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Review

Recombinant β-galactosidases – Past, present and future: A mini review

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ABSTRACT

The present review deals with current developments of novel β-galactosidases derived from recombinant vectors and by protein engineering approaches together with the use of efficient recombinant microbial production systems in order to present the applications of recombinant enzymes as a relevant synthetic tool in biotechnology. The union of specific physical and chemical properties of recombinant proteins with specific recognition of catalytic properties of biomolecules has led to their appearance in myriad novel biotechnological applications. The interest in exploiting recombinant enzymes as biocatalysts is constantly increasing nowadays. The plausible advantages involved with their use include their (1) rigidity and permeability, (2) hydrophobic/hydrophilic character, (3) ease of purification and large-scale production, (4) immediate separation from the reaction mixture after completion of reaction without using any chemicals or heating, (5) regenerability as they impart stability to enzymes by protecting their active sites from deactivation, and (6) recombinant enzymes can be conveniently tailored within utility limits. β-Galactosidase is one of the relatively few enzymes that have been used in large-scale processes to perform lactose hydrolysis and galacto-oligosaccharide production. Thus, the present article gives brief outline of recombinant β-galactosidases obtained from various mesophilic, psychrophilic and thermophilic sources and their potential applications in biotechnology industry.

Contents

1. Introduction

β-Galactosidases (E.C. 3.2.1.23) catalyzes the hydrolysis of lactose to glucose and galactose and transfers the galactose formed from lactose cleavage onto the galactose moiety of another lactose to yield galacto-oligosaccharides (GOS) which are utilized as growth promoting substrates of bifidobacteria in human intestine [1–3]. The parameters and price of β-galactosidases are major attributes which determine the technology and relative costs of lactose hydrolysis and galacto-oligosaccharide production as ascertained by its direct addition to the substrate which is economically unacceptable due to low value of whey as its waste product [4,5]. The enzymatic properties of recombinant β-galactosidases suggest that they bring efficient conversion of lactose in dairy products via their structural modification. Studies are therefore in progress on the large-scale application of recombinant β-galactosidases for degradation of lactose [6]. The drive for cost-cutting efficiencies in the heating/cooling steps of biotechnological processes and for increase in the recovery of the products of enzymatic reactions has led to an increased interest in the use of β-galactosidases isolated from psychrophilic and thermophilic microorganisms [7,8]. Compared to animal and plant sources, β-galactosidases from microorganisms are produced in

Abbreviations: *E. coli*, *Escherichia coli*; GOS, galacto-oligosaccharides; ONPG, o-nitrophenylβ-D-galactopyranoside; ORFs, open reading frames.

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higher yields and thus are more technologically important. The major enzymes of commercial interest are isolated mainly from the yeast *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus*, *Candida kefyr*, *Saccharomyces cerevisae* and the fungi *Aspergillus niger*, *Aspergillus oryzae* [9,10]. Considerable knowledge of active sites of such enzymes will enable methods to be chosen that would avoid reaction with their essential groups protected. Thus, this technology protects enzyme inhibition by retaining their tertiary structure and provides enhanced stability to them against physical and chemical denaturants for greater production of lactose and GOS. Moreover, other recombinant enzymes that have been exploited in the recent past for other biotechnological potential include proteinases, lipases, amylases and cellulases (detergent formulations), dehydrogenases (environmental biosensors), peroxidases (bioremediation), and methylases and aminotransferases (biotransformations).

2. Thermostable β-galactosidases

Enzymes from thermophilic microorganisms are particularly attractive for their thermostability and resistance to organic solvents thereby giving favorable equilibrium for trans-glycosylation reactions [11,12]. High temperature resulted in increased initial productivity of enzyme and higher solubility of substrates in aqueous phase. The reduced water activity resulting from high substrate concentration and added organic solvent increases the trans-glycosylation reaction. Thermostable enzymes are thus gaining considerable interest in industries since they give better yields at higher temperatures (Table 1).

A number of thermostable β-galactosidases have been isolated previously from both mesophilic eubacteria and archaeabacteria. They were characterized and employed to hydrolyze lactose and in producing GOS [13–17]. However, most thermostable enzymes are synthesized at very low levels by thermophilic bacteria or archaeabacteria and are therefore cumbersome to purify. Thus, their large scale production was achieved at industrial scale by producing them in mesophilic hosts using recombinant techniques [18,19]. The efficiency of recombinant thermostable β-galactosidases obtained from *Thermus* sp., *Pyrococcus furiosus*, *Thermotoga maritima*, *Sulfolobus solfataricus* and *Geobacillus stearothermophilus* had been reported in pursuit of GOS production at high temperatures. They exhibited several advantages over native enzymes including their ease of purification, large-scale production and improvements in their activity [16,20–23].

LacZ gene from *Thermotoga maritima* β-galactosidase was cloned on 11 kb fragment by complementation of an *Escherichia coli* LacZ deletion strain. The nucleotide sequence of structural gene and two other open reading frames (ORFs) found within a 6317-bp region were investigated. The deduced amino acid sequence of *T. maritima* β-galactosidase exhibited a 1037 amino acid polypeptide with a calculated M_r of 122312. The translated sequence was 30% similar to nine other β-galactosidase sequences from bacteria and yeast. Alignment of *T. maritima* β-galactosidase with other sequences revealed that the residues responsible for Mg^{2+} binding, catalysis and substrate recognition are conserved in thermophilic enzymes. Sequence analysis also exhibited the presence of a divergently transcribed operon containing two other genes 5′ to LacZ. These ORFs encode proteins homologous to a second family of β-galactosidase found in *Bacillus* sp. and to an ATP-dependent family of bacterial oligopeptide transport proteins [24]. BgaA gene for *T. maritima* β-galactosidase was also cloned and characterized by Gabelsberger et al. [25]. However, *T. maritima LacZ* gene (TM1193) was considered as a putative gene since it was not cloned correctly and its product was not characterized due to the difficulty of its expression in an active form in *E. coli* [26]. Another thermostable β-galactosidase gene, BgaA, from *Thermus* sp. was expressed in *E. coli* as a fusion protein of BgaA with a histidine tag [27,28]. A β-galactosidase isoenzyme, β-GalI, from *Bifidobacterium infantis* HL96, was expressed in *E. coli*. It exhibited strong transgalactosylation activity. The optimum temperature and pH for ONPG and lactose were 60 °C, pH 7.5 and 50 °C, pH 7.5, respectively. The enzyme showed inhibition in presence of *p*-chloromercuribenzoic acid, divalent metal cations, Cr^{3+}, EDTA and urea. K_m and V_{max} values with ONPG and lactose as substrate were 2.6 mM, 262 U/mg and 73.8 mM, 1.28 U/mg, respectively. The rate of GOS production from 20% and 30% lactose solution was 120 mg/mL and 190 mg/mL, respectively [29].

A thermostable β-galactosidase gene bgaB from bacterium, *Bacillus stearothermophilus* was cloned and expressed in *Bacillus subtilis* WB600. It exhibited pH and temperature optimum at pH 7.0 and 70 °C, respectively, and K_m and V_{max} values of 2.96 mM and 6.62 μM/min/mg, respectively. Half-life for this thermostable enzyme was 50 h and 9 h at 65 °C and 70 °C, respectively. Findings of the work suggested that this enzyme was suitable for both hydrolysis of lactose as well as in the production of GOS in milk processing [30]. Several recombinant proteins were successfully expressed using δ-integrative systems. Oliveira et al. [31] constructed stable flocculent *Saccharomyces cerevisiae* strains producing *Aspergillus niger* β-galactosidase which was later on employed in a continuous bioreactor for lactose hydrolysis. Moreover, Slepak et al. [32] had shown a mechanism in which production of yeast β-galactosidase was used to measure estrogenic activity of some chemical compounds. They showed that yeast estrogen assay makes use of recombinant yeast cells that have estrogen receptor expression cassette and a reporter construct coding for β-galactosidase. The induction mechanism starts with the binding of estrogenic compounds to the estrogen receptor and this complex activates β-galactosidase production. Moreover, β-galactosidase was expressed in *Pichia pastoris* in a defined medium containing metals where magnesium and zinc ions were required to support their production. The product yield of this recombinant β-galactosidase was significantly influenced by the concentration of trace metals [33].

Di Lauro et al. [34] reported the purification and characterization of bacterial β-galactosidase from thermoacidophilic bacterium *Alicyclobacillus acidocaldarius*. The cloning, expression and the characterization of this recombinant enzyme (Aaβ-gal) were investigated and found to be optically active and stable at 65 °C. Similarly, Nguyen et al. [35,36] showed the cloning of β-galactosidase from *Lactobacillus reuteri* L103 and its expression in *E. coli*. These studies also revealed a significant improvement in lactose hydrolysis which proved useful in dairy industries. Moreover, *Pyrococcus woesei* gene

Table 1
Efficiency of GOS production by recombinant β-galactosidases.

Source of recombinant β-galactosidase	Temperature (°C)	pH	% GOS (g/L)	Productivity (g/L/h)	Reference
Thermus sp.	70	7.0	30 (91)	–	Akiyama et al. [16]
Pyrococcus furiosus	80	5.0	22 (60)	–	Bruins et al. [68]
Thermotoga maritima	80	6.0	19 (97)	18	Ji et al. [21]
Bifidobacterium infantis	60	7.5	63 (190)	13	Jung and Lee [67]
Sulfolobus solfataricus	80	6.0	53 (315)	5.6	Park et al. [22]
Geobacillus stearothermophilus R109W	37	6.5	23 (41)	6.9	Placier et al. [23]