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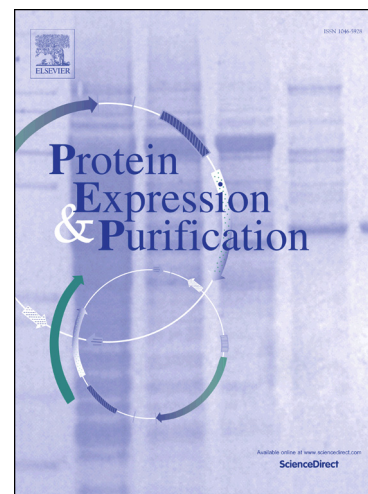
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1 Expression, Activation and Characterization of Porcine 2 Trypsin in *Pichia pastoris* GS115

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9 Abstract

10 Trypsin is a typical member of serine protease families, specifically cleaving the carboxyl group
11 of peptides at the basic amino acids arginine and lysine. The DNA fragment of porcine trypsin with its
12 propeptide coding sequence was optimized and synthesized according to codon usage bias of *Pichia*
13 *pastoris*. The optimized sequence was integrated into genome of *P.pastoris* GS115 by using the vector
14 pHBM905A. The yield of the recombinant protein was 0.48 mg/ml with a maximum activity of 19.2
15 U/ml after 96h induction in a 5L fermenter. An optimum activity for the recombinant trypsin was
16 observed at 35°C and pH 8.5. This is the first time to express the porcine trypsinogen with *P.pastoris*
17 expression system. This report also found that the propeptide was cleaved from the recombinant protein
18 and the enzymogen was transferred into trypsin at the later phase of the fed-batch cultivation. In
19 particular, the activation process can be initiated by changing pH.

20 **Keywords:** porcine trypsin; autocatalysis; High-level expression *Pichia pastoris*; high density
21 fermentation

22 Introduction

23 Trypsin (EC 3.4.21.4) is a serine protease, which exists widely in nature, and has been discovered
24 in bacteria, fungus and mammals[1, 2]. In mammals, Trypsin is closely linked with metabolism,
25 digestion, coagulation [3]. Trypsin is secreted as a zymogen by pancreas without activity. Trypsinogen
26 must be transported to duodenum and subsequently activated by enterokinase [4]. Otherwise, the active
27 trypsin causes the self-damage of the body. Therefore, the mechanism of trypsinogen activation has
28 been studied intensively. The previous reports indicated that the propeptide of trypsin contains 6 amino
29 acids (VDDDDK) and this sequence is highly conserved in mammals [5]. Self-activation or
30 hydrolyzation with enterokinase activates trypsinogen by removing the N-terminal peptide propeptide
31 [6]. Similarly, previous studies proved that trypsin is also initially produced as trypsinogen in
32 *Streptomyces griseus*, which contains a propeptide (4 amino acid residues, APNP) and the active
33 trypsin [7]. There are subtle differences between different species; the activation process is strictly
34 followed.

35 Trypsin catalyzes a hydrolytic cleavage of peptides at the carboxyl group of the basic amino acids
36 arginine and lysine and generally used in leather processing, biotechnological processing, medicine
37 field, and food processing[8]. Traditionally, trypsin was mainly extracted from animal organs and
38 tissues. However, the demand of trypsin was increasing in recent years and the yield is limited. In
39 addition, the traditional method presented some problems, such as complex production process and the
40 enzymes from animal potential contamination with infectious agents. For these reasons, heterologous

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