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Characterization and preparation of highly stable aggregates of a novel type of hydrolase (BL28) from *Bacillus licheniformis*

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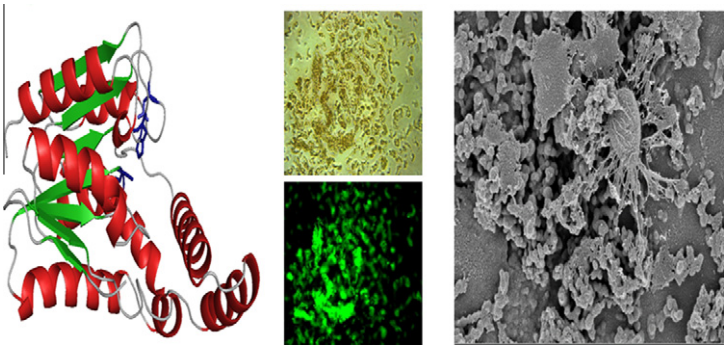
HIGHLIGHTS

- Novel type of hydrolase (BL28) from *Bacillus licheniformis* was characterized.
- Highly selective and specific properties of BL28 were investigated.
- CLEAs of BL28 exhibited improved stabilities and high reusabilities.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel type of hydrolase (BL28) from *Bacillus licheniformis* was identified, expressed in *Escherichia coli*, characterized, and immobilized for industrial applications. Biochemical characteristics of BL28 were investigated by performing SDS–PAGE, mass spectrometry, enzyme assays, CD spectroscopy, intrinsic fluorescence, and *in silico* analysis. Furthermore, cross-linked enzyme aggregates (CLEAs) of BL28 were prepared. These CLEA-BL28 aggregates exhibited improved catalytic efficiencies and stabilities compared to free BL28 against harsh conditions of thermal or chemical stress as well as high reusability. The characteristics of the CLEA-BL28 aggregates highlight their great potentials in pharmaceutical and chemical industries.

1. Introduction

Hydrolases, which are widely recognized in all three domains of life, are one of the most important enzymes for a variety of biotechnological applications. They display high stability and good enantioselectivity for a broad range of substrates without any cofactors, even in the presence of organic solvents or under nonpolar conditions (Yoo et al., 2011; Tan et al., 2010; Schmidt et al., 2009). To date, hydrolase have been classified into eight families based on sequence homologies and biological properties (Arpigny and Jaeger, 1999). These enzymes, though low sequence similarities have been identified, share a characteristic α/β hydrolase fold (β-strands surrounded by α-helix) with a highly conserved catalytic triad. A typical catalytic triad is composed of a nucleophilic serine in conjunction with a histidine and an acidic residue (aspartic- or glutamic acid). The pentapeptide consensus sequence G-X-S-X-G is usually found around the active site serine (Carr and Ollis, 2009; Qian et al., 2007). With increasing demands for hydrolases in specific biotechnological applications, novel enzymes of microbial origin have been continuously identified, e.g., EstGtA2 from *Geobacillus thermodenitrificans* (Charbonneau et al., 2010), EstD from *Thermotoga maritima* (Levisson et al., 2007), Est30 from *Geobacillus stearothermophilus* (Liu et al., 2003), and LipG from a metagenomic library (Lee et al., 2006). In accordance, using microbial genome sequences, we applied bioinformatic tools for the discovery of novel

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