High-level expression of the native barley α-amylase/subtilisin inhibitor in **Pichia pastoris**

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High-level expression of the native barley alpha-amylase/subtilisin inhibitor in Pichia pastoris.

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Abstract: An expression system for high-level expression of the native Hordeum vulgare alpha-amylase/subtilisin inhibitor (BASI) has been developed in Pichia pastoris, using the methanol inducible alcohol oxidase 1 (AOX1) promoter. To optimize expression, two codon-optimized coding regions have been designed and expressed alongside the wild-type coding region. To ensure secretion of the native mature protein, a truncated version of the alpha mating factor secretion signal from Saccharomyces cerevisiae was used. In order to be able to compare expression levels from different clones, single insertion transformants generated by gene replacement of the AOX1 gene was selected by PCR screening. Following methanol induction, expression levels reached 125 mgL(-1) from the wild-type coding region while expression from the two codon-optimized variants reached 65 and 125 mgL(-1), respectively. The protein was purified and characterized by Edman degradation, liquid chromatography mass spectrometry and insoluble blue starch assay, and was shown to possess the same characteristics as wild-type protein purified from barley grains.

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