Chapter

Propionibacterium freudenreichii: General Characteristics and Probiotic Traits

Vinícius de Rezende Rodovalho, Diego Lucas Neres Rodrigues, Gwénaël Jan, Yves Le Loir, Vasco Ariston de Carvalho Azevedo and Eric Guédon

Abstract

Propionibacterium freudenreichii is a Gram-positive dairy probiotic bacterial species that has been used as a ripening starter in the production of Swiss-type cheese for a long time. It has been exploited for the optimization of cheese production, including ripening capacities and aroma compounds production, but also for the production of vitamin B12 and organic acids. Furthermore, it has emerged in the probiotics landscape owing to several beneficial traits, including tolerance to stress in the gastrointestinal tract, adhesion to host cells, anti-pathogenic activity, anticancer potential and immunomodulatory properties. These beneficial properties have been confirmed with *in vitro* and *in vivo* investigations, using several omics approaches that allowed the identification of important molecular actors, such as surface proteins, short-chain fatty acids and bifidogenic factors. The diversity within the species was shown to be an important aspect to take into consideration, since many of these properties were strain-dependent. New studies should dive further into the molecular mechanisms related to the beneficial properties of this species and of its products, while considering the complexities of strain diversity and the interactions with the host and its microbiota. This chapter reviews current knowledge on the possible impact of *P. freudenreichii* on human health.

Keywords: *Propionibacterium freudenreichii*, propionibacteria, probiotics, immunomodulation, food microbiology

1. Introduction

The denomination "probiotics" comprises living microorganisms, including bacteria and yeasts, with health-promoting properties and suitable for safe consumption, as confirmed by their dietary uses for thousands of years of human history [1–3]. Lactic acid bacteria and bifidobacteria comprise traditional probiotic bacteria species, widely documented and commercialized [3, 4]. However, different species have emerged in the probiotics landscape, such as the dairy species *Propionibacterium freudenreichii* [4, 5], which is phylogenetically related to bifidobacteria (**Figure 1**) [4].
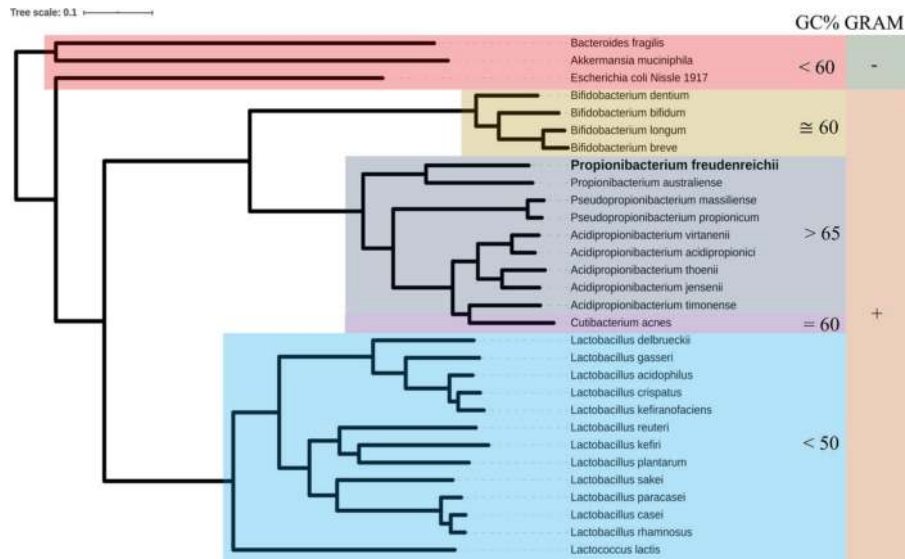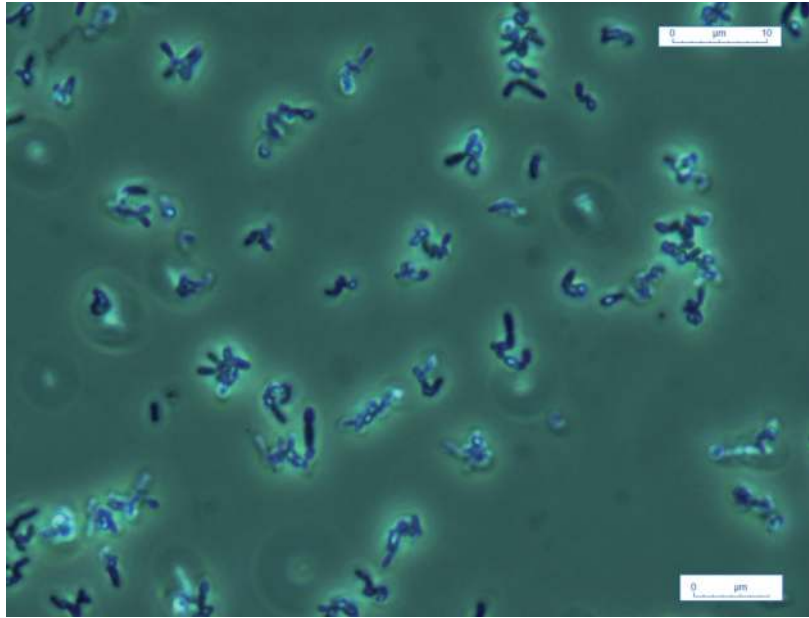
Figure 1.
Phylogenetic tree showing genomic similarity between health-promoting P. freudenreichii *species, other probiotic or closely-related species.*

The former *Propionibacterium* genus encompassed a group of microorganisms with importance in industry and health, due to the production of valuable metabolites, food, cosmetic and pharmacological products [6]. Previously, this genus included classic dairy propionibacteria species and skin-associated pathogenic propionibacteria [7]. However, a genome-based taxonomy reevaluation suggested the reclassification of cutaneous bacteria into the *Cutibacterium* genus, together with the inclusion of two other new genera for formerly classic propionibacteria, *Acidipropionibacterium* and *Pseudopropionibacterium* [7]. *P. freudenreichii*, which is one of the most notable dairy propionibacteria species, kept its former taxonomic classification [4, 7].

P. freudenreichii is a Gram-positive, high GC-content, mesophilic, aerotolerant, non-motile, non-spore forming bacterium, that shows low nutritional requirements and survives in harsh environments [5, 8, 9]. Regarding morphology, it is a pleomorphic rod microorganism, with aggregation tendency, forming clusters that resemble Chinese characters [5] (**Figure 2**). This bacterium, isolated from samples of Emmental cheese, was first described by Orla Jensen and von Freudenreich in 1906 [10]. Recently, *P. freudenreichii* strains have been identified in fecal samples from a discrete cohort of human preterm breastfed infants, suggesting that it could be a component of the healthy human gut microbiota [11].

P. freudenreichii is able to use several carbon sources (e.g., glycerol, erythriol, L-arabinose, adonitol, galactose, D-glucose, D-fructose, D-manose, inositol, arbutine, esculine, lactose, lactate and gluconate) in the fermentation process to produce propionate, together with acetate, succinate and carbon dioxide (CO_2) [9, 12, 13]. Unlike other species, *P. freudenreichii* is able to reduce pyruvate into propionate via the transcarboxylase cycle (also referred to as Wood–Werkman cycle), which is a cyclic process coupled to oxidative phosphorylation, that allows a higher ATP yield than in other propionate-producing bacteria [9]. In its turn, pyruvate is a metabolic node molecule, which may be used either for the NADH-generating synthesis of acetate, or for the NADH-consuming synthesis of propionate [14]. In a strain-dependent manner, the bacterium modulates the proportions of pyruvate that are reduced into propionate or oxidized into acetate and CO_2, thus maintaining the

Figure 2.
Optical microscopy image showing the morphological aspect of a P. freudenreichii *CIRM-BIA129 culture, with typical aggregates resembling Chinese characters.*

redox equilibrium [9]. Therefore, this species encompasses biochemically versatile strains, that find different applications in several contexts [5].

2. Technological importance

P. freudenreichii is widely used for the production of Swiss-type cheeses, such as Emmental [5, 15] (**Figure 3**). In such dairy matrices, the CO_2 gas that is produced during fermentation forms bubbles that diffuse slowly, creating characteristic holes, or "eyes", in cheese architecture [9, 12]. Cheese flavor is related to propionate and acetate, as well as the products of amino acids catabolism and fat hydrolysis by propionibacteria [16, 17]. Importantly, these dairy products containing *P. freudenreichii* displayed anti-inflammatory properties *in vivo* [18–20], increasing the recognition of this bacterium and of its products as health-promoting. Therefore, dairy propionibacteria are considered 2-in-1 bacteria, with both fermentative and probiotic properties, which makes them ideal for the development of health-promoting fermented food [5, 18].

This bacterium is also well recognized to encompass a pathway for vitamin B12 (cobalamin) synthesis [8, 9]. Vitamin B12 is a water-soluble vitamin, which plays a key role in the functioning of the brain, of the nervous system and in the

Figure 3.
Emmental cheese produced using P. freudenreichii *CIRM-BIA129, in conjunction with* Streptococcus thermophilus *and* Lactobacillus delbrueckii.

production of blood [21]. It is also a co-factor of methylmalonyl-CoA mutase, which catalyzes a crucial step in the fermentative route to produce propionate [22]. Therefore, the growth conditions of *P. freudenreichii* have been optimized for the production of vitamin B12, using substrates such as cereal matrices [23, 24], waste frying sunflower oil [25], tofu wastewater [26] and soybean agroindustry residue [27]. Moreover, *P. freudenreichii* has been genetically engineered to enhance vitamin B12 and propionate production [6, 28].

The production of vitamin B12, organic acids, trehalose and other metabolites, together with the safe use as cheese ripening starter and probiotic characteristics, make this bacterium attractive for several biotechnological and industrial applications [5, 6, 29, 30]. A wide range of genetic and environmental optimizations have been conducted to improve these properties [6, 29]. Moreover, some optimizations of the growth and processing conditions allowed the improvement of resistance towards storage and towards several industrial processes, such as freeze-drying and spray-drying [30–33].

3. Strain variability

The interesting properties of this bacterium, such as health-promoting features, and participation to industrial vitamin B12 and cheese production, were shown to be strain-dependent, suggesting the need for analysis that account for that variability [9]. As an example, some strains presented differences in nitrogen and sugar degradation, which had a genetic origin, probably resulting from horizontal transfers, duplications, transpositions and other mutations [13]. This strain diversity was confirmed at the genomic level by another study and attributed to transposable elements, in such a way that genome plasticity enabled bacterial adaptation to several environments [34].

In view of this strain-related variability, there have been efforts to specify criteria for the selection of probiotic strains. These criteria include tolerance to stresses encountered within the gastrointestinal tract, adhesion to host cells, antipathogenic activity, anticancer potential, immunomodulatory properties, industrial requirements and molecular characterization using omics methodologies [3]. Mounting evidence shows that *P. freudenreichii* fulfills these criteria [5].

4. Stress tolerance

Regarding stress tolerance and adaptation to the gastrointestinal tract (GIT), some *P. freudenreichii* strains presented adaptations, including morphological and proteomic modifications [35–37]. For example, those modifications were verified during the acid tolerance response in the strain *P. freudenreichii* SI41, which was investigated using a kinetic study of stress proteins production during acid adaptation [35]. As a result, biotin carboxyl carrier and proteins involved in DNA synthesis and repair were associated to early acid stress response, whereas chaperonins GroEL and GroES were associated to late acid stress response [35]. Analysis with the same strain showed that bile salts (a mixture of cholate and deoxycholate) triggered drastic morphological changes and induced proteins related to signal sensing and transduction, general stress and an alternative sigma factor [36]. The same strain was used in a follow-up comprehensive study that included heat, acid, and bile salts conditions to study *P. freudenreichii* tolerance. As a result, each form of stress induced specific proteins, but six of them were common to all stresses, including chaperones and proteins involved in energetic metabolism and oxidative

stress remediation [37]. An *in vitro* study that involved 13 strains of *P. freudenreichii* showed that most of them had high capacity of tolerance to simulated gastric juices with varying pH and small intestine conditions [38].

Moreover, this resistance was also evidenced *in vivo*. The mRNA of *P. freudenreichii* methylmalonyl-transcarboxylase was detected in human fecal samples using real time reverse transcriptase polymerase chain reaction (RT-PCR) [39]. Methylmalonyl-transcarboxylase is a key enzyme of the transcarboxylase cycle, only expressed when propionic fermentation is active, therefore its detection in fecal samples indicated that the bacterium survived and remained metabolically active, transcribing genes within the human digestive tract [39]. A multi-strain study using human microbiota-associated rats monitored intestinal microbiota composition and short-chain fatty acids production, confirming that *P. freudenreichii* stress tolerance in the GIT is also strain-dependent [40]. *P. freudenreichii* CIRM-BIA1 was shown to adapt metabolically and physiologically to the colon environment of pigs, with changes in carbohydrate metabolism, down-regulation of stress genes and up-regulation of cell division genes [41]. Furthermore, the use of food vehicles for *P. freudenreichii* delivery, such as cheese and fermented milk, improved its resistance towards the GIT stressing environment [15, 18, 19, 42, 43].

Other aspects of *P. freudenreichii* resistance to stress conditions have also been studied, such as long-term nutritional shortage [44, 45]. A screening was performed with eight *P. freudenreichii* strains, which were incubated for several days after the beginning of the stationary phase, without further supplementation of nutrients. They displayed high survival rates and no lysis, indicating that these strains adapt to long-term nutritional shortage, using a viable, yet nonculturable state [45]. The strain *P. freudenreichii* CIRM-BIA138 was further studied in these conditions of incubation, and it was shown that a high population was maintained, even after exhaustion of lactate, the preferred carbon source. RNA-seq analysis showed that several metabolic and information processing pathways were down-regulated [44].

Another important feature of *P. freudenreichii* during stress response is the accumulation of trehalose. A study that investigated this bacterium during adaptation to osmotic, oxidative and acid stress, showed that the trehalose-6-phosphate synthase/phosphatase (OtsA–OtsB) pathway, related to trehalose synthesis, was enhanced in these conditions [46]. Another study focused in stress response to low temperature (4°C), a condition that mimicked cheese ripening conditions. As a result, seven *P. freudenreichii* strains displayed a slowed-down cell machinery, cold stress response and the accumulation of trehalose and glycogen [47]. *P. freudenreichii* also accumulated glycine betaine, glycogen, trehalose and polyphosphates when cultured in hyperconcentrated media [48]. The accumulation of trehalose, together with glycine betaine, was further verified in a technological context, when bacterial viability was increased during spray drying and storage, through the optimization of the growth medium composition and thermal adaptation [31]. The ratio of the concentrations of these intracellular osmoprotectants, trehalose and glycine betaine, was further shown to modulate the stress tolerance during the technological processes of freeze-drying and of spray-drying [32].

5. Adhesion properties

Adhesion to host cells is another important feature of probiotics, which favors their local beneficial action. Early studies revealed the ability of several probiotic bacteria, including *P. freudenreichii*, to adhere to glycoproteins and mucus from human intestinal tract [49, 50]. In the case of *P. freudenreichii*, the adhesion of some

strains to pig ileum cells (IPEC-J2) was between 25 and 35%, and that proportion was higher with the addition of $CaCl_2$ [51]. In the case of human host, several bacterial strains were tested for adhesion to HT-29 colon cells *in vitro*; the most adhesive strain was *P. freudenreichii* CIRM-BIA129 and surface layer protein B (slpB) key role in adhesion was demonstrated by using gene inactivation [52, 53]. Another study demonstrated that bacterial adhesion to immobilized mucus could be synergistically improved by administration of strains combinations, such as *P. freudenreichii* ssp. *shermanii* JS in combination with *Bifidobacterium breve* or *Lactobacillus rhamnosus* strains [54].

6. Anti-pathogenic activity

There are also several evidences of an anti-pathogenic activity in this species. *P. freudenreichii* JS reduced by 39% the adhesion of *S. aureus* to human intestinal mucus and by 27% its viability, probably due to the production of organic acids [55]. *P. freudenreichii* PTCC 1674 was reported to secrete a lipopeptide biosurfactant with antimicrobial activity mainly against *Rhodococcus erythropolis*, and anti-adhesive activity mainly against *Pseudomonas aeruginosa* [56]. Moreover, *P. freudenreichii* DSM 20270 significantly inhibited *E. coli* O157:H7 growth *in vitro* [51].

P. freudenreichii also showed anti-pathogenic properties in animals. *P. freudenreichii* B-3523 and B-4327 impacted *Salmonella* strains multiplication, motility and adhesion to avian epithelial cells *in vitro* [57]. The follow up study indicated that the cell-free culture supernatants of the same probiotic strains were bactericidal against multidrug-resistant *Salmonella enterica* serovar Heidelberg [58]. *In vivo* assays further showed that the probiotic strains reduced the pathogen cecal colonization and dissemination to the liver in turkey poults [58]. *P. freudenreichii* consumption was furthermore shown to limit and to delay colonization of the mice intestinal tract by the pathogen *Citrobacter rodentium* [59].

In line with the synergies observed in terms of adhesion, probiotic combinations were proposed to improve anti-pathogenic activity, such as a combination of *P. freudenreichii* JS, *L. rhamnosus* GG and LC705, and *B. breve* 99, which promoted the inhibition, displacement and competition with several pathogenic species, such as *S. enterica*, *Listeria monocytogenes* and *Clostridium difficile* [60]. In another study, *P. freudenreichii* JS decreased the adhesion of *Helicobacter pylori* to Caco-2 intestinal cells when used individually, but also inhibited membrane leakage, improved epithelial barrier function and modulated inflammatory cytokines when used in combination with *L. rhamnosus* and *B. breve* strains [61].

7. Anticancer potential

Promising results, in the context of intestinal carcinogenesis, were also reported in this species. A pioneer study showed that *P. freudenreichii* ITGP18 and *P. freudenreichii* SI41 could induce apoptosis of cultured human colorectal carcinoma cell lines *in vitro* and this effect was mediated by short-chain fatty acids (SCFAs), such as propionate and acetate, acting on cancer cells mitochondria [62]. Following up, it was further clarified that the effect of SCFAs was modulated by extracellular pH shifts; and in acidic pH, cell death mode changed from apoptosis to necrosis in human colon HT-29 cells [63]. These effects were confirmed *in vivo*, with *P. freudenreichii* TL133 inducing the apoptosis of colon cells in human microbiota-associated rats treated with 1,2-dimethylhydrazine, yet not in healthy rats [64].

Another strain, *P. freudenreichii* ITG P9, was also employed for the development of a fermented milk with anti-oncogenic potential, since it induced apoptosis in cultured HGT-1 human gastric cancer cells *in vitro* [43]. Next, this fermented milk was proposed as an adjuvant in colorectal cancer therapy based on TNF-related apoptosis-inducing ligand (TRAIL), due to possible synergistic effect between the bacterium and TRAIL, which was confirmed with the enhancement of cytotoxic activity in HT-29 cells [65]. Another study investigated the crosstalk between bacterium and cancer cells: the latters produce lactate as a result of the metabolic shift referred as "aerobic" glycolysis or "Warburg effect"; lactate may then be used by this bacterium as a carbon source, stimulating its production of SCFAs [66].

8. Modulation of microbiota composition

Regarding the modulation of microbiota composition, consumption of dairy propionibacteria was shown to enhance intestinal populations of bifidobacteria in humans [67, 68]. In line with this, the stimulation of bifidogenic growth was observed in cell-free filtrate and cellular methanol extract derived from *P. freudenreichii* 7025 cultures [69]. Following analysis with the same strain allowed the purification of a bifidogenic growth stimulator component, the identification of its chemical structure (2-amino-3-carboxy-1,4-naphthoquinone, ACNQ) and the demonstration of its bifidogenic activity in the concentration of 0.1 ng/mL [70]. Another strain, *P. freudenreichii* ET-3 was reported to produce 1,4-dihydroxy-2-naphthoic acid (DHNA) in concentrations of 10 μg/mL, which also stimulated the growth of bifidobacteria [71]. The beneficial effect of DHNA was later confirmed *in vivo*, using mice with colitis induced by 2.0% dextran sodium sulphate (DSS). DHNA attenuated inflammation, through the modulation of intestinal bacterial microbiota and suppression of lymphocyte infiltration [72].

The bifidogenic growth stimulator derived from *P. freudenreichii* was also orally administrated to human patients in a pilot study, being promising for the treatment of ulcerative colitis [73]. Subsequent studies included optimizations of the production of bifidogenic growth stimulators, including an increased production by switching to aerobic growth conditions [74] and the use of lactic acid as a carbon source in a bioreactor system with a filtration device [75].

9. Immunomodulatory properties

There is mounting evidence, both *in vitro* and *in vivo*, that *P. freudenreichii* exerts immunomodulatory effects by several mechanisms, in a strain-dependent manner. For example, a screening for IL-10 induction in human peripheral blood mononuclear cells (PBMCs) was performed in 10 strains of *P. freudenreichii*, resulting in the selection of the two most anti-inflammatory strains: *P. freudenreichii* ITG P20 (equivalent to CIRM-BIA129) and SI48 [59]. In the same study, the strain *P. freudenreichii* SI48 was further tested *in vivo*, in mice with acute colitis induced by trinitrobenzenesulphonic acid (TNBS), lowering significantly inflammatory and histological markers of colitis [59]. Other studies also showed that the immunomodulatory properties were strain-dependent within the species *P. freudenreichii* [76]. An integrative strategy encompassing comparative genomics, surface proteomics, transcriptomics, assays of cytokines induction and genes inactivation, identified relevant proteins and strains specificities in immunomodulation [77]. Remarkably, surface proteins of the S-layer type were shown to be crucial in immunomodulation, but the immunomodulatory properties varied among strains, due to complex

Strain	Protein	Name/Description	Accession	Evidence level	Ref.
CIRM-BIA129 (ITG P20)	Ensemble of surface proteins			proteomic, *in vitro*	[82]
	GroL2	60 kDa chaperonin 2	CDP49125	genomic, proteomic	[77]
	HsdM3	Type I restriction-modification system DNA methylase	CDP48267	genomic, transcriptomic, mutant studies *in vitro*	[77]
	LacI1	Arabinose operon repressor	CDP47860	transcriptomic	[77]
	MerA	Pyridine nucleotide-disulphide oxidoreductase	CDP48574	genomic, proteomic	[77]
	Pep	Hypothetical protein	CDP48241	genomic, mutant studies *in vitro*	[77]
	PFCIRM129_04790	Hypothetical protein	CDP48736	genomic, transcriptomic	[77]
	PFCIRM129_10785	Hypothetical protein	CDP49252	transcriptomic	[77]
	PFCIRM129_10930	Hypothetical protein	CDP48242	genomic, transcriptomic	[77]
	SlpB	Surface layer protein B	CDP48273	genomic, transcriptomic, proteomic, mutant studies *in vitro*	[77]
				mutant studies *in vitro* and *in vivo*	[83]
				mutant studies *in vitro*	[52]
	SlpE	Surface protein with SLH domain	CDP48858	genomic, transcriptomic, proteomic, mutant studies *in vitro*	[77]
	SlpF	Surface protein with SLH domain	CDP49687	proteomic, mutant studies *in vitro*	[77]
CIRM-BIA 121	Acn	Aconitase, Aconitate hydratase	CEG89374	transcriptomic	[77]
	DcuA	C4-dicarboxylate transporter	CEG91776	genomic	[77]
	Eno1	Enolase 1	CEG91483	proteomic, mutant studies *in vitro*	[77]
	HtrA4	Serine protease	CEG91080	genomic, transcriptomic, proteomic, mutant studies *in vitro*	[77]
	PFCIRM121_08235	Hypothetical protein/unknown function	CEG91253	genomic, mutant studies *in vitro*	[77]
	SlpC1	Surface layer protein C	CEG91216	genomic, transcriptomic	[77]
UF1	LspA	Large surface layer protein A	n.a.	proteomic, *in vivo*	[84]
	DlaT	Dihydrolipoamide acetyltransferase	n.a.	proteomic, mutant studies *in vivo*	[11]

Legend: Ref.: references, n.a.: not available.

Table 1.
Proteins from P. freudenreichii related to its immunomodulatory properties.

combinations of molecular features [77]. The strain-specific export of surface proteins, adhesins and moonlighting proteins was confirmed in a different subset of *P. freudenreichii* strains [78]. Additionally, acute colitis induced by dextran sodium sulfate (DSS) in rats was ameliorated by *P. freudenreichii* KCTC 1063, which stimulated in intestinal cells the expression of MUC2, a main component of mucus [79].

The roles of *P. freudenreichii* in the modulation of host immunological response became even more relevant when a human commensal strain was identified. *P. freudenreichii* UF1 was demonstrated to be a component of the gut microbiota of preterm infants that were fed with human breast milk and to mitigate intestinal inflammatory diseases [11]. Moreover, this strain modulated the intestinal immunity of mice against pathogen challenge, specifically against systemic *L. monocytogenes* infection, by regulating Th17 cells [80]. This beneficial effect was confirmed in newborn mice, which were susceptible to intestinal pathogenic infection, but had their defense enhanced by this strain, particularly by the increase in protective Th17 cells and regulatory T cells [81].

Regarding the bacterial factors involved in immunomodulation, evidence points out mainly to surface proteins (**Table 1**). The strain *P. freudenreichii* CIRM-BIA129 had its proteome investigated, with the identification of surface-exposed proteins and their role in induction of IL-10 and IL-6 release by PBMCs [82]. Among the identified proteins, there were cell wall-remodeling proteins, transport proteins, moonlighting proteins and other proteins involved in interactions with the host [82]. The multi-strain and multi-omics study conducted by Deutsch et al. [77] clarified that cytoplasmic proteins might also be relevant in immunomodulation, but confirmed the key role of surface-layer proteins B (SlpB) and E (SlpE), particularly in strain *P. freudenreichii* CIRM-BIA129. SlpB was then shown to be crucial for bacterial adhesion to epithelial intestinal cells [52], and a mutation in its gene had pleiotropic effects, suggesting this protein could have a central role in cellular processes [53]. Additionally, *in vivo* assays that were conducted in mice with mucositis induced by 5-Flourouracil (5-FU), showed that SlpB protein is crucial for the cytokine modulation triggered by *P. freudenreichii* CIRM-BIA129 [83]. Moreover, the glycosylated large surface layer protein A (LspA) of the commensal strain *P. freudenreichii* UF1 was shown to regulate the interaction with SIGNR1 receptor, which regulates dendritic cells and counteracts pathogenic-driven inflammation, maintaining gut homeostasis [84]. Interestingly, some of these immunomodulatory proteins, including SlpB and SlpE, were recently identified in association with extracellular vesicles produced by the strain *P. freudenreichii* CIRM-BIA129, which serve as an alternative export system [85].

In addition to surface proteins, DHNA was also associated to immunomodulation. Beside its bifidogenic properties, DHNA inhibited the production of proinflammatory cytokines in intestinal macrophages of IL-10(−/−) mice treated with piroxicam [86]. Moreover, DHNA was also described as an activator of aryl hydrocarbon receptor (AhR), which is involved in the detoxification of xenobiotics and inflammation regulation [87, 88].

10. Functional foods

Importantly, the immunomodulatory properties of *P. freudenreichii* were preserved when food matrices were used as delivery vectors, including cheese [18, 19, 42, 89] and fermented milk [90–92], indicating a great potential for developing probiotic-based functional foods with immunomodulatory properties. As an example, a dairy product fermented by strain CIRM-BIA129 reduced the secretion of pro-inflammatory cytokines by colonic mucosa, improved food intake and growth of

piglets [92]. *P. freudenreichii* CIRM-BIA129 was also employed in the production of an immunomodulatory single-strain cheese, whose consumption by mice ameliorated colitis induced by TNBS, restoring the expression of tight-junction proteins and reducing the expression of markers of inflammation and of oxidative stress [89]. Similar protection, in the same colitis model, was observed using a two-strain model cheese containing *P. freudenreichii* and *L. delbrueckii* [20]. An industrial Emmental cheese was then produced using *S. thermophilus, P. freudenreichii* and *L. delbrueckii* [18] (**Figure 3**). Its consumption protected mice against colitis induced by DSS [18]. In healthy piglets, the consumption of the same CIRM-BIA129 strain associated to a cheese matrix was crucial in the preservation or enhancement of the immunomodulatory properties of the bacterium, including the induction of Th2 and Treg phenotypes [19]. The importance of the cheese matrix was also related to the protection of immunomodulatory protein SlpB against proteolysis in simulated gastrointestinal tract conditions [42]. These examples unveiled how appropriate food matrices protected or enhanced the beneficial properties of these traditional dairy propionibacteria, while establishing perspectives for the design of novel functional foods [91].

11. Safety assessments

The long history of safe production of fermented food, such as Emmental cheese, and the bacterium status of "generally recognized as safe" (GRAS) and "qualified presumption of safety" (QPS) assure the safety of *P. freudenreichii* consumption [5, 93]. However, additional assessments need to be conducted in different matrices and contexts. Probiotics included in humans trials are most frequently from genus *Lactobacillus* or *Bifidobacterium*; nevertheless, propionibacteria have also been tested [93]. For example, two clinical studies evaluated *P. freudenreichii* ET-3 culture medium safety in human adult subjects, the first one reported no differences in gastrointestinal symptoms between the groups and the other one reported differences in hematological parameters, although within the normal ranges [94]. *P. freudenreichii* strains SI 26 and SI 41 were given to adult healthy human volunteers without adverse effects, while a modulation of fecal bifidobacteria and of segmental colonic transit was observed [67]. In another study, *P. freudenreichii* strain SI 41 was given in capsules at the same dose to human volunteers without adverse effects, while an increase in fecal propionibacteria, concomitant with enhanced short chain fatty acids, was observed [95].

Moreover, several clinical trials tested multispecies probiotic supplementation containing propionibacteria. A complex formula that included *P. freudenreichii* JS, together with *L. rhamnosus* GG, *L. rhamnosus* Lc705, *B. breve* 99, and galactooligosaccharides prebiotics has been tested in several randomized, double-blind, placebo-controlled setups. The probiotic intervention was conducted in pregnant women and newborn infants, being safe and effective in the prevention of atopic eczema in children [96], increased children resistance to respiratory infections [97], protected Cesarean-delivered children from IgE-associated allergic disease [98], restored microbiota composition in children treated with antibiotics or born by cesarean procedure [99] and protected Cesarean-delivered children from allergic disease in a 13-year follow-up [100]. Finally, an integrative study analyzed adverse events associated with this probiotic combination in some of these trials, concluding that there was no association with adverse events in young and elderly subjects [101].

Importantly, probiotics supplementation is not recommended in cases of immunosuppression, such as during anticancer treatment [93]. Moreover, their beneficial effects and safety are conditioned to a complex interplay between peculiarities

of the host and of the probiotic strain or strains, which both encourages further research and suggests caution in some of its applications [93].

12. Postbiotics and beyond

As previously detailed, *P. freurenreichii* probiotic effect has been associated to several factors, including cytoplasmic and surface-exposed proteins [52, 77, 82, 83], short chain fatty acids [62, 63], metabolites [71, 72, 75] and culture supernatants [58, 94] (**Figure 4**). These probiotic-derived factors, which exert a beneficial effect on the host, have been referred as postbiotics [102]. "Postbiotic" is an emerging denomination that encompasses probiotic-derived cell-free metabolic products with health-promoting properties, including proteins, lipids, organic acids, vitamins, supernatants, among others [102–104]. The advantages of postbiotics over probiotics include purity, easy production and storage, industrial scalability, higher specificity in the mechanism of action and less adverse effects [102, 103].

In the case of *P. freudenreichii*, a remarkable example of postbiotic is SlpB protein, which was purified and exerted an immunomodulatory effect, i.e. induction of IL-10, in cultured human intestinal epithelial cells [83]. Another example that would fit into postbiotics definition is extracellular vesicles, which are membranous spherical nanostructures that transport molecules between cells [105, 106]. In probiotic bacteria, such as several *Lactobacillus* and *Bifidobacteriaum* strains, extracellular vesicles have been reported as immunomodulatory [107]. In the case of *P. freudenreichii*, we recently described their production by the strain CIRM-BIA129, which has been the first report with physicochemical, proteomic and functional characterization of extracellular vesicles in the species [85]. We identified relevant proteins in their cargo, including SlpB, and demonstrated their anti-inflammatory activity via the modulation of NF-κB pathway in cultured human intestinal epithelial cells [85].

Postbiotics hold promising perspectives for developing novel probiotic-derived products with enhanced safety and functionality [107]. Moreover, yield and cargo loading optimization are promising for modulating their properties, enhancing their beneficial effect and biotechnological applications [108]. Finally, clinical trials should be conducted in the near future to assure the
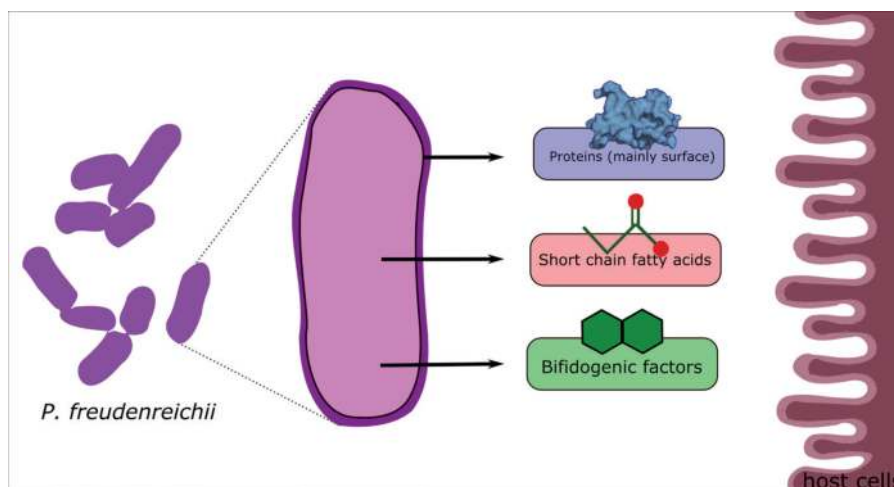
Figure 4.
Schematic summary of P. freudenreichii *probiotic traits at the molecular level.*

suitability of postbiotics and probiotics for therapy and prophylaxis, since they might exert a great impact in human health [93, 107, 109].

13. Conclusion

Overall, research on *P. freudenreichii* is consolidating its role as a probiotic, due to several outstanding features, such as the tolerance to stresses encountered in the gastrointestinal tract, adhesion to host cells, the anti-pathogenic activity, the anticancer potential and the immunomodulatory properties. Moreover, this species holds technological importance, due to long-established applications in the production of food, vitamin B12 and organic acids. Therefore, this is a promising 2-in-1 bacterium, with both fermentative and probiotic properties. New research on *P. freudenreichii* should allow the development of novel health-promoting fermented foods and should dive further into the characterization of strain diversity and of corresponding properties, as well as employ omics approaches to dissect the molecular mechanisms of its beneficial properties. Studies on this species hold a great potential for the development of novel technological approaches and therapeutic products directly impacting human health.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

Author details

Vinícius de Rezende Rodovalho[1,2], Diego Lucas Neres Rodrigues[1], Gwénaël Jan[2], Yves Le Loir[2], Vasco Ariston de Carvalho Azevedo[1] and Eric Guédon[2*]

1 Laboratory of Cellular and Molecular Genetics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

2 INRAE, Institut Agro, STLO, Rennes, France

*Address all correspondence to: eric.guedon@inrae.fr

IntechOpen

References

[1] Ozen M, Dinleyici EC. The history of probiotics: The untold story. Benef Microbes 2015; 6: 159-165.

[2] Gasbarrini G, Bonvicini F, Gramenzi A. Probiotics History. J Clin Gastroenterol 2016; 50: S116–S119.

[3] de Melo Pereira GV, de Oliveira Coelho B, Magalhães Júnior AI, et al. How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances 2018; 36: 2060-2076.

[4] Douillard FP, de Vos WM. Biotechnology of health-promoting bacteria. *Biotechnology Advances*; 37. Epub ahead of print 1 November 2019. DOI: 10.1016/j.biotechadv.2019.03.008.

[5] Rabah H, Rosa do Carmo F, Jan G. Dairy Propionibacteria: Versatile Probiotics. *Microorganisms* 2017; 5: 24.

[6] Piwowarek K, Lipińska E, Hać-Szymańczuk E, et al. Propioni bacterium spp.-source of propionic acid, vitamin B12, and other metabolites important for the industry. Appl Microbiol Biotechnol 2018; 102: 515-538.

[7] Scholz CFP, Kilian M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus Propionibacterium to the proposed novel genera Acidipropioni bacterium gen. nov., Cutibacterium gen. nov. and Pseudopropionibacterium gen. nov. Int J Syst Evol Microbiol 2016; 66: 4422-4432.

[8] Falentin H, Deutsch SM, Jan G, et al. The complete genome of propioni bacterium freudenreichii CIRM-BIA1T, a hardy actinobacterium with food and probiotic applications. *PLoS One*; 5. Epub ahead of print 2010. DOI: 10.1371/journal.pone.0011748.

[9] Thierry A, Deutsch SM, Falentin H, et al. New insights into physiology and metabolism of Propionibacterium freudenreichii. International Journal of Food Microbiology 2011; 149: 19-27.

[10] Von Freudenreichii E, Orla-Jensen O. Uber die in Emmentalerkäse stattfindene Propionsäure-gärung. Zentralbl Bakteriol 1906; 17: 529-546.

[11] Colliou N, Ge Y, Sahay B, et al. Commensal Propionibacterium strain UF1 mitigates intestinal inflammation via Th17 cell regulation. J Clin Invest 2017; 127: 3970-3986.

[12] Ojala T, Laine PKS, Ahlroos T, et al. Functional genomics provides insights into the role of Propionibacterium freudenreichii ssp. shermanii JS in cheese ripening. Int J Food Microbiol 2017; 241: 39-48.

[13] Loux V, Mariadassou M, Almeida S, et al. Mutations and genomic islands can explain the strain dependency of sugar utilization in 21 strains of Propionibacterium freudenreichii. BMC Genomics 2015; 16: 296.

[14] Deborde C, Boyaval P. Interactions between Pyruvate and Lactate Metabolism in Propionibacterium freudenreichii subsp.shermanii: In Vivo 13C Nuclear Magnetic Resonance Studies. Appl Environ Microbiol 2000; 66: 2012-2020.

[15] Gagnaire V, Jardin J, Rabah H, et al. Emmental Cheese Environment Enhances Propionibacterium freudenreichii Stress Tolerance. PLoS One 2015; 10: e0135780.

[16] Poonam, Pophaly SD, Tomar SK, et al. Multifaceted attributes of dairy propionibacteria: A review. World Journal of Microbiology and Biotechnology 2012; 28: 3081-3095.

[17] Abeijón Mukdsi MC, Falentin H, Maillard MB, et al. The Secreted Esterase of Propionibacterium freudenreichii Has a Major Role in Cheese Lipolysis. Appl Environ Microbiol 2014; 80: 751-756.

[18] Rabah H, do Carmo FLR, Carvalho RD de O, et al. Beneficial propioni bacteria within a probiotic emmental cheese: Impact on dextran sodium sulphate-induced colitis in mice. *Microorganisms*; 8. Epub ahead of print 1 March 2020. DOI: 10.3390/ microorganisms8030380.

[19] Rabah H, Ferret-Bernard S, Huang S, et al. The Cheese Matrix Modulates the Immunomodulatory Properties of Propionibacterium freudenreichii CIRM-BIA 129 in Healthy Piglets. Front Microbiol 2018; 9: 2584.

[20] Plé C, Breton J, Richoux R, et al. Combining selected immuno modulatory Propionibacterium freudenreichii and Lactobacillus delbrueckii strains: Reverse engineering development of an anti-inflammatory cheese. Mol Nutr Food Res 2016; 60: 935-948.

[21] Calderón-Ospina CA, Nava-Mesa MO. B Vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin. CNS Neurosci Ther 2020; 26: 5-13.

[22] Takahashi-Iñiguez T, García-Hernandez E, Arreguín-Espinosa R, et al. Role of Vitamin B12 on methylmalonyl-CoA mutase activity. Journal of Zhejiang University: Science B 2012; 13: 423-437.

[23] Chamlagain B, Sugito TA, Deptula P, et al. In situ production of active vitamin B12 in cereal matrices using Propionibacterium freudenreichii. Food Sci Nutr 2018; 6: 67-76.

[24] Wang P, Shen C, Li L, et al. Simultaneous production of propionic acid and vitamin B12 from corn stalk hydrolysates by Propionibacterium freudenreichii in an expanded bed adsorption bioreactor. Prep Biochem Biotechnol 2020; 50: 763-767.

[25] Hajfarajollah H, Mokhtarani B, Mortaheb H, et al. Vitamin B12 biosynthesis over waste frying sunflower oil as a cost effective and renewable substrate. J Food Sci Technol 2015; 52: 3273-3282.

[26] Yu Y, Zhu X, Shen Y, et al. Enhancing the vitamin B12 production and growth of Propionibacterium freudenreichii in tofu wastewater via a light-induced vitamin B12 riboswitch. Appl Microbiol Biotechnol 2015; 99: 10481-10488.

[27] Assis DA de, Matte C, Aschidamini B, et al. Biosynthesis of vitamin B12 by Propionibacterium freudenreichii subsp. shermanii ATCC 13673 using liquid acid protein residue of soybean as culture medium. *Biotechnol Prog*. Epub ahead of print 2020. DOI: 10.1002/btpr.3011.

[28] Wang Z, Ammar EM, Zhang A, et al. Engineering Propionibacterium freudenreichii subsp. Shermanii for enhanced propionic acid fermentation: Effects of overexpressing propionyl-CoA: Succinate CoA transferase. Metab Eng 2015; 27: 46-56.

[29] Pillai V V, Prakash G, Lali AM. Growth engineering of Propionibacterium freudenreichii shermanii for organic acids and other value-added products formation. Prep Biochem Biotechnol 2018; 48: 6-12.

[30] Gaucher F, Bonnassie S, Rabah H, et al. Review: Adaptation of Beneficial Propionibacteria, Lactobacilli, and Bifidobacteria Improves Tolerance Toward Technological and Digestive Stresses. *Front Microbiol*; 10. Epub ahead

of print 24 April 2019. DOI: 10.3389/
fmicb.2019.00841.

[31] Gaucher F, Gagnaire V, Rabah H,
et al. Taking advantage of bacterial
adaptation in order to optimize
industrial production of dry
propionibacterium freudenreichii.
Microorganisms; 7. Epub ahead of print 1
October 2019. DOI: 10.3390/
microorganisms7100477.

[32] Gaucher F, Rabah H, Kponouglo K,
et al. Intracellular osmoprotectant
concentrations determine
Propionibacterium freudenreichii
survival during drying. Appl Microbiol
Biotechnol 2020; 104: 3145-3156.

[33] Gaucher F, Kponouglo K, Rabah H,
et al. Propionibacterium freudenreichii
CIRM-BIA 129 Osmoadaptation
Coupled to Acid-Adaptation Increases
Its Viability During Freeze-Drying.
Front Microbiol; 10. Epub ahead of print
9 October 2019. DOI: 10.3389/
fmicb.2019.02324.

[34] Deptula P, Laine PK, Roberts RJ, et
al. De novo assembly of genomes from
long sequence reads reveals uncharted
territories of Propionibacterium
freudenreichii. *BMC Genomics*; 18. Epub
ahead of print 2017. DOI: 10.1186/
s12864-017-4165-9.

[35] Jan G, Leverrier P, Pichereau V, et al.
Changes in Protein Synthesis and
Morphology during Acid Adaptation of
Propionibacterium freudenreichii. Appl
Environ Microbiol 2001; 67: 2029-2036.

[36] Leverrier P, Dimova D, Pichereau V,
et al. Susceptibility and adaptive
response to bile salts in
Propionibacterium freudenreichii:
Physiological and proteomic anlysis.
Appl Environ Microbiol 2003; 69:
3809-3818.

[37] Leverrier P, Vissers JPC, Rouault A,
et al. Mass spectrometry proteomic
analysis of stress adaptation reveals both

common and distinct response pathways
in Propionibacterium freudenreichii.
Arch Microbiol 2004; 181: 215-230.

[38] Huang Y, Adams MC. In vitro
assessment of the upper gastrointestinal
tolerance of potential probiotic dairy
propionibacteria. Int J Food Microbiol
2004; 91: 253-260.

[39] Hervé C, Fondrevez M, Chéron A, et
al. Transcarboxylase mRNA: A marker
which evidences P. freudenreichii
survival and metabolic activity during
its transit in the human gut. Int J Food
Microbiol 2007; 113: 303-314.

[40] Lan A, Bruneau A, Philippe C, et al.
Survival and metabolic activity of
selected strains of Propionibacterium
freudenreichii in the gastrointestinal
tract of human microbiota-associated
rats. Br J Nutr 2007; 97: 714-724.

[41] Saraoui T, Parayre S, Guernec G, et
al. A unique in vivo experimental
approach reveals metabolic adaptation
of the probiotic Propionibacterium
freudenreichii to the colon
environment. *BMC Genomics*; 14. Epub
ahead of print 23 December 2013. DOI:
10.1186/1471-2164-14-911.

[42] Rabah H, Ménard O, Gaucher F,
et al. Cheese matrix protects the
immunomodulatory surface protein
SlpB of Propionibacterium
freudenreichii during in vitro digestion.
Food Res Int 2018; 106: 712-721.

[43] Cousin FJ, Jouan-Lanhouet S,
Dimanche-Boitrel MT, et al. Milk
fermented by propionibacterium
freudenreichii induces apoptosis of
HGT-1 human gastric cancer cells. *PLoS
One*; 7. Epub ahead of print 19 March
2012. DOI: 10.1371/journal.
pone.0031892.

[44] Aburjaile FF, Rohmer M,
Parrinello H, et al. Adaptation of
Propionibacterium freudenreichii to
long-term survival under gradual

nutritional shortage. *BMC Genomics*; 17. Epub ahead of print 8 December 2016. DOI: 10.1186/s12864-016-3367-x.

[45] Aburjaile FF, Madec MN, Parayre S, et al. The long-term survival of Propionibacterium freudenreichii in a context of nutrient shortage. J Appl Microbiol 2016; 120: 432-440.

[46] Cardoso FS, Castro RF, Borges N, et al. Biochemical and genetic characterization of the pathways for trehalose metabolism in Propionibacterium freudenreichii, and their role in stress response. Microbiology 2007; 153: 270-280.

[47] Dalmasso M, Aubert J, Even S, et al. Accumulation of intracellular glycogen and trehalose by Propionibacterium freudenreichii under conditions mimicking cheese ripening in the cold. Appl Environ Microbiol 2012; 78: 6357-6364.

[48] Huang S, Rabah H, Jardin J, et al. Hyperconcentrated Sweet Whey, a New Culture Medium That Enhances Propionibacterium freudenreichii Stress Tolerance. Appl Environ Microbiol 2016; 82: 4641-4651.

[49] Tuomola EM, Ouwehand AC, Salminen SJ. Human ileostomy glycoproteins as a model for small intestinal mucus to investigate adhesion of probiotics. Lett Appl Microbiol 1999; 28: 159-163.

[50] Ouwehand AC, Tölkkö S, Kulmala J, et al. Adhesion of inactivated probiotic strains to intestinal mucus. Lett Appl Microbiol 2000; 31: 82-86.

[51] Campaniello D, Bevilacqua A, Sinigaglia M, et al. Screening of Propionibacterium spp. for potential probiotic properties. Anaerobe 2015; 34: 169-173.

[52] do Carmo FLR, Rabah H, Huang S, et al. Propionibacterium freudenreichii

surface protein SlpB is involved in adhesion to intestinal HT-29 cells. Front Microbiol 2017; 8: 1-11.

[53] do Carmo FLR, Silva WM, Tavares GC, et al. Mutation of the surface layer protein SlpB has pleiotropic effects in the probiotic propionibacterium freudenreichii CIRM-BIA 129. Front Microbiol 2018; 9: 1807.

[54] Collado MC, Meriluoto J, Salminen S. Development of new probiotics by strain combinations: Is it possible to improve the adhesion to intestinal mucus? J Dairy Sci 2007; 90: 2710-2716.

[55] Vesterlund S, Karp M, Salminen S, et al. Staphylococcus aureus adheres to human intestinal mucus but can be displaced by certain lactic acid bacteria. Microbiology 2006; 152: 1819-1826.

[56] Hajfarajollah H, Mokhtarani B, Noghabi KA. Newly Antibacterial and Antiadhesive Lipopeptide Biosurfactant Secreted by a Probiotic Strain, Propionibacterium Freudenreichii. Appl Biochem Biotechnol 2014; 174: 2725-2740.

[57] Nair DVT, Kollanoor-Johny A. Effect of Propionibacterium freudenreichii on Salmonella multiplication, motility, and association with avian epithelial cells. Poult Sci 2017; 96: 1376-1386.

[58] Nair DVTT, Kollanoor Johny A. Characterizing the Antimicrobial Function of a Dairy-Originated Probiotic, Propionibacterium freudenreichii, Against Multidrug-Resistant *Salmonella enterica* Serovar Heidelberg in Turkey Poults. *Front Microbiol*; 9. Epub ahead of print 12 July 2018. DOI: 10.3389/fmicb.2018.01475.

[59] Foligné B, Deutsch S-M, Breton J, et al. Promising Immunomodulatory Effects of Selected Strains of Dairy

Propionibacteria as Evidenced In Vitro and In Vivo. Appl Environ Microbiol 2010; 76: 8259-8264.

[60] Collado MC, Jalonen L, Meriluoto J, et al. Protection mechanism of probiotic combination against human pathogens: in vitro adhesion to human intestinal mucus. Asia Pac J Clin Nutr 2006; 15: 570-575.

[61] Myllyluoma E, Ahonen AM, Korpela R, et al. Effects of multispecies probiotic combination on Helicobacter pylori infection in vitro. *Clin Vaccine Immunol*; 15. Epub ahead of print September 2008. DOI: 10.1128/CVI.00080-08.

[62] Jan G, Belzacq A-SS, Haouzi D, et al. Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. Cell Death Differ 2002; 9: 179-188.

[63] Lan A, Lagadic-Gossmann D, Lemaire C, et al. Acidic extracellular pH shifts colorectal cancer cell death from apoptosis to necrosis upon exposure to propionate and acetate, major end-products of the human probiotic propionibacteria. Apoptosis 2007; 12: 573-591.

[64] Lan A, Bruneau A, Bensaada M, et al. Increased induction of apoptosis by Propionibacterium freudenreichii TL133 in colonic mucosal crypts of human microbiota-associated rats treated with 1,2-dimethylhydrazine. Br J Nutr 2008; 100: 1251-1259.

[65] Cousin FJ, Jouan-Lanhouet S, Théret N, et al. The probiotic Propionibacterium freudenreichii as a new adjuvant for TRAIL-based therapy in colorectal cancer. Oncotarget 2016; 7: 7161-7178.

[66] Casanova MR, Azevedo-Silva J, Rodrigues LR, et al. Colorectal Cancer Cells Increase the Production of Short Chain Fatty Acids by Propionibacterium freudenreichii Impacting on Cancer Cells Survival. Front Nutr 2018; 5: 44.

[67] Bouglé D, Roland N, Lebeurrier F. Effect of Propionibacteria Supplementation on Fecal Bifidobacteria and Segmental Colonic Transit Time in Healthy Human Subjects. Scand J Gastroenterol 1999; 34: 144-148.

[68] Hojo K, Yoda N, Tsuchita H, et al. Effect of Ingested Culture of Propionibacterium freudenreichii ET-3 on Fecal Microflora and Stool Frequency in Healthy Females. Biosci Microflora 2002; 21: 115-120.

[69] Kaneko T, Mori H, Iwata M, et al. Growth Stimulator for Bifidobacteria Produced by Propionibacterium freudenreichii and Several Intestinal Bacteria. J Dairy Sci 1994; 77: 393-404.

[70] Mori H, Sato Y, Taketomo N, et al. Isolation and Structural Identification of Bifidogenic Growth Stimulator Produced by Propionibacterium freudenreichii. J Dairy Sci 1997; 80: 1959-1964.

[71] Isawa K, Hojo K, Yoda N, et al. Isolation and identification of a new bifidogenic growth stimulator produced by propionibacterium freudenreichii ET-3. Biosci Biotechnol Biochem 2002; 66: 679-681.

[72] Okada Y, Tsuzuki Y, Miyazaki J, et al. Propionibacterium freudenreichii component 1.4-dihydroxy-2-naphthoic acid (DHNA) attenuates dextran sodium sulphate induced colitis by modulation of bacterial flora and lymphocyte homing. Gut 2006; 55: 681-688.

[73] Suzuki A, Mitsuyama K, Koga H, et al. Bifidogenic growth stimulator for the treatment of active ulcerative colitis: a pilot study. Nutrition 2006; 22: 76-81.

[74] Furuichi K, Hojo K ichi, Katakura Y, et al. Aerobic culture of Propionibacterium freudenreichii ET-3 can increase production ratio of 1,4-dihydroxy-2-naphthoic acid to menaquinone. J Biosci Bioeng 2006; 101: 464-470.

[75] Kouya T, Misawa K, Horiuchi M, et al. Production of extracellular bifidogenic growth stimulator by anaerobic and aerobic cultivations of several propionibacterial strains. J Biosci Bioeng 2007; 103: 464-471.

[76] Foligné B, Breton J, Mater D, et al. Tracking the microbiome functionality: Focus on Propionibacterium species. Gut 2013; 62: 1227-1228.

[77] Deutsch S-MM, Mariadassou M, Nicolas P, et al. Identification of proteins involved in the anti-inflammatory properties of Propionibacterium freudenreichii by means of a multi-strain study. *Sci Rep*; 7. Epub ahead of print 2017. DOI: 10.1038/srep46409.

[78] Frohnmeyer E, Deptula P, Nyman TA, et al. Secretome profiling of Propionibacterium freudenreichii reveals highly variable responses even among the closely related strains. Microb Biotechnol 2018; 11: 510-526.

[79] Ma S, Yeom J, Lim Y-HH. Dairy Propionibacterium freudenreichii ameliorates acute colitis by stimulating MUC2 expression in intestinal goblet cell in a DSS-induced colitis rat model. Sci Rep 2020; 10: 5523.

[80] Colliou N, Ge Y, Gong M, et al. Regulation of Th17 cells by P. UF1 against systemic Listeria monocytogenes infection. Gut Microbes 2018; 9: 279-287.

[81] Ge Y, Gong M, Colliou N, et al. Neonatal intestinal immune regulation by the commensal bacterium, P. UF1. Mucosal Immunol 2019; 12: 434-444.

[82] Le Maréchal C, Peton V, Plé C, et al. Surface proteins of Propionibacterium freudenreichii are involved in its anti-inflammatory properties. J Proteomics 2015; 113: 447-461.

[83] do Carmo FLR, Rabah H, Cordeiro BF, et al. Probiotic Propionibacterium freudenreichii requires SlpB protein to mitigate mucositis induced by chemotherapy. Oncotarget 2019; 10: 7198-7219.

[84] Ge Y, Gong M, Zadeh M, et al. Regulating colonic dendritic cells by commensal glycosylated large surface layer protein A to sustain gut homeostasis against pathogenic inflammation. Mucosal Immunol 2020; 13: 34-46.

[85] Rodovalho V de R, Luz BSR da, Rabah H, et al. Extracellular Vesicles Produced by the Probiotic Propionibacterium freudenreichii CIRM-BIA 129 Mitigate Inflammation by Modulating the NF-κB Pathway. Front Microbiol 2020; 11: 1544.

[86] Okada Y, Tsuzuki Y, Narimatsu K, et al. 1,4-Dihydroxy-2-naphthoic acid from Propionibacterium freudenreichii reduces inflammation in interleukin-10-deficient mice with colitis by suppressing macrophage-derived proinflammatory cytokines. J Leukoc Biol 2013; 94: 473-480.

[87] Fukumoto S, Toshimitsu T, Matsuoka S, et al. Identification of a probiotic bacteria-derived activator of the aryl hydrocarbon receptor that inhibits colitis. Immunol Cell Biol 2014; 92: 460-465.

[88] Cheng Y, Jin UH, Davidson LA, et al. Microbial-derived 1,4-Dihydroxy-2-naphthoic acid and related compounds as aryl hydrocarbon receptor agonists/antagonists: Structure-activity relationships and receptor modeling. Toxicol Sci 2017; 155: 458-473.

[89] Plé C, Richoux R, Jardin J, et al. Single-strain starter experimental cheese reveals anti-inflammatory effect of Propionibacterium freudenreichii CIRM BIA 129 in TNBS-colitis model. J Funct Foods 2015; 18: 575-585.

[90] Foligné B, Parayre S, Cheddani R, et al. Immunomodulation properties of multi-species fermented milks. Food Microbiol 2016; 53: 60-69.

[91] Moslemi M, Mazaheri Nezhad Fard R, Hosseini SM, et al. Incorporation of Propionibacteria in Fermented Milks as a Probiotic. Crit Rev Food Sci Nutr 2016; 56: 1290-1312.

[92] Cousin FJ, Foligné B, Deutsch SM, et al. Assessment of the probiotic potential of a dairy product fermented by propionibacterium freudenreichii in piglets. J Agric Food Chem 2012; 60: 7917-7927.

[93] Dudek-Wicher R, Junka A, Paleczny J, et al. Clinical Trials of Probiotic Strains in Selected Disease Entities. *Int J Microbiol*; 2020. Epub ahead of print 2020. DOI: 10.1155/2020/8854119.

[94] Uchida M, Tsuboi H, Takahashi Arita M, et al. Safety of high doses of Propionibacterium freudenreichii ET-3 culture in healthy adult subjects. Regul Toxicol Pharmacol 2011; 60: 262-267.

[95] Jan G, Leverrier P, Proudy I, et al. Survival and beneficial effects of propionibacteria in the human gut: in vivo and in vitro investigations. Lait 2002; 82: 131-144.

[96] Kukkonen K, Savilahti E, Haahtela T, et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: A randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol 2007; 119: 192-198.

[97] Kukkonen K, Savilahti E, Haahtela T, et al. Long-term safety and impact on infection rates of postnatal probiotic and prebiotic (synbiotic) treatment: Randomized, double-blind, placebo-controlled trial. Pediatrics 2008; 122: 8-12.

[98] Kuitunen M, Kukkonen K, Juntunen-Backman K, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. J Allergy Clin Immunol 2009; 123: 335-341.

[99] Korpela K, Salonen A, Vepsäläinen O, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome*; 6. Epub ahead of print 16 October 2018. DOI: 10.1186/s40168-018-0567-4.

[100] Kallio S, Kukkonen AK, Savilahti E, et al. Perinatal probiotic intervention prevented allergic disease in a Caesarean-delivered subgroup at 13-year follow-up. Clin Exp Allergy 2018; 49: 506-515.

[101] Tapiovaara L, Lehtoranta L, Poussa T, et al. Absence of adverse events in healthy individuals using probiotics - analysis of six randomised studies by one study group. Benef Microbes 2016; 7: 161-169.

[102] Żółkiewicz J, Marzec A, Ruszczyński M, et al. Postbiotics—A Step Beyond Pre- and Probiotics. Nutrients 2020; 12: 2189.

[103] Nataraj BH, Ali SA, Behare P V., et al. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microb Cell Fact 2020; 19: 168.

[104] Tsilingiri K, Rescigno M. Postbiotics: what else? Benef Microbes 2013; 4: 101-107.

[105] Rohde M. The Gram-Positive Bacterial Cell Wall. Gram-Positive Pathog 2019; 3-18.

[106] Briaud P, Carroll RK. Extracellular Vesicle Biogenesis and Functions in Gram-Positive Bacteria. Infect Immun 2020; 88: 1-37.

[107] Molina-Tijeras JA, Gálvez J, Rodríguez-Cabezas ME. The Immunomodulatory Properties of Extracellular Vesicles Derived from Probiotics: A Novel Approach for the Management of Gastrointestinal Diseases. Nutrients 2019; 11: 1038.

[108] Liu Y, Alexeeva S, Defourny KA, et al. Tiny but mighty: bacterial membrane vesicles in food biotechnological applications. Curr Opin Biotechnol 2018; 49: 179-184.

[109] Rad AH, Abbasi A, Kafil HS, et al. Potential Pharmaceutical and Food Applications of Postbiotics: A review. *Curr Pharm Biotechnol*; 21. Epub ahead of print 17 May 2020. DOI: 10.2174/1389 201021666200516154833.