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Effect of dairy-based protein sources and temperature on growth, acidification and exopolysaccharide production of *Bifidobacterium* strains in skim milk

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ABSTRACT

The aim of the present study was to find out the best growing conditions for exopolysaccharide (EPS) producing bifidobacteria, which improve their functionality in yoghurt-like products. Two *Bifidobacterium* strains were used in this study, *Bifidobacterium longum* subsp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205. In the first part of the study the effect of casein hydrolysate, lactalbumin hydrolysate, whey protein concentrate and whey protein isolate, added at 1.5% w/v in skim milk, was evaluated in terms of cell growth and EPS production; skim milk supplemented with yeast extract served as the control. Among the various nitrogen sources, casein hydrolysate (CH) showed the highest cell growth and EPS production for both strains after 18 h incubation and therefore it was selected for subsequent work. Based on fermentation experiments using different levels of CH (from 0.5 to 2.5% w/v) it was deduced that 1.5% (w/v) CH resulted in the highest EPS production, yielding 102 and 285 mg L^{-1} for *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486, respectively. The influence of temperature on growth and EPS production of both strains was further evaluated at 25, 30, 37 and 42 °C for up to 48 h in milk supplemented with 1.5% (w/v) CH. The temperature had a significant effect on growth, acidification and EPS production. The maximum growth and EPS production were recorded at 37 °C for both strains, whereas no EPS production was observed at 25 °C. Lower EPS production for both strains were observed at 42 °C, which is the common temperature used in yoghurt manufacturing compared to that at 37 °C. The results showed that the culture conditions have a clear effect on the growth, acidification and EPS production, and more specifically, that skim milk supplemented with 1.5% (w/v) CH could be used as a substrate for the growth of EPS-producing bifidobacteria, at 37 °C for 24 h, resulting in the production of a low fat yoghurt-like product with improved functionality.

1. Introduction

Bifidobacteria are an important group of probiotic cultures commonly used in fermented dairy products. In addition, some strains of *Bifidobacterium*, which produce exopolysaccharides (EPS) have been isolated and characterised (Audy, Labrie, Roy, & LaPointe, 2010; López et al., 2011; Prasanna, Grandison, & Charalampopoulos, 2011; Ruas-Madiedo et al., 2007; Salazar et al., 2009). EPS are considered to be important in the dairy industry because of their functional role, such as the improvement of textural properties and the reduction of syneresis of fermented dairy products (De Vuyst et al., 2003; Khurana & Kanawjia, 2007). In general, various hydrocolloids originating from plant (pectin, guar gum and locust bean gum) and animal (gelatine and casein) sources have been used to produce the desired texture of fermented dairy products (De Vuyst, De Vin, Vaningelgem, & Degeest, 2001; Lucey, 2004). However, the addition of plant and animal extracts to fermented dairy products is prohibited in some countries (Grobben et al., 1998; Hallemeersch, De Baets, & Vandamme, 2005). There is also no assurance of constant price and supply of plant hydrocolloids due to drought and other environmental factors existing in the areas where these plants are grown (Iyer, Mody, & Jha, 2006). Furthermore, the quantities available are not sufficient to fulfil the demand (Laws, Gu, & Marshall, 2001). In addition, these hydrocolloids do not always produce the desired rheological properties (Saija, Welman, & Bennett, 2010). Furthermore, religious and vegetarian lifestyle choices restrict some consumers from eating foods (yoghurt, ice cream and whipped desserts) containing animal-based hydrocolloids; gelatine is an example (Karim & Bhat, 2008). In addition, there is a rapid increase of demand for smooth and creamy yoghurt products, which is commonly achieved by increasing the content of fat, sugars, proteins or stabilizers (pectin, starch or gelatin). However, consumer

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demand for products with low fat or sugar content and low levels of additives, as well as cost factors, make EPS a viable alternative (Jolly, Vincent, Duboc, & Neeser, 2002). Also, as it has no taste, EPS could be used to develop food products without hindering their original distinctive flavours (Duboc & Mollet, 2001). Therefore, EPS produced by starter cultures in fermented dairy products in situ are potential sources of food hydrocolloids which could be used to overcome the above shortcomings related to plant and animal hydrocolloids (Ayala-Hernández, Hassan, Goff, de Orduña, & Corredig, 2008; Saija et al., 2010). To this end, in situ EPS produced by strains of *Bifidobacterium* could be used as natural additives in fermented dairy products, which are favoured by some consumers over plant or animal hydrocolloids (Audy et al., 2010).

Most strains of *Bifidobacterium* grow slowly in milk due to their poor proteolytic activity, which hinders their possible application as starter culture in fermented milk products (Oliveira, Sodini, Remeuf, & Corrieu, 2001; Yonezawa et al., 2010). There are many reports that in situ production of EPS by lactic acid bacteria (LAB) can improve the rheological properties of dairy products, such as yoghurt and cheese (Canquil, Villarroel, Bravo, Rubilar, & Shene, 2007; Patel, Michaud, Singhania, Soccol, & Pandey, 2010; Vaningelgem, Zamfir, Adriany, & De Vuyst, 2004). On the other hand, there is a lack of literature concerning the application of EPS producing bifidobacteria in fermented milk production (Kohno et al., 2009; Salazar et al., 2009).

To circumvent the above, many ingredients have been evaluated to stimulate the growth and activity of bifidobacteria in milk, for example various sugars (glucose and galactose), protein sources (yeast extract, liver extract, peptones, and corn steep liquor) and different vitamins (Gomes, Malcata, & Klaver, 1998; Sodini, Lucas, Tissier, & Corrieu, 2005; Zhao, Wang, Zhao, Jiang, & Chun, 2006). However, most of these protein sources cannot be used in milk products due to their characteristic undesirable flavours (McComas & Gilliland, 2003). As a result, much interest has been focused in utilising milk-derived compounds as additives, such as whey protein concentrate, whey protein isolate and casein hydrolysate, studying mainly the effect of these compounds on the growth of *bifidobacteria* in milk either singly or as part of a mixed starter culture (Dave & Shah, 1998a; Janer, Peláez, & Requena, 2004; Marafon, Sumi, Alcântara, Tamime, & Nogueira de Oliveira, 2011; Marafon, Sumi, Granato et al., 2011; Oliveira, Perego, Oliveira, & Converti, 2011). Furthermore, temperature has been shown to effect the growth and metabolism of some strains of *Bifidobacterium* in milk (Baron, Roy, & Jean-Christophe, 2000; Garro, de Valdez, & de Giori, 2004; Ostlie, Treimo, & Narvhus, 2005).

In terms of EPS, there is much published literature describing how the growth medium composition (carbon source, amino acid composition, minerals and vitamins) (Ayala-Hernández et al., 2008; De Vuyst & Degeest, 1999; Grobben et al., 1998; Looijesteijn, Boels, Kleerebezem, & Hugenholtz, 1999; Macedo, Lacroix, Gardner, & Champagne, 2002) and the temperature (Gamar Nourani, Blondeau, & Simonet, 1998; Vaningelgem et al., 2004) can influence EPS production of LAB. However, there are no reports on the effect of different protein sources and temperatures on EPS production by *Bifidobacterium* strains in milk. Therefore, the aim of this study was to examine the effect of different dairy-based protein sources including casein hydrolysate (CH), lactalbumin hydrolysate (LH), whey protein concentrate (WPC) and whey protein isolate (WPI), and temperature on the EPS production by *Bifidobacterium longum* subsp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205 in skim milk. These two strains were selected as in our previous study *B. longum* subsp. *infantis* CCUG 52486 produced 138.0 mg L^{-1} of EPS (1.3×10^6 Da) whereas *B. infantis* NCIMB 702205 produced 77.9 mg L^{-1} of EPS (7.4×10^4 Da) when they were grown in skim milk supplemented with 0.5% yeast extract for 18 h at 37 °C (Prasanna et al., 2011).

2. Materials and methods

2.1. Bacterial strains

B. longum subsp. *infantis* CCUG 52486 and *B. infantis* NCIMB 702205 were obtained from the culture collection of the University of Göteborg in Sweden and the UK National Collection of Industrial, Food and Marine Bacteria (NCIMB), respectively. Stock cultures were maintained at −80 °C in trypticase phytone yeast extract (TPY) medium containing 15% (v/v) glycerol. Frozen stocks were initially propagated in Wilkins Chalgren anaerobe agar (WC) (Oxoid, Hampshire, UK) under anaerobic conditions at 37 °C for 72 h. Two successive growths of bacteria were carried out in 10 mL TPY broth under anaerobic condition for 18 h. The bacterial cells were harvested after the second growth cycle at 10,000×*g* for 15 min, at 4 °C. The pellet was washed with sterile phosphate buffered saline (PBS) (Oxoid, UK) and then resuspended in 10 mL of commercial pasteurised skim milk to prepare the pre-culture.

2.2. Preparation of fermented milks

Milk fermentation was carried out in glass bottles (250 mL) with screw caps. Commercial pasteurised skim milk (200 mL) was supplemented with various protein sources and was heat treated at 85 °C for 30 min. It was then cooled to 37 °C and inoculated with 1% (v/v) of the pre-culture. During incubation, samples were collected aseptically for enumeration of bacteria, pH measurement and EPS quantification.

2.3. Effect of different protein sources on cell growth and EPS production

Casein hydrolysate (Sigma-Aldrich, Dorset, England, UK), lactalbumin hydrolysate (Sigma-Aldrich), whey protein concentrate (Bulk powders, Essex, UK), whey protein isolate (Bulk powders) and yeast extract (YE) (as control protein source) (Oxoid, UK) were used to evaluate the influence of different protein sources on growth and EPS production of both *Bifidobacterium* strains. The concentration of protein sources was set at 1.5% (w/v). The milk fermentations were carried out as described in Section 2.2, at 37 °C, for 18 h.

2.4. Effect of concentration of casein hydrolysate on cell growth and EPS production

Casein hydrolysate was selected as the best protein source for both *Bifidobacterium* strains in terms of cell growth and EPS production (see Results and discussion section). Following this, the influence of its concentration (0.5%, 1%, 1.5%, 2% and 2.5%) on cell growth and EPS production was evaluated in milk fermentations, carried out as described in Section 2.2, at 37 °C for 18 h.

2.5. Effect of temperature on cell growth and EPS production

Having identified the optimum casein hydrolysate level to be at 1.5% for both *Bifidobacterium* strains, milk fermentations were carried out with 1.5% casein hydrolysate supplementation at 25, 30, 37 and 42 °C as described in Section 2.2, for 48 h. Samples were collected aseptically for enumeration of cells, to establish growth curves, and quantify the EPS.

2.6. Enumeration of bacteria

One millilitre of fermented milk sample was diluted with 9 mL of sterile PBS (Oxoid, UK) and mixed with a vortex mixer. Subsequently, serial dilutions were prepared and viable numbers were enumerated using the spread plate technique on WC agar (Oxoid, UK). The plates were incubated anaerobically at 37 °C for 72 h. Plates containing 25 to 250 colonies were enumerated and recorded as log colony forming