used by Zhao et al. [12] in single or mixed culture for the degradation of α-casein, α-lactoalbumin, and β-lactoglobulin in reconstituted skim milk. The antigenicity of the fermented milks was compared in specific-pathogen-free female Balb/c mice. On days 0, 7, 14, and 21, the mice were sensitized with a mixture of fermented milk containing *L. helveticus* KLDS 1.8701 and *L. plantarum* KLDS 1.0386, alone or in combination, and 10 µg of cholera toxin (CT) as a Th2-polarizing adjuvant. Anaphylactic shock and diarrhea were less severe and the spleen immune indices were lower in the fermented milk groups. Histamine and MCPT-1 were significantly lower in the fermented milk groups, lungs showed a normal alveolar structure without infiltrating inflammatory cells, and the structure of jejunum villi did not present remarkable edema and inflammatory cell infiltration [12].

2.4. Amelioration of Metabolic Syndrome

The study by Makwana et al. [13] reported that probiotic fermented milk alone (T1) or enriched with 2% whey proteins and soy proteins (T2) and containing 10^8–10^9 CFU/mL of lactobacilli could effectively control obesity when administered for 4 weeks to adult male Wistar rats fed with high fat diets (HFD). The fermented milks were prepared with *L. helveticus* MTCC V3, *S. thermophilus* MD2, and *L. rhamnosus* MTCC NS6 isolated from milk and Indian style yogurts.

In the fermented milk group, weight gain was 46–55% while it increased by 71% in the group receiving only the HFD. The liver weight and the weight of abdominal fat in treatments T1 and T2 were considerably reduced. Treatment with T1 and T2 produced a significant decline in triglycerides (TG) and total cholesterol but did not influence the levels of low-density lipoprotein (LDL). Among alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) indicators of liver disease, associated more frequently with obese individuals than the normal population, ALT and ALP significantly decreased in T1 and T2. Release of adipocyte leptin, a regulator of food intake and energy utilization associated with hyperleptinemia, was delayed in T1 and T2. Liver sections stained with eosin and hematoxylin for T1 showed fewer and smaller lipid vacuoles. Viable counts of lactobacilli and *S. thermophilus* in feces during the feeding phase were of the order of 7 Log CFU/mL and increased progressively during the feeding period, indicating that the probiotic lactobacilli stably colonized the intestinal tract [13].

2.5. Antihypertensive Effects

The benefits of milk fermented by dairy LAB are exerted also by their ability to form substances with the potential to ameliorate disease conditions [14]. An example is given by angiotensin converting enzyme (ACE) inhibitors (ACE-I) that exert antihypertensive effects and the best studied ACE-I-containing foods are fermented dairy products. The formation of these compounds depends on the proteolytic and peptidolytic activity of LAB that generates small peptides from milk proteins, mainly α, β, and κ-caseins, during fermentation. However, the proteolytic systems in different LAB vary not only between species but also between different strains of the same species. Consequently, starter strains producing fermented milk with higher ACE-I properties must be appropriately selected [15].

A spontaneously hypertensive rat (SHR) model was used by Glazunova et al. [15] to evaluate the effect on blood pressure of reconstituted skim milk (RSM) fermented by 8 dairy starter LAB (SLAB) strains or non-fermented negative control for four weeks. Rats fed with RSM fermented with *Lactobacillus delbrueckii* Lb100 isolated from commercial yogurt and *Lactococcus lactis* AM1 isolated from amasi showed a significant decrease of systolic pressure (Psyst) down to 154 mmHg at the end of experiment with a ΔPsyst of approximately −17 mmHg. In addition, the *L. delbrueckii* Lb100 group showed also significantly lower levels of total cholesterol and high-density lipoprotein cholesterol (HDL) [15].

The antihypertensive effects of fermented milks were reviewed by Beltrán-Barrientos et al. [16], who reported that, beyond ACE inhibitory activity, these effects may also depend on the presence of compounds with antioxidant, nitric oxide production enhancing, and opioid receptor binding activities and on the presence of γ-aminobutyric acid that binds to GABAB receptors. Moreover, antihypertensive effects can derive from compositional changes in gut microbiota. Among naturally fermented milks, kefir was examined in different studies and it was reported that kefir grains administered to SHRs for 60 days determined a significant decrease in blood pressure and an improvement in endothelial dysfunction for a partially restored imbalance between reactive oxygen species and nitric oxide. Moreover, blood pressure was reduced in SHR after nine weeks of treatment with kefir for a reduced cardiac hypertrophy, improved cardiac contractility and regulation of calcium proteins, and signals from the cardio-regulatory regions of the central nervous system (CNS) [16].

In addition, kefir treatment at the intestinal level normalized the number of Paneth cells, producing antimicrobial peptides and active in the regulation of the intestinal microbiota [17], and decreased thickening of the tunica muscularis, thus reducing alterations of the intestinal barrier. Levels of the lipopolysaccharide (LPS) endotoxin, involved in the pathogenesis of hypertension for its ability to activate the Toll-like receptor 4 (TLR4) with production of proinflammatory cytokines, chemokines, and ROS in CNS [18], decreased in the kefir-treated SHR rats. Kefir assumption normalized the levels of inflammatory cytokines TNF-α and IL-6 and reduced neuroinflammation with consequent mitigation of hypertension [16].

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