Endoplasmic reticulum-retention C-terminal sequence enhances production of an 11S seed globulin from Amaranthus hypochondriacus in Pichia pastoris.

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Source

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Abstract

The methylotrophic yeast Pichia pastoris was used to express an 11S seed globulin from Amaranthus hypochondriacus. Three different plasmids were tested for expression of amarantin. One of them, which included the untranslated regions (UTR) of the full cDNA, failed to express the amarantin under tested conditions, whereas the other plasmids, one without UTR and the other similar but including the endoplasmic reticulum-retention signal KDEL, were able to express the proamarantin in P. pastoris. After 48 h of induction, KDEL-proamarantin had accumulated quite significantly compared to unmodified proamarantin. Different solubilization patterns were also obtained from both proamarantin versions; only soluble protein was obtained from the system that included the KDEL retrieval signal. Protein fractionation was carried out by differential precipitation with ammonium sulfate, and proamarantin purification was performed using an HPLC ion exchange column. The endoplasmic reticulum-retention C-terminal sequence (KDEL retrieval signal), not commonly employed in this heterologous expression system, can therefore be used to enhance accumulation of recalcitrant protein in P. pastoris. The results obtained here also suggest that this expression system is suitable for expression and evaluation of engineered seed globulin proteins.