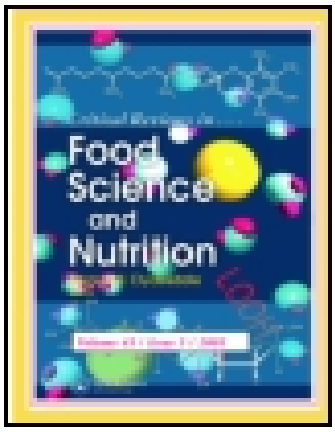

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A Review: Incorporation of Propionibacteria in Fermented Milks as a Probiotic

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A Review: Incorporation of Propionibacteria in Fermented Milks as a Probiotic

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Abstract

Propionibacteria are mainly found in dairy products and fermented milks but are found in other foods as well. Dairy propionibacteria have recently shown to exert potential probiotic activities such as production of propionic acid, vitamins, bacteriocins, essential enzymes and other vital metabolites. Furthermore, stimulating the immune system and lowering the blood cholesterol level are some of their favorable effects. They have a wide spectrum of antimicrobial activities,

inhibiting the growth of gram positive and some gram negative bacteria, as well as some yeasts and molds. At industrial scale, they are used in cheese making, especially Swiss (hard) cheeses, as dominant starter cultures. There is a rising trend to use propionibacteria in fermented milks as probiotic. The current article reviews the characteristics of propionibacteria related to their use in fermented milks either as starter culture or probiotic, methods for the enumeration of propionibacteria and their functional (*in vivo*) efficiency.

Keywords

Fermented milk, probiotic, propionibacteria

Introduction

Probiotics are microorganisms that facilitate prevention and treatment of gastrointestinal pathologic conditions. Probiotics beneficially affect human health by improving the balance of intestinal microflora and improving mucosal defense against pathogens. Several health benefits are linked to probiotics including anti-mutagenic and anti-carcinogenic effects, immune system stimulation, anti-infection properties, serum cholesterol reduction, alleviation of lactose intolerance symptoms and nutritional enhancements. To provide health benefits related to probiotic microorganisms, food industries apply the recommended consumption level of 10^6 to 10^7 CFU/g (based on the product) of probiotic bacteria (Mohammadi and Mortazavian, 2011; Mohammadi et al., 2011; Karimi et al., 2011). Survivability of probiotic bacteria in foods is very dependent on the species and strain used, metabolic interaction with lactic acid starters, fermentation conditions, pH of products, presence of oxygen, storage temperature and presence of protective compounds such as protein and fat droplets (Vinderola et al., 2000).

Probiotic microorganisms used in food industries generally belong to lactobacilli (e.g., *Lactobacillus acidophilus, L. casei, L. plantarum* and *L. rhamnosus*), bifidobacteria, propionibacteria, *Bacillus coagulans* (*Sporolactobacillus*) and some strains of yeasts (e.g., *Saccharomyces boulardii*) (Adami and Cavazzoni, 1993; Mortazavian et al., 2012). In 1997, Europeans spent about $900 million on probiotic yogurts and milks (Hamilton-Miller, 2004). The global market for probiotics was worth $14.9 billion in 2007, reached $16 billion in 2008 and is estimated more than $19 billion on sales in 2013. The application of probiotics is mostly limited to dairy products; mainly including yogurts, kefir and cultured drinks. Among them,

yogurt is the most sold product, representing the higher level of the total sell (Granato et al., 2010a,b; Cruz et al., 2010a, 2009; Karimi et al., 2011).

Propionibacteria have a wide spectrum of antimicrobial activities, inhibiting the growth of gram positive and some gram negative bacteria as well as some yeasts and molds. They are used in several fermented dairy products and have the probiotic potency (Thiel et al., 2004). Propionibacteria strains have been isolated from soil, silage, fermenting olives, intestine of rats (known as classical propionibacteria) and dairy products (Mantere-Alhonen, 1982). The economic value of the latter propionibacteria (of dairy origin) derives from their important role in cheese making, especially in Swiss (hard) cheeses. Other industrial applications are in acid and vitamin B_{12} production. A less studied characteristic within this genus is the bacteriocin production. Three bacteriocins have been identified from propionibacteria. This ability may be important in their action as probiotic. It should be noted that propionibacteria are primarily and commonly combined with lactic acid and/or bifidobacteria in probiotic food products (Mantere-Alhonen, 1995). However propionibacteria are not extensively commercialized as probiotic bacteria at the present time, there is an extensive trend for its commercialization. To the best knowledge of the authors, there is no comprehensive review article on general, industrial or health aspect of propionibacteria and their application as probiotic in fermented milks. The current article reviews the highlighted aspects of propionibacteria application in dairy industries.

Occurrence of propionibacteria in natural foods

Fermented milks are frequently considered as probiotic sources (Leverrier et al., 2005). Furthermore, the presence of probiotic propionibacteria and lactobacilli can improve the

significance of living foods, including vegetables, fruits, nuts, germinated seeds, beans and certain fermented items. The following species of propionibacteria have been identified from 24 living food items: *Propionibacterium acidipropionici*, *P. freudenreichii* ssp. *shermanii*, *P. jensenii* and *P. thoenii*. The occurrence of *P. acidipropionici* was apparent in fermented products, but non-fermented living foods also contained propionibacteria, especially *P. jensenii* and *P. thoenii*. *P. freudenreichii* ssp. *shermanii* occurs only in mushroom pickles (Mantere-Alhonen, 1995).

However classical propionibacteria have been found in various compounds such as soil, silage and vegetable fermentations, comprehensive studies have only been carried out on the natural habitats of these bacteria. The bacteria have also been isolated from anaerobic environments. Within the rumen, *Propionibacterium* spp. is responsible for urea breakdown and ammonia release. Classical propionibacteria are also found in raw milk. Some studies state a concentration of 1.3×10^4 CFU/ml in French raw milk, and an average contamination of 7×10^2 CFU/ml and 2.5×10^2 CFU/ml in raw milk used for Italian Grana cheese and Swiss raw milk, respectively. This concentration is closely related to the hygienic quality of milking facilities. Propionibacteria can grow in milk because although they preferentially use lactate as carbon substrate, they can use lactose as well. However, the bacterial growth in milk is not extensive due to their weak proteolytic activity. The presence of propionibacteria in raw milk leads to their development in certain types of cheeses, where they can reach 10^9 CFU/g in cheese. They are predominantly found in Swiss cheese due to the temperature of long ripening period (several weeks at 24°C), low salt concentration and relatively high pH (5.2). The bacterial development may also result in defects in other cheese varieties where propionic fermentation is not favorable

(late blowing in Grana cheese making and abnormal gas formation in mozzarella cheese) (Robinson et al., 2000).

Propionibacteria advantages in relation to other probiotic

Propionibacteria that are used at various products including dairy and dairy-like products has a promising role related to functionality and probiotic potentialities. For example, combined application of propionibacteria and bifidobacteria in milk and soymilk resulted in a good compatibility and reduced flatus (due to raffinose and stachyose digestion) after consumption (Wu et al., 2012).

Beside this, dairy propionibacteria are the only food grade bacteria that have a dramatic capability in trehalose (a low calorie sugar) biosynthesis. Due to positive role of trehalose in energy supply and stress protection, its gene was cloned and produced in *L.lactis*. Results showed that recombinant strains had a remarkable tolerant to environmental stress condition. So, using trehalose producing probiotics could lead to their improved viability and desired functionality against stress conditions and they could reach at a high number into human gastrointestinal system (Poonam et al., 2012).

It is shown that propionibacteria has a tremendous potential in CLA (conjugated linoleic acid) production other than various starter cultures that CLA is a health promoting fatty acid in human body (Jiang et al., 1998).

Antifungal properties of propionibacteria are proved against pathogenic fungi such as *Aspergillus fumigatus* and *Rhodotorula mucilaginosa* so that some strain could produce potent

component such as 3-phenyllactic acid and any other peptides in contribution to antifungal activity (Lind et al., 2007).

On the other hand, whey fermentation by *P. freudenreichii* increased bifidobacteria population as a potent probiotic organism and also resulted in alleviation of ulcerative colitis symptoms in animal and human model. studies showed that this positive effect is related to DHNA production by propionibacteria (Poonam et al., 2012).

It showed that propionibacteria in some cases have a distinct immunemodulatory behavior in human body. Some studies relate this clinical role to specific cell-wall associated proteins and exo-polysaccharides (EPS) that are strain specific. It is indicated that both of them are contributed in bacterium-host interaction. Although, these components are known in other probiotics, too (Foligne et al., 2010).

Microbiological characteristics of propionibacteria

Cell morphology

Propionibacteria are classified as gram positive bacteria in the Subdivision *Actinomycetes*. The propionibacteria are pleomorphic rods, but the bacterial cells may be present in coccoid, bifid or branched forms as well. The bacterial cells form singles, pairs (V or Y shape) or short chains or often grouped in "Chinese character" patterns. These bacteria are non-motile and non-spore forming (Robinson et al., 2000). The bacterial components of propionibacteria cell wall identified as adhesion determinants include acidic mucopolysaccharides (Beachey, 1981), carbohydrate capsule polymers (Wadstroum et al., 1987), glycoproteins, carbohydrates (Boris et

al., 1998), complexes of proteins and teichoic acids (Sherman and Savage, 1986), secreted proteins (Conway and Kjelleberg, 1989; Henriksson et al., 1991; Coconnier et al., 1992) and combinations of carbohydrates and proteinaceous factors of the cell surface (Greene and Klaenhammer, 1994).

It has been shown that *P. acidipropionici* CRL 1198 cells have a specific protruding structure consisting of exopolysaccharide capsules and teichoic acids which can participate in adhesion processes in intestinal epithelium cells along with proteins. These compounds are responsible for the immune stimulation property of the bacteria (Zarate et al., 2002b). Similar structures have been found in lactobacilli as well (De Ambrosini et al., 1998). This is of particular importance for the bacteria administered orally as probiotic. Generally, they can survive under the actual conditions present in the intestines (Bezkorovainy, 2001; Huang and Adams, 2004).

Biochemical characteristics

Propionibacteria are anaerobic to aerotolerant bacteria and generally catalase positive (Robinson et al., 2000). All strains produce acids from glucose and are negative for ɓ-methyl-D-xyloside, D- and L-fucose, lecithinase, oxidase, H_2S and DNase production. Aesculin hydrolysis is positive for all classical propionibacteria and strains of *P. avidum*. Other cutaneous propionibacteria are aesculin negative (Britz and Riedel, 1991). Supplemental direct fed microbes such as propionibacteria may reduce rumen acidosis and increase gluconeogenesis. Propionibacteria primarily convert lactate to propionate; therefore, *P. shermanii* (10^6 CFU/ml) increases proportion of propionate in batch cultures with mixed rumen microorganisms (De Ondarza, 2008). Propionic acid has a variety of industrial uses, including production of cellulose

plastics, herbicides and perfumes. *P. avidum* and *P. acnes* are positive for gelatin liquefaction (Vorobjeva, 1999). Certain tests (nitrate reduction and acid production from lactose, mannitol and trehalose) give variable results (Britz and Riedel, 1991). Table 1 shows the biochemical characteristics of dairy propionibacteria.

Genetic attributes

The G+C content of propionibacteria is 65ï 67%, based on the species. The species have been categorized into eight groups. The classical species, also called the dairy species (*P. freudenveichii, P. acidipropionici, P. thoenii* and *P. jenseinii*) are involved in ripening of Swiss cheeses. A new species, *P. cyclohexanicum* belonging to the classical group but not found in dairy products, has been described recently. This species was isolated from spoiled orange juice and the 16S rRNA sequence showed that it is closely related to *P. freudenreichii* (Robinson et al., 2000).

Dairy propionibacteria plasmids have repeatedly been isolated and characterized using restriction analysis; no sequences of plasmids exist so far. Different functional characteristics have been screened (e.g. metabolism of carbohydrate, proteolytic activity, inhibitors production and resistance to drug/inorganic ions) to determine plasmid encoded properties of the bacteria. The function of most plasmids cannot be elucidated at the molecular level. There is weak evidence for plasmid associated cell aggregation and only the ability of lactose fermentation has been described. The genome size of the following species has been estimated using pulsed field gel electrophoresis (PFGE): *P. acidipropionici* (3.05 mb), *P. freudenreichii* (2.5 mb), *P. jensenii* (2.41 mb) and *P. thoenii* (2.44 mb) (Dasen, 1998a).

The most often sequenced genes of propionibacteria are 16S rDNA genes. Parts of these genes have been used for the determination of at least the strains of each species. In cutaneous species, especially *P. avidum*, *P. granulosum* and *P. lymphophilum*, the already characterized sequences of 16S rDNA are relatively short (approx. 350 bp), which limit the determination of exact phylogeny of the species. Genes which encode vitamin B_{12} are also important (Dasen, 1998a). Several strains of the Genus *Propionibacterium* have been treated with various mutagenic agents (e.g., ethyl methanesulfonate, dimethyl sulfate, N-methyl-N'-nitro-N-nitrosoguanidine, X-rays and UV) to obtain stable mutants. The strains were selected mainly for an increased production of vitamin B_{12}. Other strains were selected for auxotrophy, cold sensitivity and aspartase deficiency. The former mutant strains are involved in various patent applications, especially for the production of vitamin B_{12} (Dasen, 1998a).

Resistance and sensitivity to environmental factors

The most important regulatory factors are access to nutrients, adequate water activity and appropriate pH and temperature. Water activity, pH and temperature are relatively easily controlled factors. In many food products, the presence of antimicrobial constituents restricts the bacterial growth. Spices and pigments can enhance the organoleptic experience and prevent growth of spoilage microorganisms (Lind, 2010).

Temperature

Propionibacteria grow at 15ï 40°C and neutral pH (Saarela, 2007). In general, the optimal growth temperature for most *Propionibacterium* species is 25ï 32°C (average of 30°C) (Robinson et al., 2000). Pretreatment at moderately high temperatures resulted in higher cell survival after

treatment at 85°C, 90°C and 95°C (Lind, 2010). Fermented milk processing is efficient at tolerance response of propionibacteria. This process comprises incubation at 42°C. This temperature was shown recently to induce a tolerance in *P. Freudenreichii*, leading to protection towards heat stress (Leverrier et al., 2005). The optimum temperature for dairy propionibacteria is normally around 30°C, but the bacterial growth is possible between 15°C and 40°C, depending on the strain and other growth conditions. A study with ten isolates of *P. freudenreichii* ssp. *shermanii* showed that clearly different thermotolerance could be identified for different strains within the same subspecies (Lind, 2010). The capacity to grow at low temperatures depends on the species and strains. It has been reported that the majority of strains of *P. freudenreichii* are able to grow at 7°C, possibly due to modification of the fatty acids of the cytoplasmic membrane (Robinson et al., 2000).

Temperature has a significant linear effect on propionic and acetic acid production. A decrease in temperature produces a decrease in growth rate and cell wall polymer biosynthesis, making more precursor molecules available for the exopolysaccharide (EPS) biosynthesis. At 30°C, a shift between growth and EPS production was seen while the shift seemed to disappear at the lower temperatures. A decrease in temperature led to slow down of growth and decrease in organic acid production, making more essential factors of the media and precursors of EPS biosynthesis available. In addition, a decrease in temperature induced the best synchronization of growth and EPS production; suggesting the possibility of continuous EPS production in reactors. Therefore, temperature conditions favoring high EPS production, though not optimal, could be defined as 23°C (Gorret et al., 2001).

pH

During grow of certain bacteria, organic acids such as lactic, acetic and propionic acids are produced. These are effective in decreasing pH, which can be an effective way for the inhibition of other microorganisms (Lind, 2010). Probiotics are resistant to low pH, have native association with traditional fermented foods and have adaptation to milk and other food substrates (Mortazavian et al., 2012). It is difficult to define an optimum pH for propionibacteria because this pH depends on the growth media, temperature and water activity (Robinson et al., 2000). However , some scientists reported the optimal range of 8.5 to 5.1 (Saarela, 2007). As mentioned in temperature condition, fermented milk process is efficient at inducing acid tolerance response in propionibacteria. Hence, a multi tolerance response in *P. freudenreichii* can be induced leading to efficient protection towards bile salts parallel to heat stress (Leverrier et al., 2005). In YEL medium, the optimal pH for propionibacteria is between 6.5 and 7.0 but the bacteria may survive at much lower pH values in rumen. *Propionibacterium* species, amongst other species, are responsible for urea breakdown and ammonia release, as this bacterial genus has been found in anaerobic digesters (Robinson et al., 2000). Acid challenge at pH 2 led to a loss of viability of *P. freudenreichii* comprised between 3 and 4 log cycles (Leverrier et al., 2005). This result was similar to another study on probiotics that 0.5ï 4 log cycle decrease was observed when various strains were exposed to pH 3 (Madureira et al., 2005).

Age of bacteria

Age of the culture greatly determines the digestive stress tolerance of propionibacteria. Indeed, stationary phase cells were shown to be more resistant to different stresses than

exponentially phase ones. In *P. freudenreichii,* certain strains exhibit a highly autolytic behavior when subjected to starvation (Lemee et al., 1994; Jan et al., 2001), while others acquire long lasting multi stress tolerance (Leverrier et al., 2005).

Nutrient source of media and presence of other microorganisms

Several nutrients are known to have useful effects on metabolic activity of propionibacteria. Casein fortification shows a marked increase in the growth of propionibacteria in fermentation media containing starter cultures (Baer, 1995). Yeast extract is one of the most satisfactory nitrogen sources for propionic acid bacteria (PAB). The medium contains alanine, which can be converted to pyruvate by oxidative deamination. Then, the pyruvate can be utilized by the bacteria (Harper, 1953). The presence of D-lactic acid, alanine and other amino acids might explain the marked effect of yeast extract medium on the acid production of *P. Shermanii* (El-Hagarawy et al., 1957).

Presence of other microorganisms can influence the metabolic and fermentation activities of propionibacteria. The favorable action of other species on fermentation by propionibacteria can be related to availability of lactic acid. A combination of *P. freudenreichii* ssp. *shermanii* MTCC 1371, *Bifidobacterium bifidum* NDRI and *Lactobacillus acidophilus* R can be suggested to have a moderate proteolytic activity on probiotic acidophilus milk (Sarkar and Misra, 2006). Proteolysis causes two effects in the product, increase in the digestibility (dietetic criterion) and decrease in firmness or viscosity (technological criterion) (Sarkar and Misra, 2010). A disparity in the behavior of *P. freudenreichii* ssp. *shermanii* MTCC 1371 in skim milk (SM) and formulated milk (FM) was observed. Furthermore, lowering of lactic acid content was observed,

which possibly utilized by *P. freudenreichii* ssp. *shermanii* MTCC 1371 (Parker and Moon, 1982). Therefore, incorporation of *B. bifidum* NDRI and *P. freudenreichii* ssp. *shermanii* MTCC 1371 with *L. acidophilus* R may be recommended to manufactures of probiotic acidophilus milk for infant feeding means without the risk of high acid loads (Sarkar and Misra, 2006). The microbiological characteristics of propionibacteria are represented in Figure 1.

Health advantages of propionibacteria as probiotic

Increased public request for natural and biological products and investigation of novel fermentation processes that are more efficient have forced industries to restart investigations for biological propionic acid sources. Furthermore, combination of propionic, lactic and acetic acids has been recommended for the preservation of foods. The United State Food and Drug Administration (FDA) has listed acids and sodium, calcium and potassium salts used as food preservatives in its summary of Generally Recognized as Safe (GRAS) additives and no upper limits are imposed except for breads, rolls and cheeses (0.30ï 0.38%) (Boyaval and Corre, 1995).

Dairy propionibacteria have recently been shown to possess potential probiotic effects, such as production of propionic acid, bacteriocins, tetrapyrrole compounds (Kiatpapan et al., 2001), nitric oxide (Robinson et al., 2000), vitamin B_2 (Leblanc et al., 2006) and vitamin B_{12} (Skupin et al., 1974; Holo et al., 2002; Hugenholtz et al., 2002), synthesis of β-galactosidase (enzyme used in lactose intolerant patients) and ϐ-glucuronidase (Kujawski et al., 1990; Zarate et al., 2000; Robinson et al., 2000), growth stimulation of bifidobacteria (Kaneko et al., 1994), proteolytic activity (Langsrud et al., 1978) and antibacterial properties (Al-Zoreky et al., 1993). These characteristics suggest their use as dietary supplements and reveal their favorable effects on lipid

metabolism (the presence of propionibacteria in intestine also reversed the hyperlipemic effect of a diet with high lipid content) (Chaia, 1995), immune system of hosts (exerted an immune-stimulating activity) (Zarate et al., 2002a; Perez-Chaia et al., 1995) and antagonism activity to other pathogens (e.g. a partial protective effect against infection with *Salmonella typhimurium*) (Alvarez et al., 1996; Al-Zoreky et al., 1993). Other beneficial effects of propionibacteria on the human health are based on changes in the composition and improvement of metabolic activities of the intestinal microflora (Perez-Chaia et al., 1995; Perez Chaia et al., 1999) and anti-mutagenic activities (Vorobjeva et al., 2001). Dairy propionibacteria reach concentration between 10^9 and 10^{10} CFU/g at the end of ripening (and of storage) of Swiss cheeses such as Emmental. As the ingestion of this cheese is a frequent and safe event, the innocuity of these food-grade bacteria is hence obvious (Leverrier et al., 2005). The main health advantages of probiotic dairy propionibacteria along with their involved mechanisms are discussed below. The frameworks are summarized in Figure 2.

Anti-carcinogenic effects

Various evidences support the idea that colonic microflora is involved in the etiology of cancer. Modification of the gut microflora may interfere with the process of carcinogenesis and lower the risk of colon cancer. Probiotics and prebiotics, which modify the microflora by increasing numbers of lactobacilli and/or bifidobacteria in the colon, have particularly been focused in this regard (Burns and Rowland, 2000). Possible mechanisms by which probiotics show their anti-carcinogenic effects include an enhancement of immune response in the host, binding to or degradation of potential carcinogens, production of anti-tumurigenic components,

alteration of intestinal microbials and their metabolic activities and alteration of physiological status of colon (such as pH and concentration of bile acids) (Lan et al., 2008).

A study by Burns and Rowland (2000) has shown that lactic acid bacteria and prebiotics may have protective effects against the early stages of colon cancer (Burns and Rowland, 2000). A cocktail of probiotic bacterial strains was demonstrated to increase the colonic apoptotic index in normal rats. Multiple mechanisms have been identified, including binding to potential mutagens and reduced activity of enzymes involved in carcinogen formation. Probiotics are capable of reducing the incidence of colonic tumor formation and aberrant crypt formation, suppressing unfavorable bacterial enzyme activities and reducing DNA damage (Ewaschuk et al., 2006). The biological effect of aflatoxins can be reduced by administrating subjects, who had detectable aflatoxin exposures, a probiotic source twice a day for five weeks. A statistically significant decrease in the concentration of urinary AFB-N7-guanine (the predominant form of aflatoxin-DNA complex) was observed in the probiotic group. Results of the probiotic studies encourage for additional studies on protective effects of probiotic microorganisms against toxicokinetics of aflatoxins and other natural and environmental carcinogens. Therefore, probiotic food products may be used in many regions of the world to prevent the development of liver cancer or other environmentally induced cancers (El-Nezami et al., 2006). Types and doses of consumed probiotic bacteria possibly have a significant effect on carcinogenic studies (Sanders, 1999). It has been reported that *P. freudenreichii* can act as a growth stimulator of other friendly bacteria (Chukeatirote, 2003). *Propionibacterium* spp. could kill *in vitro* human colorectal carcinoma cell lines by apoptosis (Jan et al., 2002); a property that could help colon cancer prophylaxis or prevention. It has been shown that feeding mice with *P. acidipropionici* CRL 1198 decreased β-

glucuronidase, azoreductase and nitroreductase activities involved in the release of carcinogenic compounds (Perez-Chaia et al., 1999). Another study showed that *P. freudenreichii* SI41 was capable of killing human colon adenocarcinoma cells under *in vitro* conditions. The bacteria induced apoptosis via their metabolites such as propionate that act on cancer cells and induce cell cycle arrest in the G2/M phase, generation of reactive oxygen species, Bax translocation as pro-apoptotic protein, mitochondrial depolarisation, activation of caspases (enzyme associated with apoptosis initiation) and chromatin condensation and fractionation. These abilities confer the bacterial anti-cancer potency. Furthermore, the bacterial anti-carcinogenicity effect specifically targets damaged cells and dairy PAB may help to eliminate damaged cells by inducing apoptosis in the colon epithelium (Lan et al., 2008).

Metabolites formed by term propionibacteria (e.g. folic acid) could protect body against some forms of cancer (Hugenholtz et al., 2002). Production of conjugated linoleic acids (CLAs) is also documented for *P. freudenreichii*. Amongst all biological effects described for CLAs, the anti-carcinogenic properties of rumenic acid (the cis-9,trans-11 stereoisomer of CLA) are highly promising. *P. freudenreichii* has been shown to convert free linoleic acid into rumenic acid (Thierry et al., 2011). Antitumor properties of propionibacteria seem to be related to three basic effects, immune prophylaxis (prevention of tumor induction or recurrence), inhibition of tumor growth and total/partial tumor regression (Roszkowski et al., 1990). In addition to these beneficial properties, *P. acne* (cutaneous species known as infectious bacteria) is proposed as one of the human carcinogens. Several characteristics of prostate cancer suggest the involvement of infectious species. *P. acnes* is well suited to cause persistent, low-grade infections inducing a

marked inflammatory response. *P. acnes* subtypes have frequently been associated with prostate cancer (Shannon et al., 2006).

Immune system modulation

The bacterial therapeutic effect and influence on human immune system have not been investigated thoroughly yet, but evidence of its positive effects in treatment of allergies, rheumatic and infectious diseases exist (Mantere-Alhonen, 1995). An increase in phagocytic activity of peritoneal macrophages and carbon clearance activity was observed in mice fed with *Propionibacterium*. Carbon clearance rates ($t_{1/2}$) obtained with *Propionibacterium* diets were lower than those observed in controls, indicating an enhanced phagocytic function of the reticuloendothelial system in mice at the end of the first week of feeding. On the seventh day of feeding, the phagocytic activity in mice receiving SMP (skim milk with propionibacteria) and MCP (skim milk with cream and propionibacteria) was decreased after returning to the normal diet. It has been demonstrated that some lactic acid bacteria may induce stimulation of the host immune system when administered orally. This stimulation may involve macrophages, which play an important role in the host resistance to infections and tumors. According to published results, addition of selected strains of propionibacteria to diets, which can survive and grow in the intestine, may lead to the activation of immune competent cells (Perez-Chaia et al., 1995).

Bacteria are known to be highly effective in the stimulation of non-specific cellular immune responses. Relatively, propionibacteria can be shown amongst the most potent immune modifiers effective against a variety of animal and human neoplasm. Stimulation of macrophages by propionibacteria resulted in the enhancement of adhesive properties of phagocytes, appearance of

vacuoles in the cytoplasm, activation of lysosomal enzymes and increased bactericidal and tumoricidal activities of the phages. Interestingly, all strains of the bacteria are susceptible to phagocytosis by macrophages, but only strains which are resistant to intracellular degradation display immune modulating activity (Pringle et al., 1982; Beuth et al., 1987). In contrast, passive strains are quickly degraded. Resistance to intracellular degradation and immune stimulatory properties seem to be related to the cell wall composition, particularly to the structure of peptidoglycan and carbohydrates, which obviously is strain dependent. Concerning the mechanism of immune modulation, propionibacteria were shown to possess properties to enhance natural immune response, and interferon and neopterin induction as well as specific immune responses. However, it is commonly believed that these phenomena are mainly a consequence of MPS (mononuclear phagocyte system) stimulation. Further investigations are in progress and may clarify the molecular basis of immune modulation induced by propionibacteria (Roszkowski et al., 1990).

Antibacterial and antiviral properties

Bacteriocin production may be important in the bacterial action as probiotic (Mantere-Alhonen, 1995). Production of bacteriocin-like compounds, designated as Microgard™ (Al-Zoreky et al., 1993) by *P. freudenreichii* ssp. *shermanii* has been reported (Ekinci and Barefoot, 2006). Jenseniin G, a bacteriocin that is produced by *P. jensenii* P126, is stable during heat treatment (15 min at 100°C) and in pH 3ï 12 (Grinstead and Barefoot, 1992). Jenseniin G inhibited related *Propionibacterium* species (*P. acidipropionici* P5 and *P. jensenii* P54), lactococci and selected lactobacilli (*L. helveticus* NCDO 87, *L. delbrueckii* ssp. *lactis* ATCC

4797 and *L. delbrueckii* ssp. *bulgaricus* NCDO 1489) (Weinbrenner et al., 1997). The results demonstrated that jenseniin G effectively limited acid production by *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in yogurt and could be used to extend shelf life by maintaining pH within a favorite range (pH 4.2ï 4.3) (Oberman and Libudzisz 1998; Vedamuthu, 1982). Jenseniin G is the only bacteriocin from dairy propionibacteria that is active against yogurt cultures and can be used to control acid formation and post-acidification during refrigerated storage of yogurt through marketing channels (Weinbrenner et al., 1997).

Non-specific immune modulators apparently enhance antiviral resistance in experimental animals and humans by various mechanisms such as interferon synthesis and macrophage-monocyte system activation. Accordingly, a significantly higher serum titer of antiviral antibodies had been detected in treated animals immunized with viral antigen alone. Therefore, experimental evidence suggests that propionibacteria could be applied as a safe and effective adjuvant in antiviral vaccination procedures (Siwicki et al., 1998). The exact mechanism of the antiviral activity of propionibacteria still has to be determined. Furthermore, high serum levels of interferon could be detected by *in vivo* or *in vitro* administration of propionibacteria (Okamura et al., 1982). The ability of propionibacteria to induce interferon synthesis in human and murine lymphocytes could also be seen *in vitro*. Further studies with microbial pathogens such as *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium* in experimental mouse models confirmed the importance of activated mononuclear cells in host defense since immune modulation with propionibacteria caused increased resistance to bacterial challenge in experimental mice (Roszkowski et al., 1990).

Serum cholesterol reduction

A small quantity of casein hydrolysate peptides enters the circulatory system from intestinal lumen and affects the lipid metabolism. It has been shown that propionic acid, but not propionate, infused into the stomach or cecum moderates the increase of cholesterol concentration in rats fed with casein diets. As a rule, a less efficient absorption of lipid components in the intestine may result in a lower serum cholesterol level. Thus, deconjugation of bile acids by probiotic bacteria and assimilation of cholesterol by lactobacilli and bifidobacteria have been suggested to be involved in the hypocholesterolemic effect of fermented milks (Perez-Chaia et al., 1995).

Different proteolytic activities of dairy bacteria could be the reason for the different effects observed in dairy products. It has been declared that there is a lipid lowering effect because of the milk components, as well as the presence of propionibacteria in the gut. The hypolipemic effect is more noticeable in diets with high lipid content. The SM and SM yogurt exerted a hypocholesterolemic effect to a greater extent when cholesterol was added to the diet. The liver-body weight ratio and the microscopic morphology of the liver were not different between the treatments, indicating that there was no induction of fatty liver. Therefore, the hypolipemic effect of the MCP diet may not be due to a redistribution of lipid from plasma to liver, but to a lower intestinal absorption or higher lipid catabolism (Perez-Chaia et al., 1995).

Improving lactose intolerance symptoms

An important way in which probiotics beneficially affect the health of the host is by providing enzymatic activities that improve the utilization of nutrients within the intestine (Rowland and Fuller, 1992). The ability of different microorganisms included in dairy products

to improve lactose absorption in people suffering from lactose intolerance has widely been reported (Montes et al., 1995; Jiang et al., 1996; Mustapha et al., 1997). Two possible mechanisms are involved, reduction of lactose content in the product prior to consumption and the intra-intestinal hydrolysis of lactose by the bacterial ß-galactosidase.

Studies have demonstrated that strains of dairy propionibacteria possess ß-galactosidase that resists the adverse environmental conditions of the gastrointestinal tract and could be considered for the manufacture of a probiotic product assigned to intolerant individuals (Zarate et al., 2000). β-galactosidase of propionibacteria was described as an intracellular enzyme resistant to intestinal environmental conditions (Zarate et al., 2000). The pH of the intestinal content is close to the optimum pH of the enzyme. Furthermore, the human body temperature is included in the range of temperatures that allow high enzymatic activity (Zarate et al., 2002a). Growth in the presence of bile salts in a concentration close to those found in the intestine increased the synthesis and activity of β-galactosidase. In the presence of lactose or its monosaccharides, glucose and galactose, β-galactosidase activity was higher at the exponential phase of growth than the stationary phase. The optimum pH of the enzyme activity was 6.8ï 7.0 at 37°C for both strains of *P. acidipropionici*. The influence of temperature on the enzyme specific activity was determined at the optimum pH and the maximum enzymatic activity was found at 50°C. The cations Ca^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+} and Co^{2+} inhibited the activity to different extents at all concentrations tested (0.25ï 5.0 mM), whereas Na^{+}, Mg^{2+} and K^{+} acted as enzymatic activators, increasing the specific activity at all concentrations. Thermal stability was determined at 60°C as a function of time, suggesting that the bacterial β-galactosidase is a heat-resistant protein. Regarding to low temperatures, after ten days storage at 4°C and -20°C, cell-free extracts of the

strains retained over 60% of their activity, showing the high stability of β-galactosidase of propionibacteria during the storage at low temperatures (Zarate et al., 2002a)

Nutritional enhancement

Inoculation of *P. freudenreichii* ssp. *Shermanii* increases folic acid content in cultured milk products (Hettinga and Reinbold, 1972). Folates represent an essential nutrition components involved in many metabolic pathways, mainly in carbon transfer reactions such as purine and pyrimidine biosynthesis and amino acid interconversion (Hugenholtz et al., 2002; Holasova et al., 2004).

Riboflavin (vitamin B_2) is a water soluble vitamin belonged to the B-complex group that is important for optimal body growth, red blood cell production and release of energy from carbohydrates and fatty acids (Hustad et al., 2002). In the body, riboflavin is found primarily as an integral component of the flavin adenine dinucleotide (FAD) and flavin mononucleotide coenzymes. These coenzymes participate in redox reactions in numerous metabolic pathways such as metabolism of carbohydrates, fats and proteins. In addition, flavin containing coenzymes are associated with the metabolism of folate, cobalamin, vitamin B_6 and other vitamins. Plasma riboflavin is one of the determinants of plasma homocysteine levels, a factor known to influence the risk of cardiovascular disease, pregnancy complications and cognitive impairment (Hustad et al., 2002). Riboflavin is important in the early postnatal development of the brain and gastrointestinal tract (Yates et al., 2003; Williams et al., 2007) and is able to modulate carcinogen induced DNA damage (Pangrekar et al., 1993; Webster et al., 1996). It plays a role in iron absorption and utilization (Butler and Topham, 1993; Powers et al., 2007) and can modulate

inflammatory responses (Lakshmi et al., 1991). *P. freudenreichii* B2336 could be used for the production of yogurt or fermented milk to increase levels of riboflavin; thus, increasing their commercial and nutritional values and eliminating the need for subsequent food enrichment with this essential vitamin. Such novel products could be used to decrease the number of people with clinical and subclinical riboflavin deficiency. Riboflavin deficiency is common in many regions of world, not only in developing countries but also in many industrial countries (Leblanc et al., 2006). Use of riboflavin producing strains of *P. freudenreichii* supplied as an adjunct culture in yogurt fermentation process could improve the nutritional value of yogurt. Inoculation of milk with *P. freudenreichii* B2336 (a spontaneous roseoflavin-resistant mutant derived from *P. freudenreichii* B374 that indicates increased riboflavin production) before the inoculation with commercial yogurt cultures significantly increased the riboflavin content (almost two folds), compared to a similar protocol using *P. freudenreichii* B374 (a low riboflavin producing strain) or protocols lack adjunct cultures. Administration of a fermented milk produced with a spontaneous riboflavin overproducing strain of *P. freudenreichii* (strain B2336) improved the animalôs growth compared to conventional yogurt and to the same product fermented with the parental wild-type strain, which produces low levels of riboflavin (Leblanc et al., 2006). *Propionibacterium* strains contain uniquely high concentrations of vitamin B_{12}. Propionibacteria are often used for vitamin B_{12} enrichment of cultured milk products and fermented beverages. Vitamin B_{12} is essential for the human growth and health. It is required for the production of red blood cells and nerve cell myelin and acts as a coenzyme in the metabolism of fatty acids, carbohydrates and proteins. Microbial synthesize of vitamin B_{12} also occurs in the human colon (Adams and Huang, 2005).

Other health benefits

In humans, propionibacteria have been used in combination with *Lactobacillus* spp. and *bifidobacterium* spp. for the treatment of intestinal disorders and regulation of gut flora and motility. It has been demonstrated that feeding Propioni-Acido-Bifido (PAB) milk to infants and children decreased coliforms, increased the population of lactobacilli and bifidobacteria and improved the higher weight gain and feeding efficiency ratio (Sarkar and Misra, 1998). In adults, propionibacteria supplementation promoted the intestinal growth of bifidobacteria and slowed down left colon transit time (Bougle et al., 1999). These health benefits could be related to the ability of propionibacteria to survive in the gastrointestinal tract in high numbers (Chaia, 1995; Bougle et al., 1999; Zarate et al., 2000) and to adhere to the intestinal epithelium that prolongs their maintenance in the gut (Zarate et al., 2002a).

Metabolic products of probiotic bacteria, such as bacteriocins, organic acids, diacetyl and other low molecular weight metabolites, may contribute to the control of unfavorable microorganisms and thus to a prolonged shelf life of foods (Glatz, 1992). Selected strains of these bacterial genera can be applied as protective cultures with an *in situ* production of antimicrobials (Ekinci and Gurel, 2008). Yogurts containing *P. freudenreichii* B2336 were able to prevent hepatomegaly (abnormal enlargement of the liver due to riboflavin deficiency). Furthermore, these products were able to revert morphologic changes (especially hair loss) observed in riboflavin deficient animals (Leblanc et al., 2006). In addition, propionate produced by propionibacteria may enhance absorption of calcium and iron in the gut. Different studies have shown that *P. freudenreichii* ssp. *shermanii* JS is a promising probiotic strain. When the JS

strain was administered with *L. rhamnosus* LC705 in fruit juice, the combination showed a weak positive effect on defecation frequency in elder people. In a study in irritable bowel syndrome (IBS) patients, the administration of capsules containing *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99 and JS reduced the IBS symptoms by 42%, compared to 6% reduction in placebo group. Interestingly, the same probiotic mixture containing JS strain improved patientsô tolerance to *H. pylori* eradication therapy (Suomalainen et al., 2008).

Factors influencing propionibacteria adherence to epithelial cells

The possibility of probiotic bacteria to colonize the gut depends on their ability to survive against gastric digestion and reach to a high number in the small bowel following adhesion to intestinal cells. Bacterial adhesion to intestinal cells and mucus is generally considered as the initial step in the colonization of the gut (Beachey, 1981; Coconnier et al., 1992). However, if a strain fails to adhere, its persistence in the intestine is dependent on the growth rate. Digestive enzymes and bile in gastric and pancreatic secretions (as well as other factors such as peristalsis and competition with the indigenous flora) can affect the bacterial adhesion ability (Zarate et al., 2002b). It has been reported that the bacterial adhesion ability was slightly affected by gastrointestinal digestion and metabolic and immune effects in the host. A great increase in the adhesion rate was obtained when propionibacteria were suspended in MRS medium acidified with propionic acid to pH 4.8. However, this could be the result of alterations in surface components of epithelial cells produced by acidic pH, which led to elevated levels of bacterial adherence (independent of the bacterial adhesion capabilities and nature of adhesions or host receptors) (Greene and Klaenhammer, 1994).

In dairy propionibacteria, the adhesion ability becomes an important property for the selection of strains for probiotic purposes, since they have a slow growth rate (Perez-Chaia et al., 1995; Zarate et al., 2000). Bacterial adhesion is based on nonspecific physicochemical interactions followed by specific and irreversible interactions mediated by adhesion factors of the bacterial surface and complementary receptors of the host cell (Beachey, 1981; Ofek et al., 1994). Addition of the cation chelating agent EDTA to the reaction mixture significantly reduced bacterial adherence, indicating that adhesion of propionibacteria to intestinal epithelium is affected by one or more divalent cations (Zarate et al., 2002b). Calcium was one of the cations involved in adherence, since it enhanced adhesion by increasing the adhesion rate and the number of bacteria adhered per epithelial cell. In *Propionibacterium,* adhesion was calcium independent but enhanced by the addition of this cation. Calcium (like other cations) possibly acted by providing ionic bridges between the negatively charged surfaces of bacterial and epithelial cells (Kleeman and Klaenhammer, 1982). This enhanced adhesion, promoted by calcium, also represents an interesting characteristic as propionibacteria are usually delivered to the host by being included in dairy products (such as Swiss cheese) with high calcium content.

Application of propionibacteria in dairy products and fermented milks as probiotic

History of use in dairy products as probiotic

There is presently much more experimental data on the probiotic applications of bacteria belonging to the *Lactobacillus* and *Bifidobacterium* species than propionibacteria. However, several reports suggest that they may have a positive impact on the health situation, function rate and comfort of the gut. The main application of these bacterial species is the ripening of Swiss

cheeses, characterized by round eyes. In addition, propionibacteria participate in microbiological safety and nutritional quality of cheeses. The bacteria inhibit a variety of gram negative bacteria (*Pseudomonas, Salmonella* and *Yersinia*) and several fungi, but not gram positive bacteria. Propionibacteria were effective at protecting fermented milks against spoilage by yeast and moulds.

The yogurt starter *Streptococcus thermophilus* was shown to produce vitamin B_9 which is consumed by lactobacilli. Different strains of dairy propionibacteria produce various amounts of vitamin B_9, which could be higher than that produced by *S. thermophilus* (Saarela, 2007). *P. freudenreichii*, produces large amounts of riboflavin and has been incorporated into yogurt to enhance concentration of riboflavin in some researches. By adding propionibacteria, riboflavin enriched yogurt suppressed the symptoms of riboflavinosis in rats, while the conventional yogurt failed to have such a significant health benefit (Leblanc et al., 2006). UHT milk with 1.5% fat was selected as the substrate for propionibacterium inoculation to synthesis folates. This substrate with minimal residual microflora and a relatively low content of natural folates allowed a better recognition of changes caused by the fermentation. Fermentation was conducted at 30°C and 37°C without agitation (Holasova et al., 2004).

During the production of probiotic acidophilus milk, the application of *P. freudenreichii* ssp. *shermanii* MTCC 1371 and *B. bifidum* NDRI (as microbial additives) with *L. acidophilus* R is suggested, based on a variety of technical and dietetic factors such as titrable and volatile acidity, diacetyl and acetoin production, extent of proteolysis, lactic acid content, lactose hydrolyzing activity, antibacterial activity and viable populations of lactobacilli, propionibacteria and

bifidobacteria. This probiotic acidophilus milk maintained its dietetic characteristics up to seven days of storage at 8±1°C. The advantages of the milk may be related to its enhanced prophylactic features, compared to plain acidophilus milk (Sarkar and Misra, 2010).

Technological aspects of incorporating propionibacteria in dairy products as probiotic

Technological aspects in dairy products can be divided into three categories, including viability of propionibacteria in fermented milks, fermentation time in products inoculated by propionibacteria and sensory attributes of final product (flavor, texture and appearance). Figure 3 shows technological aspects of propionibacteria in dairy products and fermented milks as probiotic. Table 2 includes selected publications on application of propionibacteria in dairy products as probiotic.

Viability of propionibacteria in dairy products and fermented milks and gastrointestinal tract

To achieve the health benefits related to probiotic microorganisms, recommendations for the minimum viable counts of each probiotic strain per gram or milliliter of probiotic products are quite variable from 10^5 CFU/g to 10^7 CFU/g. In general, the food industry has applied the recommended level of 10^6 CFU/g at the time of consumption for probiotic bacteria. This standard appears to provide bacterial concentrations that are technologically attainable and cost effective rather than to achieve a specific health effect in humans (Karimi et al., 2011).

Many intrinsic and extrinsic factors in food products significantly affect viability of probiotic microorganisms in fermented milks. Some of these factors include pH, titrable acidity, molecular oxygen, redox potential, hydrogen peroxide, bacteriocins, biorelationship amongst starter

cultures and microbial competitions, short chain fatty acids, flavoring agents, antimicrobial preservatives, packaging materials and conditions, rate and proportion of inoculation, stepwise/stagewise fermentation, microencapsulation, supplementation of milk with nutrients, heating the product, incubation and storage temperatures, carbonation, addition of salt, sugar and sweeteners, cooling rate of product and scale of production (Heydari et al., 2011). Most of the research regarding viability of propionibacteria in food products has been accomplished in cheese. The main factors reported to affect viability of propionibacteria in fermented milk are explained below.

Sodium chloride

Sodium chloride is an important ingredient in cheese and possesses several functions such as flavor, flavor enhancer, texture modifier and starter culture regulator (Karimi et al, 2012). However, high consumption of sodium chloride could be harmful and some regulatory agencies have determined some limits for the sodium content of products. For example, "US Department of Agriculture food" reported that the category "milk products" should not introduce sodium more than 8-8.9% to the diet of consumers 20 years old and over. On the one hand, substitution of NaCl with other ionic alternatives in sodium reduction strategies have not been popular because of the more organoleptic acceptability of NaCl compared to the others (Agarwal et al., 2011). For example, Grummer and his colleagues studied the effect of NaCI replacer in cheddar cheese. They showed that $CaCl_2$ and $MgCl_2$ had a significantly off-flavors in the final product. However, KCl induced taste changes were not significantly different from control. Therefore, they proposed that potassium chloride could be used only as a partially NaCl replacer in Cheddar

cheese (Grummer et al., 2012). Same results observed in the study of Cruz and his colleagues in partial replacement of sodium chloride by potassium chloride in Minas fresh cheese (Cruz et al., 2011b) and Karimi and his colleagues in Iranian UF Feta cheese (Karimi et al., 2012). It should be noted that salt can affect bacterial viability via its direct effect on cells as well as increasing the osmotic pressure of the media (Karimi et al, 2012a,b; Cruz et al., 2011a).

Swiss type cheeses are classified as relatively low sodium products (average 400 mg/100 g of cheese) (Agarwal et al., 2011). Effect of sodium chloride on Swiss cheeses requires brine salting of the cheeses just before ripening. In cheese, salt is an important factor which influences the growth of propionibacteria, as they are sensitive to sodium chloride. The inhibiting effect of sodium chloride on the bacterial growth is significant since the pH is low. In YEL medium at pH 7, the great majority of propionibacteria strains are able to grow at a maximum concentration of 6ï 7% of salt. In cheese, however, the bacterial sensitivity is increased in pH 5.2ï 5.4, just before ripening starts. Their growth decreases drastically at a concentration of 3% of salt in cheese, just near the cheese rind. *P. freudenreichii* ssp. *shermanii* has been shown to respond to changes in high osmolarity media by varying the concentration of specific solutions, especially glycine-betaine, in order to maintain the turgor pressure. This feature enables the bacteria to adapt to the rising osmotic pressure due to the salting stage in the cheese process (Robinson et al., 2000).

Storage at low temperatures

Ability of some propionibacteria strains to grow at low temperatures (about 7°C) presents technical difficulties in cheese technology. This occurs when the ripened cheeses are stored in cold rooms after ripening; hence, the growth of propionibacteria can lead to excessive swelling

of the cheese loafs (Robinson et al., 2000). Swiss cheese was manufactured with single strains of *Propionibacterium shermanii* and *Propionibacterium arabinosum*. At 7.2°C, a temperature at which Swiss cheeses are often stored commercially, the bacterial strains grew well and poor, respectively (Park et al., 1967). Moreover, a red spotting defect is sometimes seen caused by the growth of pigmented PAB strains (Rossl et al., 1998). In *Propionibacterium*, as many other microorganisms, trehalose accumulation has been shown to occur as a result of different stress conditions such as low temperature and high osmolarity. In fermentation processes using propionibacteria, high amounts of trehalose were found. This demonstrates the actual potency of propionibacteria for the production of low-calorie trehalose in fermented foods (Hugenholtz et al., 2002).

It was found that reducing the incubation temperature by 10°C or lower than that needed for optimal growth slowed down the development of propionibacteria. In Grana cheese, a moderate number of these bacteria produce the organoleptic characteristics that make it typical. The combination of a low temperature (22°C), a high concentration of NaCl (> 2%) and a pH of less than 5.5 effectively slowed down the development of these bacteria in the first 30 to 60 days of ripening, which would allow the curd to become hard enough to resist the pressure of carbon dioxide (CO_2) better than a soft curd does (Carcano et al., 1995).

Gastrointestinal tract and pH profile

The main reasons for the dramatic decline in viability of the delivered cells into the gastrointestinal tract include very low pH of the stomach and the presence of bile salts in the small intestine (Mortazavian et al., 2008). Effect of various pH of simulated gastric juices on the

viability of 13 dairy propionibacteria strains was studied during 180 minutes of transit time. There was no loss of viability for all strains at pH 4. In contrast, at pH 3 ten strains retained the same level of viability, while at pH 2 all strains showed reduced viability. The variability of dairy propionibacteria strains to survive at pH 2ï 3 suggests that the acid tolerance of dairy propionibacteria is strain specific, and that these pH could be considered as critical factors for the selection of potential probiotic dairy propionibacteria.

Although pH could be used as an appropriate direct factor for the selection of probiotic strains, most of the probiotics are freely consumed in food products. The presence of food ingredients has been reported to improve the bacterial viability during gastric transit. The suggested mechanism for the beneficial effect of food ingredients is the increase in pH of the gastric contents resulted from the addition of foods (Huang and Adams, 2004). Addition of 5 g of cheese to 10 ml of gastric juice has been reported to increase the pH from 2 to 4.74, whereas 5 g of yogurt only increased the pH to 3.65 (Karimi et al., 2011). The improved viability of microorganisms during simulated gastric transit in pH 2 indicates that low acid tolerant strains need not be excluded from probiotic applications, providing they can be delivered to the intestine in high numbers preferably as part of a buffered food or encapsulated delivery systems (Huang and Adams, 2004).

Investigating the viability of probiotics after the exposure to gastrointestinal conditions should be performed as a complementary study to the current works. However, there are limited studies associated with the survival of probiotic cells in cheeses under the gastrointestinal tract conditions. *In vivo* studies regarding survival analysis of probiotics in food products are

commonly carried out under simulated gastrointestinal conditions. Dense matrix, high buffer capacity and relatively high-fat content of cheeses offer good protection to probiotic microorganisms during delivery throughout the human digestive tract. Cheeses (pH 4.8ï 5.6) have a markedly higher pH than fermented milks (pH 3.7ï 4.5), providing a much stable environment to support the long-term survival of acid sensitive probiotics, compared to fermented milk products (Karimi et al., 2011). Simulated small intestine conditions have a little effect on the viability of dairy propionibacteria. In general, dairy propionibacteria have shown a high tolerance capacity to upper gastrointestinal transit, providing an alternative source to lactobacilli and bifidobacteria for the future probiotic development (Huang and Adams, 2004).

Bacterial relationships between propionibacteria and other probiotics

A question about the interaction and possible antagonistic effects between propionibacteria and lactic acid bacteria has been raised in this connection with different viewpoints. In most cases, lactobacilli seem to dominate in the culture and even prevent the growth of propionibacteria (Parker and Moon, 1982). In mixed cultures, the growth rate of propionibacteria and the production of acids and CO_2 were lower than in pure cultures. However, lactobacilli/propionibacteria pairs sometimes had a beneficial effect on the growth of each other. An antagonistic effect of the metabolites of propionibacteria to *L. acidophilus* is reported even in much diluted substrates (Mantere-Alhonen, 1995). The growth stimulation of *B. adolescentis* by *P. freudenreichii* is a factor which could be significant when using these bacteria as probiotic agents (Kaneko et al., 1994). In mixed cultures, the growth rate of *B. adolescentis* increased from

3.7×10^6 CFU/ml to 2.2×10^8 CFU/ml and the growth rate of *P. freudenreichii* increased from 8.7×10^7 CFU/ml to 4.7×10^8 CFU/ml (Mantere-Alhonen, 1995).

Stress adaptation of propionibacteria to product media

The process of yogurt-type fermented milks was accompanied by a slow decrease in pH, down to 4.6. It was shown that pre-exposure of *P. freudenreichii* to this pH triggers an acid tolerance response, affording tolerance to a subsequent exposure to pH down to 2 without any significant loss of viability (Jan et al., 2001). It is thus possible that fermented milk process is efficient at inducing acid tolerance response in this bacterium. Moreover, this process comprises incubation at 42°C. This temperature was shown recently to induce a multiple tolerance response in *P. freudenreichii,* leading to efficient protection towards bile salts and heat stress in particular (Leverrier et al., 2004). Besides the viability of propionibacteria in fermented milks, the bacterial *in vivo* viability is also critical to provide its health benefits. This is very important for choosing propionibacteria as starter culture in dairy products.

Selected probiotic must have appropriate tolerance to low pH values and bile salts. As mentioned previously, the resistance variability of dairy propionibacteria strains at pH 2ï 3 verifies that the acid tolerance is strain specific, and this pH is considered as a critical feature in selection of potentially probiotic propionibacteria (Huang and Adams, 2004). Results have shown that *P. freudenreichii* ssp. *shermanii* MTCC 1371 failed to grow in the presence of 0.5ï 2.0% of bile salts and hence was not bile salt tolerant (Sarkar and Misra, 2006). However, certain strains of propionibacteria possess bifidogenic properties (Bougle et al., 1999; Kouya et al., 2007). The bacterial growth associated with bifidobacteria and other bacteria within the intestinal

microflora may be a good advantage in maintaining greater bacterial viability during gastrointestinal transit (Kaneko et al., 1994; Mori et al., 1997).

Oxidative stress

Oxygen molecules could be destructive agents to anaerobic bacteria because of their contribution in reactive oxygen species (ROS) formation as active free radicals that lead to oxidative stresses (McCord, 2000). In probiotic containing products, oxidative stress can lead to the lesser probiotic favorable activity and viability. However, some probiotic strains have antioxidant nature and show active-redox metal ion chelating capability in the medium. Thus, they diminish harsh effects of oxidative damages (Lee et al., 2005). Furthermore, some probiotics such as propionibacteria produce low-calorie sugars such as trehalose that can probably react with oxygen radicals and possess antioxidant activity (Hugenholtz and Smid, 2002). Interestingly, propionibacteria could produce superoxide dismutase that it is known as one of operating factor in oxidation damage remediation (Leverrier et al., 2003).

To reduce the antioxidant stress of food media to probiotics, some studies have carried out on the application of glucose oxidase enzyme in oxygen elimination. Results showed that oxidative stresses minimized by this strategy and the higher viable count of probiotics observed in comparison to control (Cruz et al., 2012a,b).

Another study about glucose oxidase application in probiotic survival improvement in yogurt carried out by Cruz and his colleagues. According to their study, enzymatic reaction in the presence of glucose and oxygen as a substrate could control any oxidative reactions and refuse using chemical preservatives. The main goal of their study was optimization of processing

condition and determination of the optimum level of glucose and glucose oxidase to achieve the desired results (Cruz et al., 2010b).

Incubation time during the production of fermented milks

Probiotic bacteria grow slowly in milk due to the lack of proteolytic activity. A usual solution is the addition of yogurt bacteria (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) to probiotic products to reduce the fermentation time (Shihata and Shah, 2000; Antunes et al., 2004). Low proteolytic activity of propionibacteria is related to their grater peptidase activity compared to proteolytic nature which limits propionibacteria in nitrogen and lactose utilization from milk. Therefore, starter lactic acid bacteria can provide lactate and peptides, from lactose and milk proteins, respectively, favoring propionibacteria growth in milk and dairy products (Cousin et al., 2012).

Because of the slow growth of propionibacteria in cultures, longer fermentation times were observed when the bacteriocin producing *P. jensenii* B1264 (2%) and *P. thoenii* (*jensenii*) P126 (2%) were used alone as starter culture for the production of a new fermented dairy product (approx. 9 h) (Ekinci and Gurel, 2008).

Combination of *P. freudenreichii* ssp. *shermanii* with *L. acidophilus* R improved acid production in FM (0.378ï 1.368% concentration of lactic acid) during incubation (Sarkar and Misra, 2006). However, improvement could be seen after 8 h of incubation in SM (1.116% concentration of lactic acid). Combined use of *P. freudenreichii* ssp. *shermanii* MTCC 1371, *B. bifidum* NDRI and *L. acidophilus* R induced a higher rate of acid production after 4 and 12 h of

incubation in SM and FM. A combination of these bacterial strains may be suggested for the production of probiotic acidophilus milk for infant feeding means (Sarkar and Misra, 2006).

Sensory characteristics of final products

There are few studies on sensory attributes of propionibacteria in fermented milks. The study on a sour milk product containing *P. freudenreichii* ssp. *shennanii* and *L. acidophilus* (3:1) showed a very good sensory property, mild flavor, good consistency and good conservation quality (Mantere-Alhonen, 1995). The diacetyl and acetoin contents along with volatile acids contributed to the typical aroma of the product. Combination of *P. freudenreichii* ssp. *shermanii* MTCC 1371 with *L. acidophilus* R significantly induced the diacetyl and acetoin production (Sarkar and Misra, 2006). It has been reported that some strains of propionibacteria have a good ability in EPS (exopolysaccharide) formation (Skogen et al., 1970; Crow, 1988; Racine et al., 1991). EPS provides appropriate functional properties in fermented milks (e.g. viscosifying, stabilizing, emulsifying, gelling and water binding properties) and can affect sensory properties of the product (Gorret et al., 2001; Mohammadi and Mortazavian, 2011).

Propionibacteria are used as secondary starter mainly in the production of Swiss cheeses. The bacterial presence, in association with other bacterial genera within the milk microflora, was positively correlated to aroma described as ñpungentò in experimental mini Swiss cheese made from raw milk. A variety of volatile branched chain acids are produced in minor amounts by propionibacteria in addition to the main fermentation products (acetic and propionic acids), including isobutyric, isovaleric and isocaproic acids. These organic acids are described as sweaty, fruity, sweet, cheesy, fatty acid like substances. Acetate and propionate play a significant role in

the definition of the typical flavor in Swiss cheeses. Furthermore, propionibacteria are able to produce volatile sulfur compounds. This ability is strain dependent. Isovaleric acid was the most potential branched chain aroma in Swiss cheeses, with a concentration nearly 600 times higher than its odor threshold in water (Demarigny et al., 1997; Thierry and Maillard, 2002).

Propionibacteria could have a significant role in increasing proteolysis in fermented milks. Proteolysis participates in flavor development, either by producing aroma precursors and compounds or by releasing flavor molecules fixed on proteins. Small peptides can interfere with calcium and magnesium ions to produce a broth-like aroma. The bitter taste of cheese is due to the appearance of small hydrophobic peptides. Concerning propionibacteria that are generally weak proteolytic bacteria, two enzymatic activities performing on ß- and ŭs$_1$-caseins are described. It is assumed that adventitious bacterial flora is responsible for these biochemical and sensory differences in various cheeses, especially facultative heterofermentative lactobacilli and propionibacteria (Demarigny et al., 1997). The ability of propionibacteria to convert branched chain, aromatic and sulfur-containing amino acids to volatile compounds has been reported.

Incorporation of propionibacteria in dairy products for functions other than probiotic

In 1906, von Freudenreich and Orla-Jensen isolated bacteria from Emmental cheese and named them *Bacterium acidi-propionici* and *Bacillus acidi-propionici*. These microorganisms were characterized by their ability to produce propionic acid. The researchers suspected them for the typical ñeyeò formation in the cheese. The Genus *Propionibacterium* was first proposed by Orla-Jensen in 1909 (Dasen, 1998a).

Propionibacteria are naturally present in the raw milk used for the production of cheese. However, this natural flora is becoming more and more depleted by the improvement of raw milk quality and the process of microbiological purification of milk, such as microfiltration and bactofugation. For this reason, propionibacteria are added at a low concentration (10^5 CFU/ml of milk) by cheese makers at the beginning of process. In fact, only *P. freudenreichii* is used as a starter, possibly because it is the most resistant species to heat treatment applied to the curd during the cheese production. However, indigenous propionibacteria are not completely eliminated by the milk thermal treatment and despite their low concentration, these genera are able to predominate during the ripening process due to the significant length of ripening period. Propionibacteria grow in cheese after the development of thermophilic lactic acid bacteria (LAB), as they can obtain their growth energy from the fermentation of lactate produced LAB (Robinson et al., 2000).

The importance of dairy and classical propionibacteria in food production is generally related to their role in Swiss cheese ripening as agents of flavor formation. In long ripening Italian cheeses such as Parmesan, Grana, Padano and Provolone, a massive development of propionibacteria may cause economic losses due to intense CO_2 production from lactate and formation of anomalous holes or cracks during the late ripening (Rossl et al., 1998). For decades, *P. freudenreichii* ssp. *shermanii* JS has been used as a starter culture in Jarlsberg cheese in Finland. Furthermore, the strain has been combined with *Lactobacillus rhamnosus* LC705 in a protective culture, Bioprofit^{TM}, which is used in biopreservation of fermented foods (Suomalainen et al., 2008).

It is difficult to determine the exact role of propionibacteria in production of flavor compounds because the presence of aromatic molecules is resulted from the activity of various bacterial species developed in cheeses. It seems that this activity, which is responsible for the development of aromatic compounds, is mostly significant during the ripening. However, it remains to be determined whether these bacteria are responsible for the production of aromatic amino acids or compounds resulted from the catabolism of amino acids. However, proteolytic activity of propionibacteria is not significant, some studies focus on their lipolytic capacities. In food industries, polysaccharides are widely used as viscosifiers, stabilizers, emulsifiers, gelling or water binding agents (Gorret et al., 2001). The main sources of polysaccharides are plants and algae. However bacteria are potential sources of polymers, only *Xanthomonas campestris* and *P. elodea* are used to produce food grade polymers. As mentioned earlier, propionibacteria are known for their EPS production ability (Skogen et al., 1970; Crow, 1988; Racine et al., 1991). Figure 4 describes the functionality of propionibacteria in dairy products for functions other than probiotic activity.

Methods for isolation, identification and enumeration of propionibacteria in dairy products

Propionibacteria are anaerobic to microaerotolerant bacteria and do not grow on solid media exposed to air. Hence, obtaining colonies requires culture of bacteria in anaerobic jars. However, the bacterial growth in liquid culture media does not require anaerobic conditions possibly due to their microaerotolerant feature (Robinson et al., 2000). The main methods for the identification and enumeration of propionibacteria are explained as follows (see Figure 5).

Selective culture media

Propionibacteria are usually a minor population in milk samples and therefore difficult to isolate using nonselective media (Robinson et al., 2000). Dairy propionibacteria can utilize many different carbon sources such as glucose, fructose and glycerol but the preferred substrate is lactate. An appropriate medium contains 1% sodium lactate, 1% tryptone, 0.5% yeast extract and 0.5% KH_2PO_4. This will support the bacterial good growth, but depend on the purpose of cultivation; other media could be more appropriate (Lind, 2010). Propionibacteria grow well on complex media such as Brain Heart Infusion (BHI) broth but due to the bacterial long generation time, contamination with other bacteria can occur. This explains why more selective media are preferred. Production of propionic acid and vitamin B_{12} requires specific conditions. Maximum yield of propionic acid requires a completely anaerobic environment at 37°C, while the production of vitamin B_{12} needs aerobic conditions at 40°C (Lind, 2010).

Several culture media have been proposed for the isolation of propionibacteria (mainly from cheeses); from which the most popular is YEL (yeast extract sodium lactate). YEL is based on lactic acid as carbohydrate source and lacks selectivity. Since propionibacteria are relatively slow growing microorganisms (1ï 14 days), contaminant microorganisms almost grow faster on this medium. Addition of 4 µg/ml of cloxacillin (MIC for propionibacteria < 8 µg/ml) to the medium is recommended to inhibit the growth of LAB commonly used as starters in dairy products (Vorobjeva, 1999). With this medium, to which agarose has been added (YELA), five to six days are required to obtain colonies of 2 mm in diameter which are cream colored for *P. acidipropionici* and *P. freudenreichii,* orange or brick red for *P. jenseinii* and brick red for *P. thoenii* (Robinson et al., 2000).

PAL (Standa Industries, France), a selective solid medium for the enumeration of propionibacteria from dairy products, is available but data on its selectivity for other systems (such as clinical isolates) are not presented. In addition, PAL is rather expensive and the identification of propionibacteria is based on a color reaction in the medium (pink to yellow). This can result in diagnostic mistakes since the detection of single-colony contaminants is no longer possible at high colony counts. No selective media is currently in use for the isolation of cutaneous strains. The isolates are incubated on standard media under anaerobic conditions and identified using classical methods (Vorobjeva, 1999).

Propionibacteria can selectively be grown in mixed cultures containing lactate (as a primary energy source) and small amount of heavy metal salts to which propionibacteria are resistant (selected from groups of water-soluble cadmium and arsenic salts). As a result, other bacteria such as *Lactobacillus* spp. in the mixed culture samples do not grow because they are resistant to neither cadmium salt nor arsenic salt. In a preferred media, the heavy metal salt is an arsenic salt combined with an antibiotic which allows the medium to be selective for *Propionibacterium* spp. (Tomes et al., 1991). These preliminary screening results suggest that the use of heavy metals has a selective potency, particularly for the arsenic and cadmium salts. The bacterial resistance to cadmium is not as general as its resistance in nature. However, some strains are much more resistant to cadmium salt than other strains within the same species. This characteristic can be used for the selective recovery of a bacterial strain of special interest. Combination of arsenic salts and netilmicin helps to selectively screen cultures for *propionibacterium* spp. Research has showed that a mixture of arsenic salt and netilmicin was effective in inhibiting all lactobacilli strains tested but not *propionibacterium* spp. (Tomes et al., 1991).

A recently developed medium now available commercially, Pal Propiobac[@], allows for the improved isolation of propionibacteria from samples with a complex flora. In addition to classical nutritional elements, this medium contains glycerol as the fermentation substrate, lithium to inhibit various lactic acid bacteria, a cocktail of antibiotics active against gram negative bacteria and purple bromocresol. The propionibacteria colonies appear brown on the medium with a diameter larger than 0.5 mm, surrounded by a yellow area caused by decreased pH resulted from glycerol fermentation (Robinson et al., 2000).

Biochemical assays

Some genera of propionibacteria can appear gram variable under the certain growth conditions (Beveridge, 1990). Usually, production of propionic and acetic acids is the simplest way to differentiate *Propionibacterium* spp. (Himmi et al., 2000). Several methods based on the comparative protein profiles, serology and immunology have been described to differentiate between the classical *Propionibacterium* spp. A method which has been proposed previously simply uses antibiotic resistance patterns to classify anaerobes to the genus level. A method for the detection of *P. acnes* has been developed using pyrolysis mass spectrometry and artificial neural networks (Goodacre et al., 1994).

In a study by Schreckenberger and Blazevic (1974), various rapid biochemical tests were used, including nitrate reduction, indole production, hydrolysis of gelatin, aesculin and starch, and hydrolysis of o-nitrophenyl-,B-D-galactopyranoside (ONPG) by beta galactosidase. The results showed that rapid testing of anaerobic bacteria is possible and in most cases can be

carried out within less than four hours. An exception might be the test for nitrate reductase (Schreckenberger and Blazevic, 1974).

Molecular methods

Reliable and fast detection of propionibacteria strains by classical methods is not as easy as those using DNA probe techniques. In raw milk, these techniques can prove the occurrence of wild-type strains which can replace the starter cultures and cause unfavorable side effects such as red spots during the cheese ripening (Dasen et al., 1998). In addition, a method that allows a fast screening procedure of clinical samples to detect medically relevant strains would help to shorten diagnosis time (Peters et al., 2004). The chromosome size of four propionibacteria strains was determined using PFGE. This technique has been used to identify some classical propionibacteria to species level as well as other bacterial species (Gautier et al., 1992). The use of RAPD (random amplified polymorphic DNA) technique has also been investigated to achieve species differentiation. However, RAPD has been shown in some cases to be a rather irreproducible technique (Gardiner et al., 1995).

Broad-range PCR (polymerase chain reaction) is a promising tool for patients with negative culture endocarditis and allows the detection of rare and non cultivable organisms (Goldenberger et al., 1997). Methods based on the amplification of target genes and restriction of the resulted fragments by PCR and RFLP (restriction fragment length polymorphisms) techniques have been designed. Restriction analysis of 16S-23S rDNA intergenic spacer regions has also been proposed (Dasen, 1998a). Some studies used restriction of 23S rDNA insertion regions typically found in high G+C content gram positive bacteria. These methods have been used for the

classical species such as *P. jreudenreichii* (Roller et al., 1992). In patients done surgical operations, microbial cultures or direct microscopy may not detect the causative microorganism in the infected valve. In such cases, slow growing or non-cultivable microbes may be the etiologic agents or the patient may have received antimicrobials before the specimen was obtained. To overcome these problems, culture independent molecular techniques using specific DNA probes and sequencing of the genes encoding for 16S rRNA may help (Goldenberger et al., 1997).

Other genetic techniques based on specific gene probes targeted 16S rDNA have been developed for use in hybridization of classical and medically relevant propionibacteria. These techniques have been used to detect and identify either cutaneous or classical strains of *Propionibacterium* spp. A technique that allows the detection of whole genus has not been described yet (Dasen, 1998a).

Conclusion

Extensive public interest in natural products has revived industrial research for biological production of propionic acid as a natural food preservative. Combination of propionic, lactic and acetic acids has been recommended for the preservation of foods. These organic acids can be produced by probiotic microorganisms. To provide health benefits related to probiotic microorganisms, food industries have generally applied the recommended level of 10^6 CFU/g to 10^7 CFU/g (depend on the type of product) at the time of consumption for probiotic bacteria. Survivability of probiotic bacteria to environmental conditions very depends on the bacterial species and strain, metabolic interaction with lactic acid starters, fermentation conditions, pH of

the product, presence of oxygen, storage temperature and presence of protective compounds such as protein and fat droplets. The most important regulatory factors are access to nutrients, adequate water activity, suitable pH and optimum temperature. Food applications of probiotics are mostly found in dairy products; with yogurts, kefir and cultured drinks as the major nutrient categories. Metabolic products of probiotic bacteria such as bacteriocins, organic acids, diacetyl and other low molecular weight metabolites may contribute to the control of unfavorable microorganisms and thus to a prolonged shelf life of foods. In probiotic food products, propionibacteria are primarily and commonly combined with lactic acid bacteria and/or bifidobacteria. Although, propionibacteria are not extensively commercialized as probiotic at the present time, there is an extensive trend for its commercialization for this usage. Selected probiotic bacteria must have appropriate tolerance to low pH values and bile salts. Resistance variability of dairy propionibacteria strains at pH 2ï 3 verifies that the acid tolerance in these bacteria is strain specific, and that this range of pH is considered as a critical factor in the selection of potential probiotic dairy propionibacteria. In summary, use of propionibacteria as a food probiotic source, especially in different dairy products, can help consumers overcome some clinical health problems and hence reduce extensive medical expenses.

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Table 1. Biochemical characteristics of dairy propionibacteria (Vorobjeva 1999; Robinson et al., 2000; Adams and Huang 2005)

	Acid production					Others						
organism	**Sucrose**	**Maltose**	**Arabinose**	**Celobiose**	**Glycerol**	**Aesculin hydrolysis**	**Indole production**	**Gelatin liquefaction**	**Reduction of nitrate**	**Hemolysis**	**Color of pigment**	**G+C in DNA (mol %)**
P. freudenreichii	-	-	+	-	+	+	-	-	d	-	Creamy	65±10
P. jenseenii	+	+	-	d	+	+	-	-	-	d	Creamy	66±05
P.thoeniii	+	+	-	d	+	+	-	-	-	+	Red-brown	67±11
P. acidipropio	+	+	+	+	+	+	-	-	+	-	Creamy to orang	67±08

nici	e-yellow

[+] 80% or more strains are positive

[d] 21ï 79% is positive[-] 11ï 20% is positive

Table 2. Selected publication on application of propionibacteria in dairy products and fermented milks as probiotic

Formulation specification	Probiotic bacteria	Storage conditions	Viability	Reference
Probiotic acidophilus milk made from reconstituted skim milk and formulated mil	B. bifidum NDRI, P. freudenreichii ssp. shermanii MTCC 1371 and L. acidophilus R	Incubated at 37.1°C for 12 hours	Inoculated at 1% level individually	Sarkar and Mirsa 2006
Probiotic acidophilus milk made from skim milk	B. bifidum NDRI, P. freudenreichii ssp. shermanii MTCC 1371 and L. acidophilus R	Incubated at 37.1°C for 12 hours and 8±1°C for 7 days	Inoculated at 1% level	Sarkar and Mirsa 2010
Conventional yogurt	P. freudenreichii NIZO B2336	Fermentation was performed for 18 h at 31°C until the pH was 4.4	5×10^8 CFU/ml	Leblanc et al., 2006
Fermented UHT milk with	B. longum, B. bifidum, S.	Then, yogurt batches were	2%, 1% and 5% of inoculums was	Holasova et al., 2004

1.5% fat content	thermophilus and P.freudenreichiissp. Shermanii	freeze dried and ground. Finally, yogurt powder was mixed with a riboflavin deficient diet at a ratio of 1:40 (w/w)	applied with Bifidobacterium, S. thermophilus and Propionibacterium, respectively	
Swiss type cheeses	P. freudenreichii strains (P843, P572, P728, P196 and P873)	Fermentation was conducted at 37°C and 30°C in the case of Propionibacterium, without agitation. Samples were taken after 6, 12 and 18 hours of fermentation, subjected to extraction and hydrolysis, stored at ï 18°C until purification and	10^{8}ï 10^{9} CFU/g	Pruitt 2005

		quantification		
French raw milk	Classical propionibacteria	Anaerobically incubated at 30, 22, 10, 7.2 and 4°C	1.3×10^4 CFU/ml	Robinson et al., 2000
Raw milk used for Italian Grana cheese	Classical propionibacteria	Cold temperature	7×10^2 CFU/ml	Robinson et al., 2000
Swiss raw milk	Classical propionibacteri	Cold temperature	2.5×10^2 CFU/ml	Robinson et al., 2000

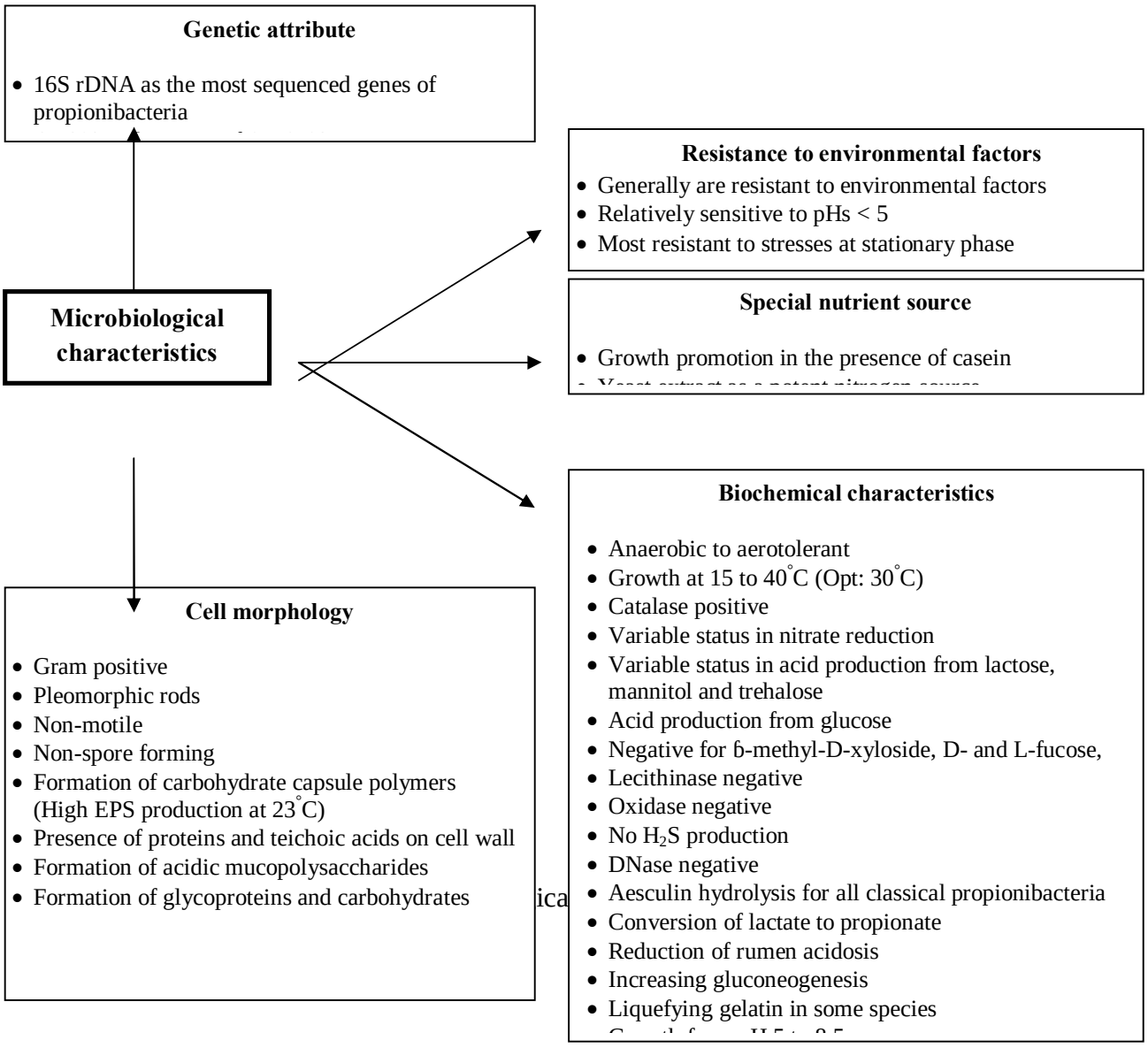

Figure 1. Main microbiological characteristics of propionibacteria

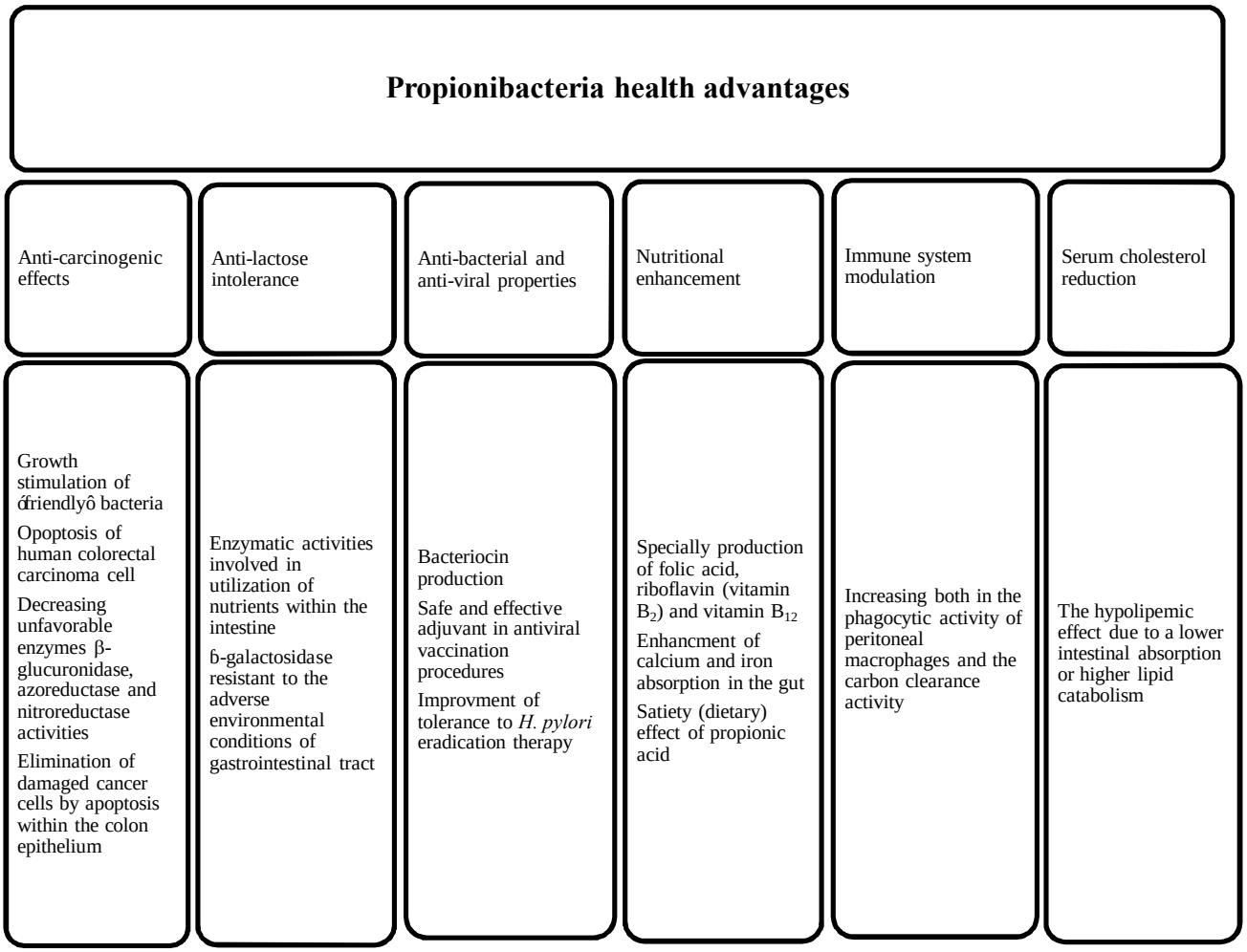

Figure 2. The main health advantages of probiotic dairy propionibacteria along with their involved mechanisms

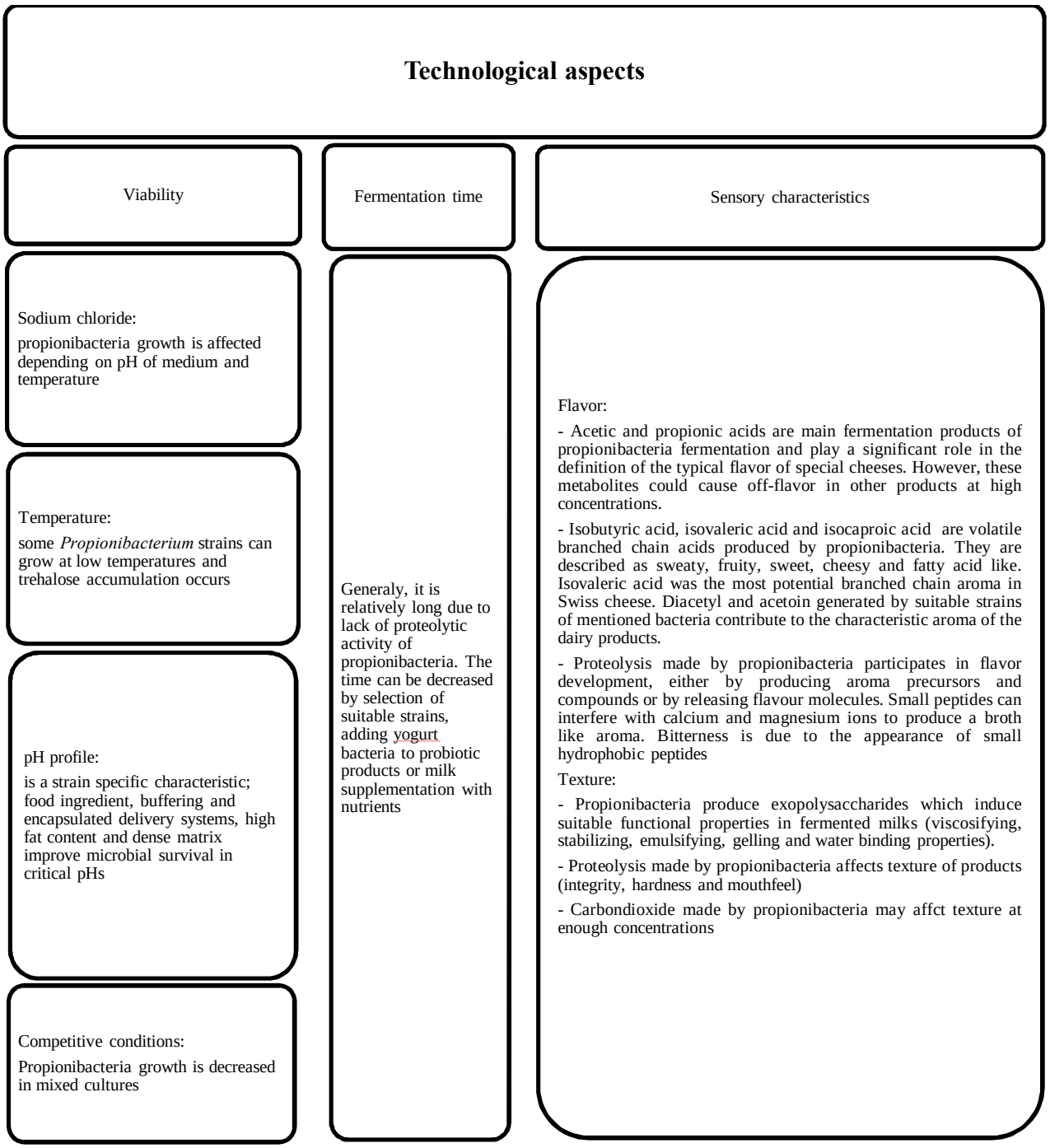

Figure 3. Technological aspects of propionibacteria in dairy products and fermented milks as probiotic

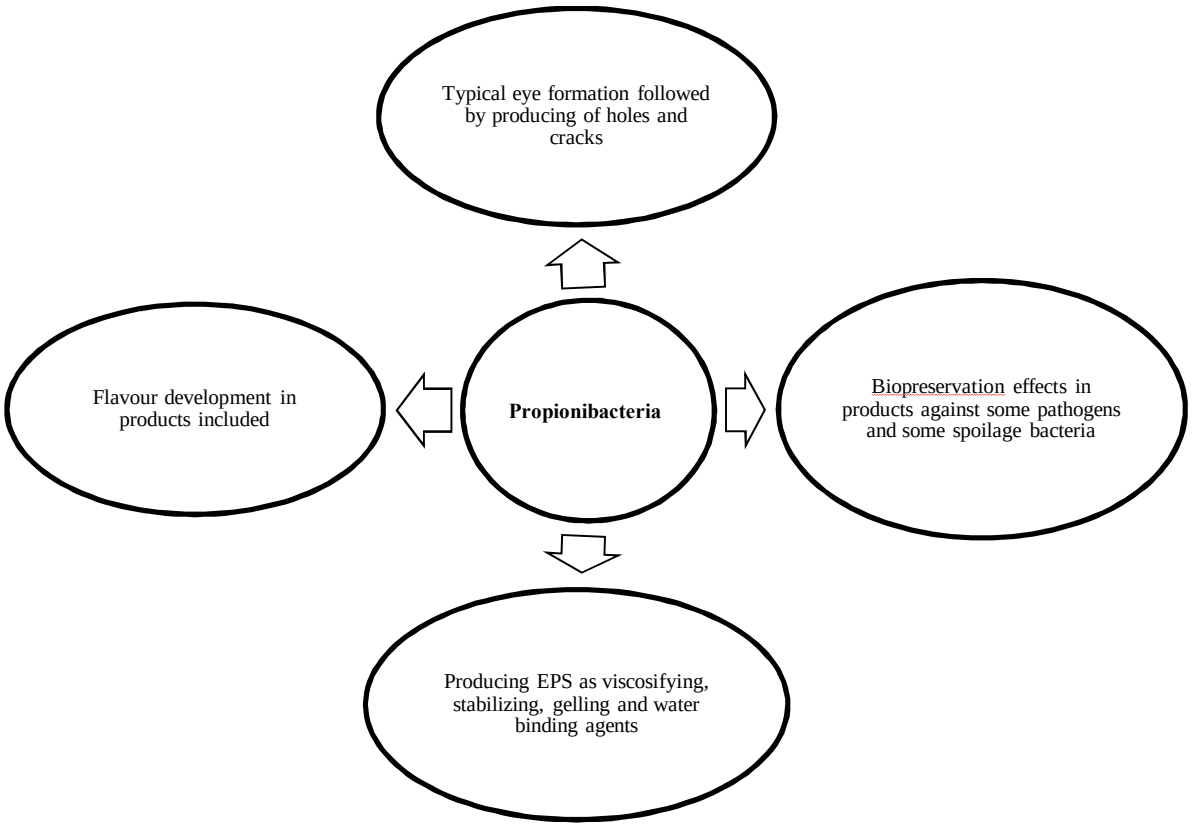

Figure 4. The functionality of propionibacteria in dairy products for functions other than probiotic

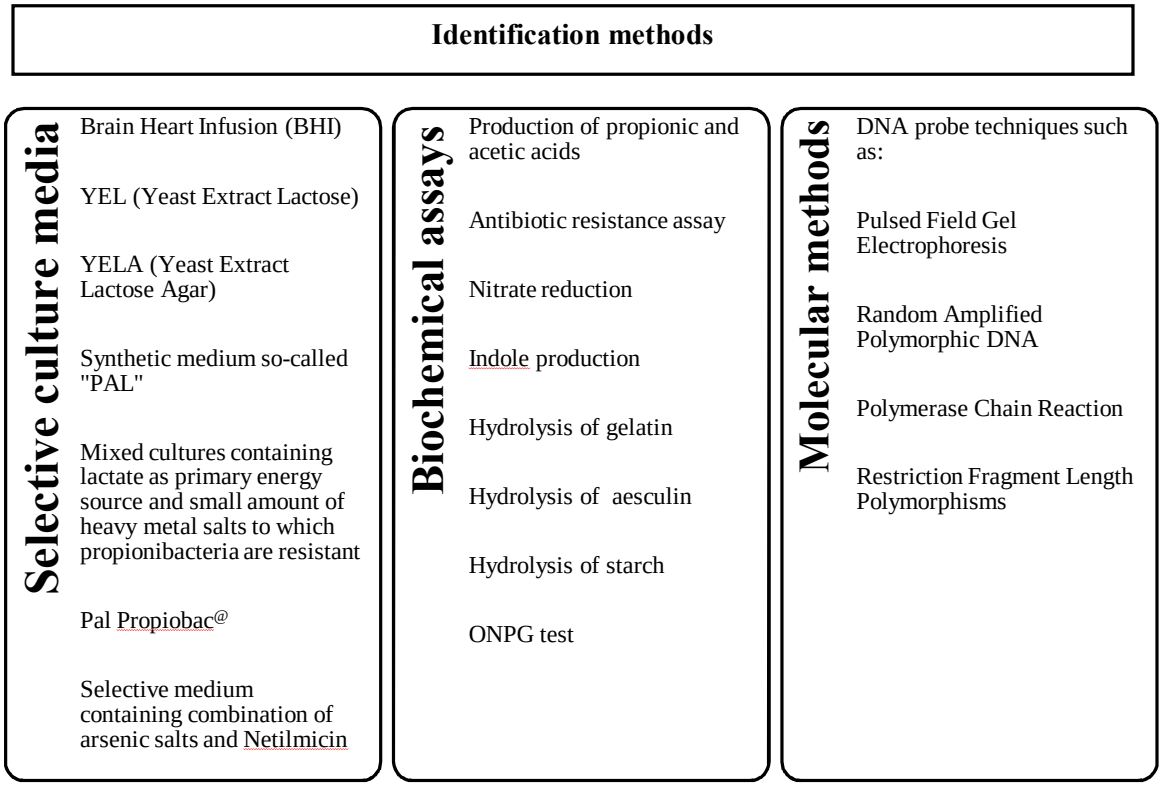

Figure 5. Main methods to identify and enumerate propionibacteria in dairy products

