Expression and biochemical function analysis of feruloyl esterase and its mutants derived from ruminal anaerobic fungus

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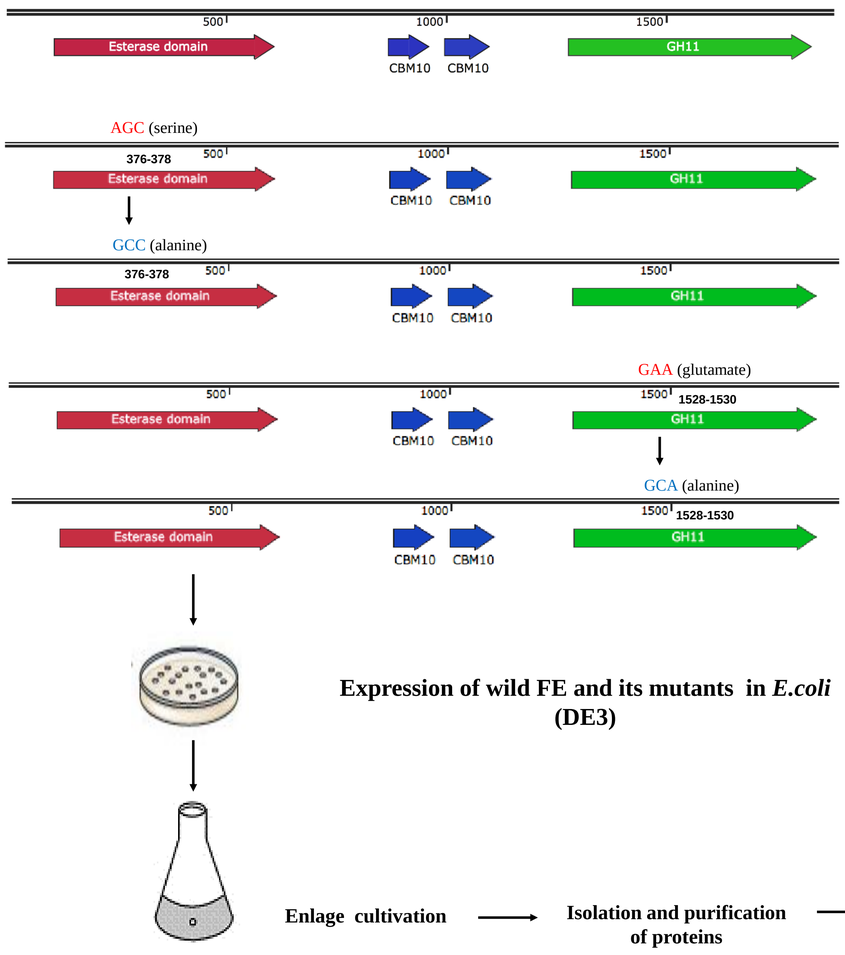
Introduction

- The genome-wide library of anaerobic fungus, *Pecoramyces* sp. F1, contains abundant genes that encode polysaccharide-degrading enzymes.
- However, only limited enzyme genes are developed, and the biological characteristics of most enzymes have still remained unknown.
- This study was aimed to express a feruloyl esterase (FE) and its mutants derived from the fungus and analyse their biochemical function.

Materials and Methods



Gene architecture of wild feruloyl esterase
Fe-WT

Gene mutated esterase domain
Fe-M1

Gene mutated GH11 domain
Fe-M2

Results

The domain organization and purification of wild FE and mutants

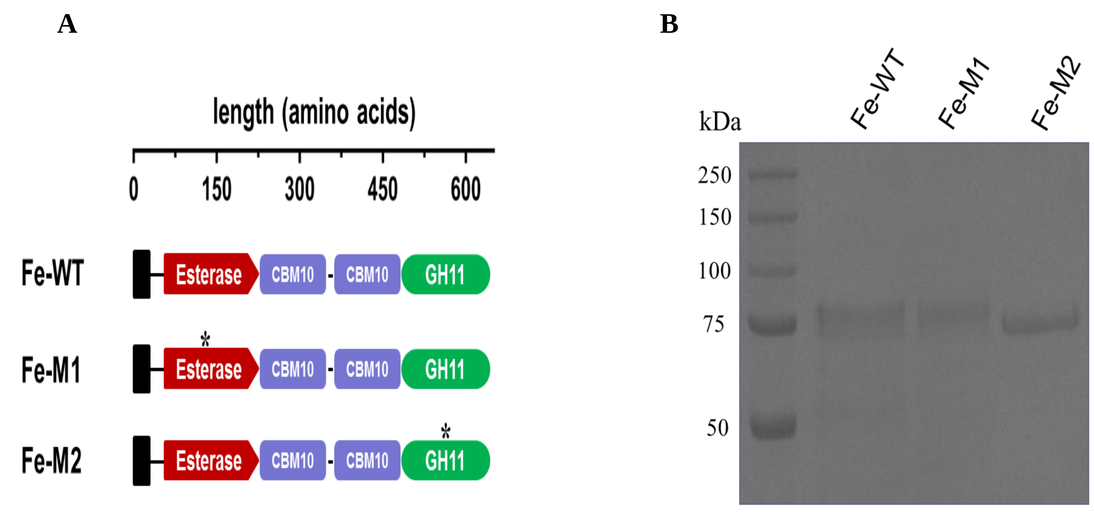


Figure. 1 (A) The amino acids domain organization of wild FE and mutants, * shows the position of mutagenesis.
(B) The SDS gel electrophoresis results of purified FE and mutants.

The optimum pH and temperature of wild FE

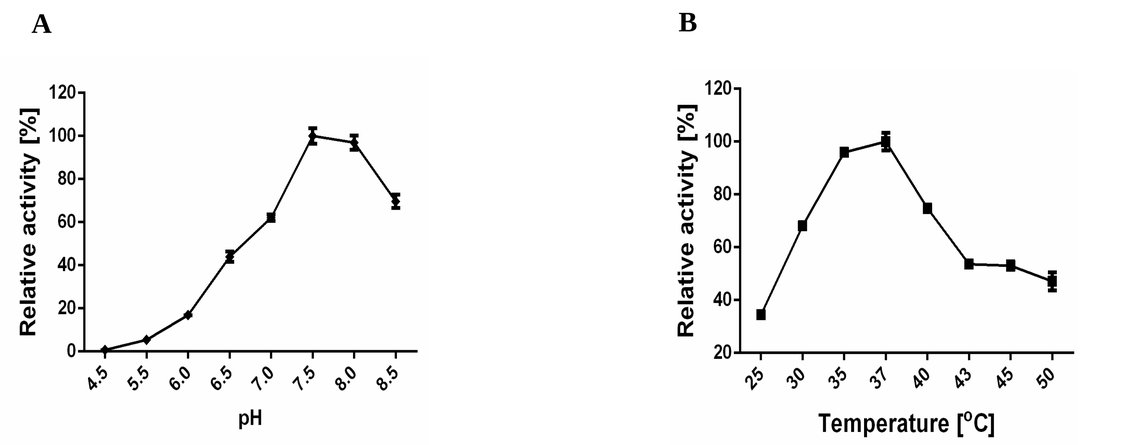


Figure. 2 (A) and (B) both using *p*NP-acetate as substrate. (A) Setting up series of pH values to determine the enzyme activity at 37 ℃ (B) Setting up series of temperature to determine the enzyme activity at pH 7.5.

Results

The degradation of polysaccharides, feruloyl oligosaccharides and esters by wild FE and mutants

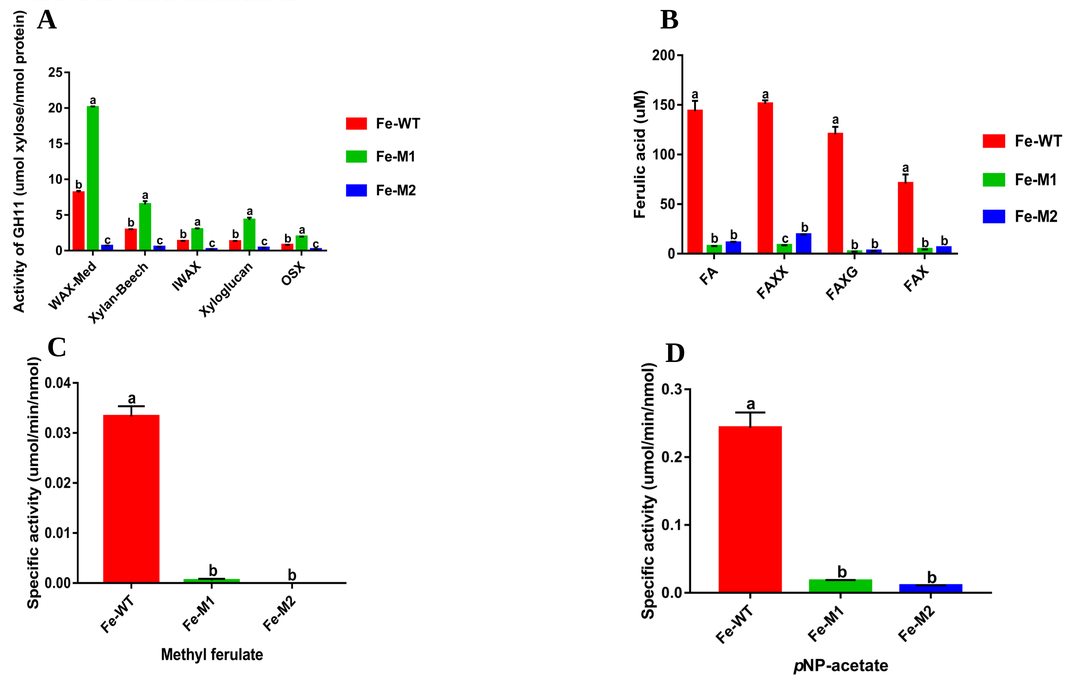


Figure. 3 (A) For the degradation of polysaccharide, the degradability of Fe-M1 was significantly higher than the other three enzymes (P<0.05), and the mutation of GH11 decreased the polysaccharide-degrading capacity of Fe-M2 significantly (P<0.05). (B)(C)(D) all showed that the mutation EST and GH11 in FE both reduce the digestibility of esters and feruloyl oligosaccharides significantly (P<0.05).

Kinetic properties of wild FE and mutants with WAX-medium viscosity as substrate

A

Protein	K_m (mg ml^{-1})	K_{cat} (s^{-1})	K_{cat}/K_m (ml mg^{-1} s^{-1})	V_{max} (mg ml^{-1} min^{-1})
Fe-WT	10.940	103.6	9.5	0.4668
Fe-M1	5.317	112.7	21.2	0.5077
Fe-M2	36.720	12.6	0.3	0.0566

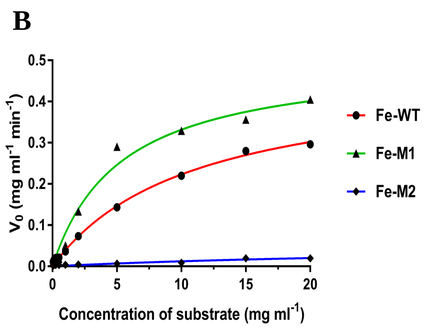


Table. 1 (A) the Km value of Fe-M1 was nearly the half of WT, it indicated that the substrate affinity of Fe-M1 is significantly increased (P<0.05). The Kcat and Kcat/Km value of Fe-M1 was also increased, showing that its catalytical efficiency of substrate is enhanced. However, the Km value of Fe-M2 was significantly improved (P<0.05), its substrate affinity was decreased. The reduced Kcat and Kcat/Km value of Fe-M2 meaned that its catalytical efficiency is significantly declined (P<0.05). **Figure. 4** (B) Kinetic curves of wild FE and mutants with WAX-medium viscosity as substrate.

Kinetic properties of wild FE and mutants with *p*NP-acetate and Methyl ferulate as substrate

A

Substrate/Protein	K_m (mM)	K_{cat} (s^{-1})	K_{cat}/K_m (mM^{-1} s^{-1})	V_{max} (mM min^{-1})
Fe-WT				
*p*NP-acetate	1.348	16.6	12.3	0.2484
Methyl ferulate	0.640	1.3	2.0	0.0195
Fe-M1				
*p*NP-acetate	2.157	0.3	0.1	0.0052
Methyl ferulate	0.458	0.1	0.2	0.0011
Fe-M2				
*p*NP-acetate	0.490	0.5	1.0	0.0080
Methyl ferulate	0.290	0.1	0.4	0.0018

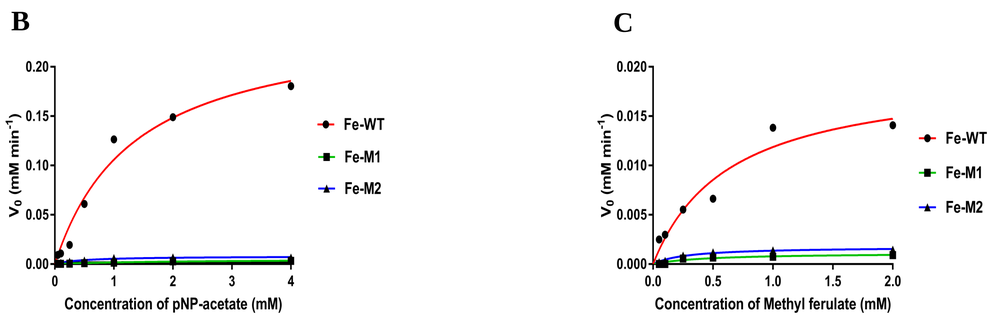


Table. 2 (A) Compared with wild FE, the Kcat values of two mutants with *p*NP-acetate and methyl ferulate as substrate were both significantly decreased (P<0.05), which meaned that their catalytical efficiency of substrate are both declined. When using the same enzyme, the Vmax of catalysing *p*NP-acetate was significantly higher than that of catalysing methyl ferulate (P<0.05). **Figure. 5** (B)(C) Kinetic curves of wild FE and mutants with *p*NP-acetate and methyl ferulate as substrates.

Conclusion

In summary, the mutation of esterase domain in wild FE increased the polysaccharide-digestibility of the enzyme. The mutations of EST and GH11 both decreased the esterase activities of FE. According to these results, we speculate that the existence of EST domain can repress the activity of GH11 in FE. However, for the degradation of esters, these two different domains show strong interaction effect.

Acknowledgements

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