Site Directed Mutagenesis of StIsa2 Gene and Expression of the Recombinant Enzyme in *E. coli*

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Abstract.

Isoamylase is a type of starch debranching enzyme (DBE) that debranches glucan polymers producing linear maltooligosaccharides during starch biosynthesis in plants. Three isoamylase isoforms exist, and each plays a distinct and important, but yet unclear role in starch biosynthesis. However, isoamylase isoform 2 (Isa2), lacking 6 of 8 conserved residues in the catalytic site, has no catalytic activity. Despite this, Isa2 plays an important role in starch biosynthesis, as can be seen in Arabidopsis where Isa2 null mutants produced 80% less starch and accumulated water soluble polysaccharides instead. The objectives of this work was to restore the catalytic residues by mutating the Solanum tuberosum isoamylase isoform 2 (Stisa2) gene, to express and purify the recombinant Stisa2 enzyme, and subsequently, test whether catalytic activity had been restored. Three PCR-based methods: overlap extension PCR, asymmetrical overlap extension, and an improved overlap extension PCR were used to introduce point mutations to substitute the identified DNA bases. After validating the mutations, the recombinant enzyme was expressed in E. coli Rosetta 2 under optimized conditions and expression of soluble Stisa2 was confirmed through western blot analysis. However, preliminary purification of the soluble enzyme showed no activity from the modified enzyme. In silico analysis was also carried out to investigate these properties.

Key words: Isoamylase, Starch Debranching Enzyme, Mutagenesis, Heterologous Expression