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Multiple amino acid substitutions significantly improve the thermostability of feruloyl esterase A from *Aspergillus niger*

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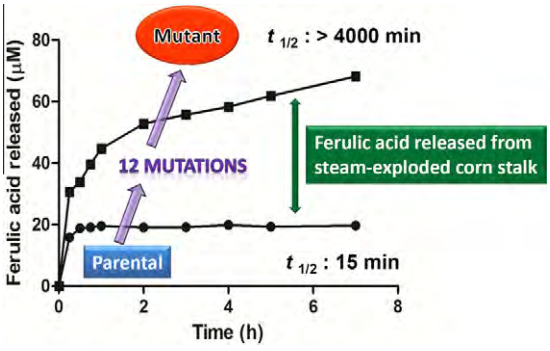
HIGHLIGHTS

- Half-life of thermal inactivation of *An*FaeA increases from 15 to >4000 min.
- Twelve beneficial mutations lead to increased thermostability.
- Release of ferulic acid from corn stalk increases to >300% using mutant *An*FaeA.

GRAPHICAL ABSTRACT



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ABSTRACT

Feruloyl esterase A from *Aspergillus niger* (*An*FaeA) is one of the most important feruloyl esterases of industrial relevance. Previous work aided by the PoPMuSiC algorithm has identified two beneficial mutants (D93G and S187F) with thermostabilization effect. In this work, twelve additional amino acid substitutions were identified to be beneficial to the thermostability of *An*FaeA after screening a random mutagenesis library constructed in *Pichia pastoris*. Combination of these mutations resulted in a mutant with 80% residual activity after heat treatment at 90 °C for 15 min and a half-life increasing from 15 min to >4000 min at 55 °C. The thermostabilized mutant displayed significantly enhanced performance compared to the parental *An*FaeA when applied to the treatment of steam-exploded corn stalk at 60 °C together with an xylanase, demonstrating its great potential for industrial application.

1. Introduction

Feruloyl esterases (FAEs) (EC 3.1.1.73) are critical enzymes involved in the complete degradation of lignocellulose, which cleave the ester bonds between hydroxycinnamic acids and arabinoxylans or certain pectins present in plant cell walls (Faulds, 2010; Wong, 2006). Four types (types A, B, C and D) of FAEs were classified based on their substrate utilization and primary sequence identity (Crepin et al., 2004), and a recent report proposed a new classification of 12 FAE families based on a combination of published experimental data and the interplay of descriptor-based computational analysis with pharmacophore modeling (Udatha et al., 2011). Up to now, FAEs have been widely applied to the degradation of lignocellulose, the manufacture of pulp and paper, as well as the food and pharmaceutical industries (Fazary and Ju, 2008).

The feruloyl esterase A (*An*FaeA) from *Aspergillus niger* is one of the most investigated FAEs of industrial significance, which has attracted much attention from researchers (Faulds, 2010; Hermoso et al., 2004; Juge et al., 2001; Mcauley et al., 2004; de Vries et al.,

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