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Review

Bifidobacteria in milk products: An overview of physiological and biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits

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ABSTRACT

Research and commercial interest in the genus *Bifidobacterium* have increased in the last decade due to their potential health benefits in probiotic functional foods, especially in dairy products. However, cultivation of bifidobacteria in milk is a difficult task compared with that of conventional starters because milk is not a good medium for growth of these nutritionally fastidious microorganisms. Therefore, suitable strains of *Bifidobacterium* for dairy products should be selected based on their safety and technological and functional properties. There are a number of milk products containing bifidobacteria in the world market and the demand for new products is increasing with the awareness of the potential health benefits of the consumption of products blended with bifidobacteria. Some strains of *Bifidobacterium*, which produce exopolysaccharide, have been isolated and characterised. This review will discuss the general characteristics of bifidobacteria, exopolysaccharide production, the selection criteria of bacterial strains for milk products, current applications of bifidobacteria in milk products, and their nutritional and beneficial health properties.

Contents

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1. Introduction

Bifidobacteria are an important group of probiotic cultures commonly used in fermented dairy products. A *Bifidobacterium* strain was first isolated by Henry Tissier (of the Pasteur Institute) from the faeces of breast fed infants; he named the bacterium *Bacillus bifidus*. Tissier showed that bifidobacteria could displace the proteolytic bacteria causing diarrhoea and recommended the administration of bifidobacteria to infants suffering from this symptom (Guarner et al., 2008). Orla-Jensen (1924), a Danish microbiologist proposed that *B. bifidus* could be classified as a separate species under the genus name *Bifidobacterium*. However, it was classified under genus *Lactobacillus* for much of the 20th century, because of some similarities such as rod shape and obligate fermentative characteristics (Leahy, Higgins, Fitzgerald, & Sinderen, 2005). However, Stackebrandt, Rainey, and Ward-Rainey (1997) proposed a unique hierarchical structure combining the genus *Bifidobacterium* with the genus *Gardnerella* into the single family of *Bifidobacteriaceae* in the order of *Bifidobacteriales* using 16S rRNA analysis.

Bifidobacterium are Gram positive, anaerobic, non-motile and non-sporulating organisms (Alp & Aslim, 2010; Wei, Mengesha, Good, & Anné, 2008). They may have various shapes including short curved rods, club shaped rods, and bifurcated Y shaped rods (Shah, 2006a). Under unfavourable conditions bifidobacteria show branching and pleomorphism, although they are predominantly rod shaped in their natural habitat (Leahy et al., 2005). The branching nature of bifidobacteria depends not only on the strains but also on the medium used for cultivation (Tannock, 1999). In addition, it was reported that depending on the composition of the culture medium, some strains of the genus *Bifidobacterium* may have a V or X shape in addition to the characteristic Y shape (Ballongue, 2004). Furthermore, the abundance of N-acetylglucosamine, alanine, aspartic acid, glutamic acid, serine and Ca^{2+} ions in the growth medium can influence the cell shape of bifidobacteria (De Dea Lindnera et al., 2007).

Bifidobacteria contribute a major part in the human intestinal microbiota in healthy humans. They are considered to provide many beneficial effects including improvement of lactose digestibility, anticarcinogenic activity, reduction of serum cholesterol level, synthesis of B vitamins and facilitation in calcium absorption (Xu, Shang, & Li, 2011). Even though *Bifidobacterium* strains are already used in dairy products, they have some inferior behavioural characteristics compared to the traditional lactic acid bacteria (LAB) used in fermented dairy products, which hinder their possible applications (Prasanna, Grandison, & Charalampopoulos, 2012b). More specifically, they have weaker growth and acid production in cows' milk and require long fermentation times, anaerobic conditions, and low redox potential for their growth (Gomes & Malcata, 1999; Janer, Peláez, & Requena, 2004; Lourens & Viljoen, 2001; Prasanna, Grandison, & Charalampopoulos, 2012a). Therefore, it is very important to select suitable strains to be incorporated into probiotic dairy products. These include strains that grow quickly in milk, as this would decrease the cost of the process and the risk of contamination (Martinez-Villaluenga & Gomez, 2007), strains that could grow in combination with traditional starter cultures (Davidson, Duncan, Hackney, Eigel, & Boling, 2000), as well as strains that do not produce unpleasant flavours or textures (Saarela, Mogensen, Fonden, Mättö, & Mattila-Sandholm, 2000) but potentially produce compounds, such as aroma compounds and/or biopolymers, that improve the organoleptic properties of the product.

Many species of bacteria have been reported to produce exopolysaccharides (EPSs) which are either loosely attached to the cell surface or completely excreted to the environment as slime. The common EPS producing bacterial species used in food application, include streptococci (Qin et al., 2011; Säwén, Huttunen, Zhang, Yang, & Widmalm, 2010), lactobacilli (Rodríguez-Carvajal et al., 2008; Wang et al., 2010), lactococci (Ayala-Hernández, Hassan, Goff, de Orduña, & Corredig, 2008; Costa et al., 2010) and bifidobacteria (Prasanna et al., 2012b; Ruas-Madiedo et al., 2007). EPS produced by different bacteria has been reported to be useful in a wide range of applications including food products, pharmaceuticals, bioemulsifiers, bioflocculants and chemical products (Wang, Ahmed, Feng, Li, & Song, 2008). There is high demand for EPS from different species of bacteria as newly emerging industrially important biopolymers, due to their generally recognised as safe (GRAS) status, environmental friendly production and higher potential activity at low concentration compared to commercially available polymers (Gutiérrez, Leo, Walker, & Green, 2009; Kanmani et al., 2011). In addition, bacterial EPSs have been shown to be effective as immunomodulatory, immunostimulatory, antitumour, anti-inflammatory, and antioxidant agents (Liu et al., 2010). There are many published review papers regarding lactic acid bacteria, EPS production and their application in dairy products. However, so far there is no comprehensive review focusing on selection of bifidobacteria for milk products and EPS production of different *Bifidobacterium* strains. Therefore, the present review covers general characteristics of bifidobacteria, EPS production, the selection criteria of *Bifidobacterium* strains for milk products, current applications of bifidobacteria in milk products, and their nutritional and beneficial health properties upon human consumption.

2. General characteristics of bifidobacteria

To date, more than 30 species have been isolated and assigned to the genus *Bifidobacterium* (Leivers et al., 2011). This section covers ecology of bifidobacteria, their role in human gastrointestinal tract and some physiological and biochemical properties.

2.1. Ecology of bifidobacteria and their involvement in the human gastrointestinal tract

Bifidobacteria have been isolated and assigned from five different ecological niches: the intestine, the oral cavity, foods, insect gut, and sewage (Ventura et al., 2007). Species of *Bifidobacterium*