# Recombinant Cytochrome *c*-type *cis/trans* Fatty Acid Isomerase from *Pseudomonas putida* KT2440 and its Catalytic Activity

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In the periplasm of *Pseudomonas putida* strain, there is a cytochrome *c*-type *cis/trans* fatty acid isomerase (CTI) containing a heme-binding motif (CXXCH). This enzyme can directly engage in the *cis/trans* isomerism of unsaturated fatty acids. In this study, a CTI gene of *P. putida* KT2440 was cloned into the pET26b(+)/pEC86 co-expression system and heterologously expressed in the *Escherichia coli* system for mass production. The CTI gene with overhangs (2,274 bp) was amplified from the gDNA of *P. putida* KT2440, and it was in-fusion cloned into a pET26b(+) vector in frame with an N-terminal *pelB* signal peptide and a C-terminal polyhistidine fusion tag. The cloned pET26b(+) plasmid was co-transformed into *E. coli* BL21(DE3) with a pEC86 vector for the cytochrome *c* maturation, and the expression of CTI was induced by adding IPTG and 5-aminolevulinic acid. The pure CTI fractions were obtained at 227.14–277.80 mM imidazole using Ni-NTA affinity chromatography, and a single CTI band (approximately 86 kDa) was confirmed by SDS-PAGE analysis. The CTI activity in the buffer solution was evaluated against three *cis*-MUFAs (oleic acid, *cis*-vaccenic acid, and palmitoleic acid), and all their *trans*-MUFAs (elaidic acid, *trans*-vaccenic acid, and palmitelaidic acid) were detected after the reaction. These results suggest that the mass production of CTI could be achieved by molecular cloning, and by extension, CTI will be applied to the enzymatic elimination of *trans* fats in lipid-related foods in the future.