Pratyoosh Shukla *Editor*

Recent Advances in Applied Microbiology

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Foreword

This important new book describes key recent advances in applied microbiology. Microbes provide abundant benefits to mankind. Our knowledge of microbial applications must continue to advance through research such as that described in this book in order for us to expand our use of microbes for the benefit of the world's growing population. Many microbial processes are sustainable and have the potential to reduce carbon emissions compared with existing chemical processes. The rapid advances in genomics and the increased use of engineering principles, computer science, and bioinformatics in applied microbiology make this a particularly exciting time for the field.

The book comprises 14 chapters that are divided into 4 sections. "Microbial Biotechnology" includes chapters discussing microbial enzymes and useful products such as polyhydroxyalkanoates that can be used in the production of biodegradable plastics and microbial surfactants with application in bioprocessing. "Microbes in Health" tackles the topics of multidrug-resistant bacteria, probiotics that can be used to improve human health, and processes of microbial pathogenesis. The section "Microbial Interactions" comprises chapters discussing the application of microbes in improving plant growth and crop yields, microbes that can tolerate metal contamination and may have application in bioremediation, plant pathogen interactions, and the use of bacteria in the transformation of isoflavones. The final section "Computational Approaches in Microbiology" includes chapters highlighting advanced studies on proteins, enzymes, and peptides.

Dr. Pratyoosh Shukla, the editor of this volume, is to be congratulated on doing a fine job of bringing together a diverse range of topics to effectively highlight some of the important contributions in applied microbiology that will lead to new microbial biotechnology ventures. The practical discoveries described in this book help to provide new products and solutions to some of the challenges facing us in the twenty-first century.

Director and Professor
Institute of Marine and Environmental Technology
University of Maryland Center for Environmental Science
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Russell T. Hill

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About the Editor

Prof. Pratyoosh Shukla, PhD, is a professor and the head of the Department of Microbiology at Maharshi Dayanand University, Rohtak, India. His primary research interests are in enzyme technology, microbial biotechnology, and protein bioinformatics. He completed his PhD in the field of microbiology and fungal biotechnology at APS University, Rewa, India. Following his PhD, he pursued his postdoctoral studies at the Department of Biotechnology and Food Technology, Durban University of Technology, South Africa. He has 15 years of research and 17 years of teaching experience. He has produced 73 scientific publications and has authored or edited 6 books. He also has filed a patent on novel β-1, 4-endoxylanase from *Thermomyces lanuginosus* SS-8 and the mode of action thereof.

He is a life member of a number of academic bodies, including the Indian Science Congress Association (ISCA), India Society for Technical Education (ISTE), Mycological Society of India (MSI), Asian Federation of Biotechnology (AFOB), American Society for Microbiology (ASM), European Federation of Biotechnology (EFB), etc. He also holds the roles of associate editor, *BMC Microbiology*; editor, *Indian Journal of Microbiology* (Springer); editor in chief, *Journal of Microbiology*, Internet Scientific Publications, USA (2007–2009); reviewer and member of the editorial board for the *Journal of Applied Sciences in Environmental Sanitation*, ITS, Indonesia; etc. He is currently the general secretary of the Association of Microbiologists of India (AMI) (since 2014). He has also been presented with a number of academic awards, such as the ASM-IUSSTF Indo-US Professorship Award in Microbiology by the American Society for Microbiology (2014); AMI Alembic Award in industrial microbiology; and the Fast Track Young Scientist by DST, Govt. of India (2012). He was also selected as a scientist/project investigator and participated in the Southern Ocean Antarctica Expedition (Ministry of Earth Sciences, Govt. of India) (January to March, 2011).

Part I

Microbial Biotechnology

1 Immobilization of *A. oryzae* β-galactosidase on Silica Nanoparticles: Development of an Effective Biosensor for Determination of Lactose in Milk Whey

Anchal Goel, Rajeshwari Sinha, and Sunil K. Khare

Abstract

The present study demonstrates the covalent immobilization of β-galactosidase on functionalized silica nanoparticles for its application in lactose and whey hydrolysis. Under optimal conditions of 1% (w/v) glutaraldehyde, protein to carrier ratio of 66.6 mg/g and pH 7.0, a very high immobilization efficiency of 94% was obtained. The pH and temperature optimum of the immobilized β-gal was 4.5 and 50 °C with ONPG as substrate. Compared to the soluble enzyme, covalently bonded nanosilica-β-gal conjugate exhibited greater stability against inhibition by galactose and a higher thermal stability at 40 °C with a $t_{1/2}$ of 15.8 h. A lower K_m and increased catalytic efficiency indicated higher substrate affinity and reactivity upon enzyme attachment to nanoparticle surface. Reusability of the immobilized preparation extended up to 14 cycles. The immobilized preparation effectively hydrolyzed whey and lactose to soluble simple sugars with 50% of hydrolysis occurring in 6 h. The rate of lactose and whey hydrolysis by immobilized β-gal was 1.5 and 2.5 times higher than that for the free enzyme, respectively. Immobilized β-gal preparation may be advantageously and commercially explored for effective bioremediation of dairy waste, devising biosensors or analytical tools for food and environmental technology or conversion of whey into value-added products.

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β-galactosidase • Immobilization • Silica nanoparticles • Lactose • Whey hydrolysis

1.1 Introduction

The worldwide production of milk whey, a by-product of the dairy industry, is estimated to be about 180–190 × 10^6 ton/year (Baldasso et al. 2011). Whey primarily comprises of 94–95% water, 5–6% dry matter, 3.8–4.3% lactose, 0.8–1.0% total protein, 0.6–0.65% whey protein, and 0.5–0.7% minerals (Tsakali et al. 2010). Release of this whey into the environment leads to deterioration of soil structure thus impacting crop yields detrimentally and also depletes the dissolved oxygen from water bodies thereby disrupting the aquatic life (Shukla and Wierzbicki 1975; Becerra and Gonzalez Siso 1996). Given the serious environmental threat associated with the disposal of whey, generated as dairy waste, it becomes essential to devise newer methods of utilizing or pretreating the whey before being disposed.

An important approach which could be employed for treatment of whey involves the enzymatic hydrolysis of lactose present in whey. β-galactosidase (β-gal) is one such enzyme, widely used in food technology as enzyme supplements for people suffering from lactose intolerance (Heyman 2006). Treatment of lactose in milk or milk-based products with β-gal has also been used in addressing problems of insolubility and low sweetening ability of lactose (Husain 2010; Oliveira et al. 2011), generation of sweet syrups for manufacture of soft drinks and pastries (Mustafa et al. 2014), and transglycosylation of lactose to galactooligosaccharides (GOS) (Colinas et al. 2014; Maischberger et al. 2008; Rosenberg 2006).

Immobilized β-gal preparations present an efficient and commonly employed approach for hydrolysis of lactose. Their importance stems from the improved stability, higher activity, and reusability, resistance to catalyst poisoning, reduced microbial contamination, easy recovery, and separation properties offered by such immobilized preparations. So far, β-gal has been immobilized on a wide range of supports like chitosan, cotton cloth, epoxy support, cellulose beads, cross-linked enzyme aggregates, as well as glutaraldehyde-agarose (Klein et al. 2013; Albayrak and Yang 2002; Marín-Navarro et al. 2014; Roy and Gupta 2003; Klein et al. 2012; Gaur et al. 2006; Li et al. 2015; Cardelle-Cobas et al. 2016). Nanomaterials, owing to their high surface to volume ratio, provide immensely attractive surfaces for enzyme immobilization and development of robust nano-biocatalytic preparations with myriad of applications (Ansari and Husain 2012). β-galactosidase, primarily sourced from *Aspergillus oryzae* and *Kluyveromyces lactis*, has been immobilized on a wide range of nanoparticles (NPs) including Fe_3O_4-chitosan, silver, ZnO, chitosan-hydroxyapatite, polystyrene nanofibers, concanavalin A layered Al_2O_3, and silica (Pan et al. 2009; Ansari et al. 2012; Husain et al. 2011; Cabuk et al. 2014; Ansari and Husain 2011; Verma et al. 2012; Misson et al. 2016).

An important strategy for improving aqueous dispersibility and preventing NP aggregation is their surface modification through functionalization (Subbiah et al. 2010). Additionally, functionalization also provides scope for high enzyme loading capacity, uniform distribution of enzyme on the NP surface, and stronger enzyme links with the NPs. Silica NPs, known for their thermal, mechanical, and chemical stability, low toxicity, biocompatibility, and resistance to microbiological attacks, provide sufficient functional groups for surface modifications that allow for control of surface chemistry and efficient enzyme attachment (Hartmann and Kostrov 2013). The use of functionalized silica nanoparticles as a viable scaffold for β-gal immobilization is being recently explored (Verma et al. 2012; Singh et al. 2011).

In consideration of the tremendous relevance of β-gal in the food industry, the development of an active, stable, reusable biocatalyst that cost-effectively addresses the treatment of whey before disposal is relevant. The present study describes the development of an efficient nano-biocatalytic biosensor system using functionalized silica nanoparticles as immobilization support for β-galactosidase, for application in hydrolysis of lactose present in whey. With improved enzymatic properties, the immobilized preparation demonstrates immense potential to facilitate effective bioremediation of dairy waste water, aid in devising biosensors and analytical tools for environmental and food technology, and also enable generation of value-added products from glucose-galactose syrup obtained upon hydrolysis of lactose present in dairy waste.

1.2 Results

1.2.1 β-galactosidase Assay and Protein Estimation

The activity of free and immobilized β-galactosidase toward ONPG (*O*-Nitrophenyl-β-D-galactosidase) was determined following the method of Craven et al. (1965) with slight modifications. One unit of β-gal activity is defined as number of micromoles of o-nitrophenol released by hydrolysis of substrate per minute per ml of enzyme.

Protein concentration was determined by Bradford assay using bovine serum albumin as standard (Bradford 1976).

1.2.2 Activation of Silica Nanoparticles

A covalent coupling-based method was used to immobilize the enzyme on functionalized silica nanoparticles (Zhang et al. 2011). Five hundred microliters (15 mg) of commercial silica nanoparticles were washed thoroughly in sodium phosphate buffer (0.2 M, pH 7.0), suspended in 1.0 ml of the same buffer, and incubated by addition of 1% (v/v) glutaraldehyde for 2.5 h at 30 °C. The activated preparation was then centrifuged, washed at least five times with sodium phosphate buffer (0.2 M,