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Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health[1]

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ABSTRACT

The capacity of strains to produce exopolysaccharides (EPS) is widespread among species of lactic acid bacteria and bifidobacteria, although the physiological role of these molecules is not yet clearly understood. When EPS are produced during food fermentation, they confer technological benefits on the fermented end products, such as improved texture and stability. In addition, some of these EPS may have beneficial effects on consumer health. These uses of EPS necessitate optimal and sufficient production of these molecules, both in situ and ex situ, not only to improve their yields but also to obtain a particular functionality. The present study reviews the commonly used methods of production, isolation, and quantification that have been used in recent studies dealing with EPS-producing lactic acid bacteria and bifidobacteria.
Key words: exopolysaccharides, lactic acid bacteria, bifidobacteria, isolation

INTRODUCTION

The present review will primarily focus on a comprehensive overview of recent studies (mostly since 2013) on recovery and quantification of exopolysaccharides (**EPS**) produced by lactic acid bacteria (**LAB**) and bifidobacteria. The last dedicated review on this topic dates back to 2005 (Ruas-Madiedo and de los Reyes-Gavilán, 2005). For literature that also encompasses aspects of the classification, biosynthesis, physiological role, and applications of EPS, the reader is referred to the reviews by Cerning (1990, 1995), De Vuyst and Degeest (1999), De Vuyst et al. (2001), De Vuyst and Vaningelgem (2003), Font de Valdez et al. (2003), De Vuyst and de Vin (2007), and Chapot-Chartier et al. (2011). For information on the genetics of EPS biosynthesis, the reviews by Jolly and Stingele (2001), Broadbent et al. (2003), Lee and O'Sullivan (2010), and Hidalgo-Cantabrana et al. (2014) can be consulted.

Strains of species of LAB and bifidobacteria often produce EPS, sharing the same main monosaccharides (Cerning, 1995; De Vuyst and Degeest, 1999; De Vuyst et al., 2001). Heteropolysaccharides are usually composed of D-glucose, D-galactose, and L-rhamnose (Ruas-Madiedo and de los Reyes-Gavilán, 2005; De Vuyst and de Vin, 2007; Hidalgo-Cantabrana et al., 2014), although much structural diversity exists (Vaningelgem et al., 2004; Mozzi et al., 2006; De Vuyst et al., 2011); for instance, leading to mannose-rich variants (London et al., 2014). These heteropolysaccharides are usually produced in amounts ranging from 0.05 to 0.60 g/L (Ruas-Madiedo and de los Reyes-Gavilán, 2005). In contrast, homopolysaccharides are either of a glucan or fructan nature and are produced in higher amounts, up to several grams per liter (Grosu-Tudor et al., 2013; Rühmkorf et al., 2013; Mazzoli et al., 2014; Miao et al., 2014; Wolter et al., 2014; Malang et al., 2015). The physiological role of these EPS molecules, which are either loosely attached to the cells or excreted as slime into the environment, is not yet fully understood, but they probably act protectively to the producer cells or facilitate their attachment to surfaces and tissues (Prasanna et al., 2014; Mazzoli et al., 2014). With respect to bifidobacteria, it has been suggested that EPS offer protection against the harsh conditions of the gastrointestinal tract (Hidalgo-Cantabrana et al., 2014) and play a role in microbial interactions (Rios-Covian et al., 2013; Chen et al., 2014). In the case of LAB, EPS may also play a role in biofilm formation, which is of particular concern if the producing strain is a human pathogen, as shown for the cariogenic *Streptococcus mutans* (Klein et al., 2015).

Strains producing EPS have been isolated from a variety of sources, both milk-based products and nondairy

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Table 1. Overview of recent food and nonfood sources for the isolation of exopolysaccharide-producing lactic acid bacteria and bifidobacteria

Origin	Reference
Milk-based products	Ahmed et al. (2013), Behare et al. (2013), Grosu-Tudor et al. (2013), Zajšek et al. (2013), Lynch et al. (2014), Zamfir and Grosu-Tudor (2014), Chen et al. (2015), Wang et al. (2015)
Soymilk-based kefir	Botelho et al. (2014)
Cereal products	Zannini et al. (2013), Lynch et al. (2014)
Wine	Dimopoulou et al. (2014)
Ropy cider	Notararigo et al. (2013)
Rice beer	Joshi and Koijam (2014)
Pickled vegetables	Park et al. (2013), Lai et al. (2014)
Fermented fish	Abdhul et al. (2014)
Animal or human feces and gut contents	Górska-Frączek et al. (2013), Shang et al. (2013), Lynch et al. (2014), London et al. (2014), Shao et al. (2014)

foods (Table 1). Frequently, EPS are obtained from nonfood sources, in particular animal or human feces and gut contents. When EPS are produced during food fermentation, they can confer technological benefits to the fermented end products, such as improved texture and stability (De Vuyst and de Vin, 2007; Ravyts et al., 2012; Prasanna et al., 2014). As such, EPS-producing LAB and bifidobacteria can be used to improve the rheology of dairy products, enhancing mouthfeel and reducing syneresis (De Vuyst and de Vin, 2007; Hahn et al., 2014; Lluis-Arroyo et al., 2014; Lynch et al., 2014), with particular applications in low-fat yogurts (Ravyts et al., 2011; Prasanna et al., 2013; London et al., 2015) and other fermented milks (Behare et al., 2013; Yilmaz et al., 2015), as well as low-fat cheeses (Şanli et al., 2013; Di Cagno et al., 2014; Oluk et al., 2014). They may also be of use to improve the texture of nondairy food matrices, as shown for starchy foods and beverages (Tamani et al., 2013; Zannini et al., 2013; Ismail and Nampoothiri, 2014; Wolter et al., 2014; Kajala et al., 2015), fermented vegetables (Juvonen et al., 2015), and fermented soymilk (Li et al., 2014a). Functionality and contribution to the microstructure network of the food may nevertheless depend on the specific structure of the EPS molecules involved (Gentès et al., 2013), the presence of milk solids (Ravyts et al., 2011), or the difference between capsular and free EPS (Mende et al., 2013). Therefore, the introduction of an EPS-producing starter culture is not always successful (Şanli et al., 2014). In addition to their textural benefits, some EPS exert positive effects on consumer health, due to their potential immunomodulatory, antitumor, antiviral, anti-inflammatory, or antioxidant capacities (Prasanna et al., 2014). During the last few years, such aspects have become a hot research topic and have been investigated in several EPS-producing strains of LAB and bifidobacteria (Ciszek-Lenda et al., 2013; Górska-Frączek et al., 2013; Guo et al., 2013; Marcial et al., 2013; Zhang et al., 2013; Abdhul et al., 2014; Hidalgo-Cantabrana et al., 2014; Lai et al., 2014; Li et al., 2013, 2014b; Shao et al., 2014; Wang et al., 2014, 2015; Chen et al., 2015). Both the textural and potential health advantages of EPS are important elements in view of the development of functional starter cultures for the food fermentation industry in general and the dairy industry in particular (Leroy and De Vuyst, 2004; De Vuyst and de Vin, 2007; Ravyts et al., 2012).

Technological use of EPS requires optimal and sufficient production of these molecules, both in situ and ex situ, not only to improve their yields but also to obtain a particular functionality. Moreover, metabolic engineering strategies often have modest or negligible effects on EPS yields, due to inherent limitations, especially in the case of heteropolysaccharides (Mazzoli et al., 2014). Therefore, process engineering remains particularly useful, and appropriate methods of production and recovery need to be established and hence require increased attention (Degeest et al., 2001; Ruas-Madiedo and de los Reyes-Gavilán, 2005). One particular difficulty relates to the development of starter cultures producing sufficiently high concentrations of EPS during food processing. Moreover, knowledge of EPS production conditions and cellular regulation mechanisms is also important for the production of starter cultures as such and their processing into ready-to-use formulations, especially when they produce high concentrations of EPS. All this requires a better understanding of EPS production and regulation mechanisms (Rühmkorf et al., 2013; Suzuki et al., 2013). Therefore, the present review will illustrate current practice and simple state-of-the-art procedures for the production, isolation, purification, and quantification of EPS produced by LAB and bifidobacteria (Figure 1).

PRODUCTION OF EPS

Successful EPS recovery relies primarily on the use of a suitable culture medium for ex situ production, not only allowing high production yields but also lacking interference of medium components with detection