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High level secretory expression of brazzein in yeast

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Brazzein, a sweet-taste protein, is a small protein derived from the African plant *Pentadiplandra brazzeana* Baillon. In previous study, we constructed the expression system for recombinant brazzein in yeast *Kluyveromyces lactis* which is recognized as GRAS. Brazzein expressed in yeast expression system shows high yield and can be simply purified due to yeast's secretion pathway. In addition, as folding procedure of proteins takes place in endoplasmic reticulum(ER) in eukaryotes, proteins expressed in yeast are automatically folded properly. However, there exist four disulfide bonds in the structure of brazzein which may lead to the misfolding of the protein in high level. Thus, by over-expressing protein disulfide isomerase which catalyzes disulfide bond formation and isomerization of protein in the ER, we induced proper protein folding so that the secretion of recombinant brazzein can be increased. As a result, the expression level of the recombinant brazzein increased approximately 1.5 times more than that of previous expression system and the amount of misfolded/unfold recombinant brazzein remaining inside the cell decreased. For much higher expression of the recombinant brazzein, we also replaced LAC4-PB I promoter existing in commercial pKLAC2 vector with wild-type LAC4 promoter. As LAC4-PB I promoter which lacks function in bacteria shows the reduced level of transcription in yeast, we restored the original promoter, LAC4, in order to increase the level of transcription of brazzein gene. The yield of the recombinant brazzein expressed in final expression system was approximately 1.7 times more than in previous expression system. We expect that diverse brazzein variants would be applied to this expression system for commercial production due to its high yield.

Biography

Jinkyung Lim earned a Bachelor's degree from Chung-Ang University in Korea. She is currently pursuing her Master course in Biochemistry. She has published a paper regarding GST enzyme in journal and has researched yeast expression system of sweet-taste protein brazzein.

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