Note

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Purification of an extracellular carboxypeptidase from *Pseudozyma hubeiensis* 31-B and its characterization as a food additive

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Abstract

A newly isolated yeast strain, *Pseudozyma hubeiensis* 31-B, produced an extracellular carboxypeptidase but no detectable proteinase activity. The carboxypeptidase was purified from filtered culture medium by ammonium sulfate precipitation and successive four column chromatography steps: TOYOPEARL DEAE-650M, TOYOPEARL Butyl-650M, hydroxylapatite, and TOYOPEARL HW-55 chromatography. The final enzyme preparation appeared homogeneous on SDS-polyacrylamide gel electrophoresis. The enzyme had a molecular mass of 70.5 kDa. The optimum temperature and pH of the purified enzyme were approximately 50°C and 5.0, respectively. The purified carboxypeptidase preferentially hydrolyzed *Z*-Phe-Leu, and its activity was inhibited by diisopropyl fluorophosphate. The carboxypeptidase produced by *P. hubeiensis* 31-B has potential applications in the food industry as a food additive.

Keywords : *Pseudozyma hubeiensis*, extracellular carboxypeptidase, purification, food additive

I Introduction

Carboxypeptidases (CPs) catalyze the release of amino acid residues from the C-terminal end of peptides and proteins. Various CPs from fungi such as *Aspergillus niger*[1], yeast such as *Saccharomyces cerevisiae*[2, 3], and bacteria such as *Pseudomonas*[4] have been reported. In particular, carboxypeptidase Y (CPY; EC 3.4.16.5) from *S. cerevisiae* has been studied in detail[5]. CPs are useful enzymes for processing foods containing proteins and/or peptides because they can efficiently eliminate the bitterness of bitter peptides generated by proteinases[6]. For example, CPs are used to improve the flavor of dairy products by enhancing flavor development in liquid seasonings and yogurt, and to aid the ripening of specific cheeses. *Aspergillus* and *Saccharomyces cerevisiae* are used to produce food additive CPs included in the list of Japanese Existing Food Additives. However, CP preparations derived from *Aspergillus* generally contain secreted proteinases that can hydrolyze proteins randomly, impacting the physical properties and imparting a bitter taste to processed foods and thus limiting the use of peptidase preparations in the food processing industry. In contrast, *S. cerevisiae* produces peptidases as intracellular or cell-bound enzymes that are not secreted into the culture medium.

The first step of food additive enzyme production involves the removal by centrifugation or filtration of fermenting microorganisms containing intracellular or cell-bound peptidases, but extraction of these enzymes from cells on an industrial process is challenging. In the next step, undesirable enzymes such as proteinases must be removed from the culture filtrate, but accomplishing this efficiently and economically on an industrial scale is also difficult.

We were therefore interested in identifying a yeast strain that produces extracellular CP with the ability to debitter

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The properties of the carboxypeptitase from *Pseudozyma hubeiensis* 31-B are available in Japanese Published Unexamined Patent Application No. P2017-86A. (Date of publication: Jan. 5, 2017)