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Research review paper

Recombinant microbial systems for improved β-galactosidase production and biotechnological applications

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ABSTRACT

β-Galactosidases (EC 3.2.1.23) constitute a large family of proteins that are known to catalyze both hydrolytic and transgalactosylation reactions. The hydrolytic activity has been applied in the food industry for decades for reducing the lactose content in milk, while the transgalactosylation activity has been used to synthesize galacto-oligosaccharides and galactose containing chemicals in recent years. The main focus of this review is on the expression and production of *Aspergillus niger*, *Kluyveromyces lactis* and bacterial β-galactosidases in different microbial hosts. Furthermore, emphasis is given on the reported applications of the recombinant enzymes. Current developments on novel β-galactosidases, derived from newly identified microbial sources or by protein engineering means, together with the use of efficient recombinant microbial production systems are converting this enzyme into a relevant synthetic tool. Thermostable β-galactosidases (cold-adapted or thermophilic) in addition to the growing market for functional foods will likely redouble its industrial interest.

Contents

1. Introduction

1.1. The β-galactosidase enzyme

β-galactosidase (commonly also known as lactase) is an enzyme (EC 3.2.1.23) that catalyzes the hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides (http://expasy.org/enzyme/3.2.1.23; Gasteiger et al., 2003). Conventionally, its main application has been in the hydrolysis of lactose in milk or derived products, particularly cheese whey. More recently, β-galactosidases with transgalactosylation activities (i.e. which can oligomerise galactosides) have been extensively exploited for the production of functional galactosylated products (Fig. 1).

Many organisms naturally synthesize β-galactosidase, including microorganisms, plant and animal cells (Husain, 2010; Panesar et al., 2006). Traditionally, the β-galactosidases most widely used in industry

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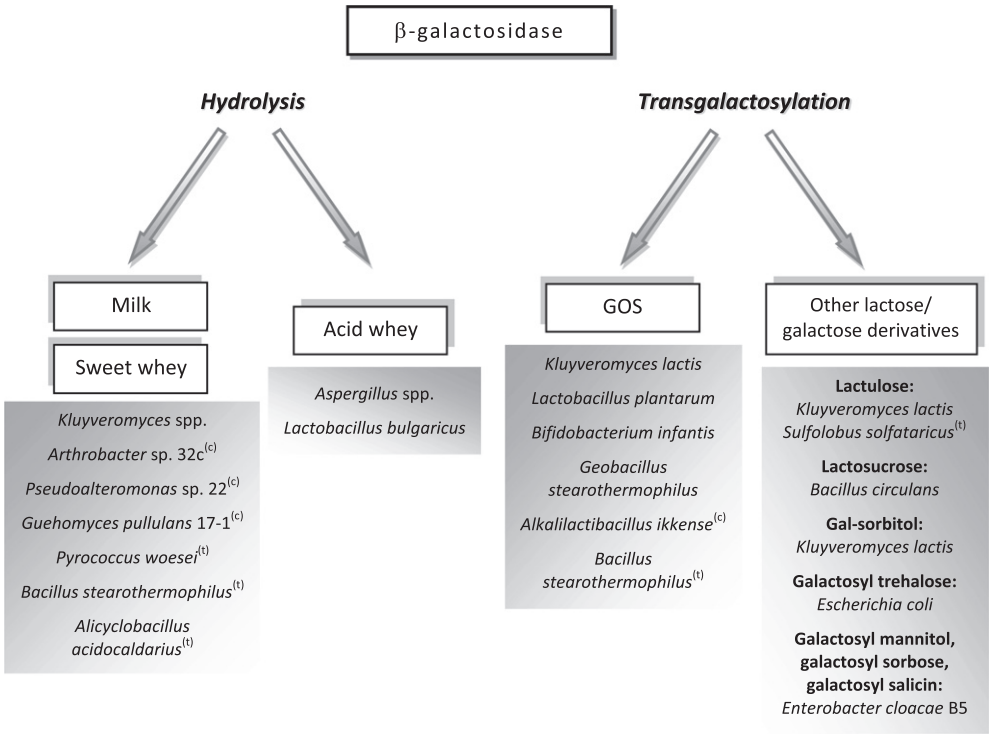

Fig. 1. Biotechnological applications of β-galactosidases: hydrolysis of lactose in milk and cheese whey and synthesis of GOS or other lactose/galactose derivatives by transgalactosylation reactions. The gray boxes show examples of microbial enzyme sources for each application. (c)Cold-active enzymes; (t)thermophilic enzymes.

were obtained from *Aspergillus* spp. and *Kluyveromyces* spp. (Husain, 2010; Panesar et al., 2006; Siso, 1996; Zadow, 1984), because these could be readily obtained with acceptable productivities and yields from cultivations of these microorganisms. Additionally, products obtained from these organisms are generally recognized as safe (GRAS status) for human consumption, which is critical for food related applications (Kosseva et al., 2009; Panesar et al., 2006; Siso, 1996). In *Aspergillus* spp. β-galactosidase is secreted to the extracellular medium. These fungal enzymes have a pH optimum in the acidic range (2.5–5.4) and a high temperature optimum that allows their use at temperatures up to 50 °C (Panesar et al., 2006; Zadow, 1984). Their main application is in the hydrolysis of acid whey, which derives from the production of fresh or soft cheeses (Yang and Silva, 1995). Conversely, in *Kluyveromyces* spp. the β-galactosidase is intracellular; lactose is first transported to the interior of the yeast cell by a permease and then hydrolyzed intracellularly to glucose and galactose, which follow the glycolytic pathway or the Leloir pathway, respectively (Domingues et al., 2010). The yeast enzyme has a near neutral optimum pH (6.0–7.0) and therefore has a broader range of applications, particularly in the hydrolysis of milk and sweet whey (derived from hard cheese manufacturing) (Panesar et al., 2006; Yang and Silva, 1995; Zadow, 1984). Because of its intracellular nature, the enzyme needs to be extracted from the yeast cells by disrupting or permeabilizing the cells using chemical and/or mechanical treatments (Panesar et al., 2006).

Panesar et al. (2006) compiled a list of β-galactosidase commercial preparations. Yeast sources are *Kluyveromyces lactis* and *Kluyveromyces marxianus* (species that now includes former species *Kluyveromyces fragilis* and *Saccharomyces fragilis* as well as its anamorph *Candida pseudotropicalis*; Lachance, 1998), while fungal sources include *Aspergillus niger* and *Aspergillus oryzae*. The β-galactosidase from *Escherichia coli* is the most extensively studied but its industrial use is hampered by the fact that it is not considered safe for food applications. Nevertheless, it is commercially available for analytical purposes (Panesar et al., 2006; Siso, 1996). Finally, a preparation obtained from *Bacillus* sp. is also commercialized (Panesar et al., 2006).

Lactic acid bacteria (include a diverse group of lactococci, streptococci and lactobacilli) and bifidobacterium, which are recognized as safe organisms, have been regarded as good sources of β-galactosidases, especially for functional food applications (Husain, 2010). More recently, there has been growing interest in a considerable number of β-galactosidases from other sources that present diverse properties of biotechnological interest (Panesar et al., 2006). Particular attention has been rewarded to thermotolerant or cold-active enzymes from yeast and bacterial sources (Husain, 2010; Panesar et al., 2006; Park and Oh, 2010b). The X-ray crystal structures of several microbial β-galactosidases have been unraveled, although none of the enzymes with solved structures is known to be used in food processing (Gosling et al., 2010).

Nowadays, recombinant DNA technology can be used to express and optimize the production of interesting β-galactosidases from the most diverse sources in microbial hosts that are recognized for their highly efficient heterologous protein production. This possibility greatly expands the range of potential applications for β-galactosidases and their economically effective utilization in industrial processes. Modern molecular biology tools combined with bioprocess engineering strategies can be used to optimize protein production, resulting in technically and economically effective enzyme production systems. Besides the wide-ranging properties offered by natural sources, new features – such as reduced product inhibition (Park and Oh, 2010b), higher product yields (Gosling et al., 2010) or secretion signals (Becerra et al., 2001b) – may be built into specific β-galactosidases using state-of-the-art protein engineering tools.

1.2. Applications of β-galactosidase

Lactose is a disaccharide formed by glucose and galactose that is found in milk. In humans, lactose intolerance or lactose malabsorption is a common problem. In fact, it is estimated that over 70% of the world's adult population have problems in digesting lactose (Adam et al., 2004; Husain, 2010; Lifran et al., 2000), resulting from absent or reduced