B-2

Characteristics of salt-tolerant protease purified from the fermented anchovy sauce

WOO JAE KIM · SANG MOO KIM

Factulity of Marine Bioscience & Technology, Kangnung National Uiversity

Introduction

Enzymes have been used as processing aids in the manufacture of food products to improve their quality, solubility and stability for centuries. About 50% of the enzymes used as industrial processing aids are protein hydrolases which have been used in a number of industrial application including laundry detergents, feed, leather treatment, silk degumming, cheese making, chill proofing, meat tenderzing, fermented sauces, and pharmaceuticals.

Recently, interest has developed to find a salt-tolerant protease for fermented foods with high amount of NaCl. shortening the fermentation period and increasing the amount of functional substances in fermented food with a high amount of salt concentration.

In this study, the characteristics of salt-tolerant protease purified from anchovy sauce with about 26% NaCl fermented at different fermentation period was investigated.

Material and Methods

Enzyme production - A salt-tolerant proteolytic bacteria, *Bacillus subtillis,* was isolated from anchovy sauce fermented at 15±3℃ for 5 years. *Bacillus subtilis* was cultured in 500 mL of proteolytic medium in a 2 L wide-mouth culture flask. Cultures were incubated at 37 ℃ for 8 days at 150 rpm.

Enzyme purification - The precipitate from the ammonium sulfate fractionation in 20mM Sodium acetate buffer (pH 5.5) was dialyzed overnight using 10K cut-off dialysis tubing at 4℃. The 40~60% fraction was loaded onto a column of DEAE-A50. The fractions with protease activity pooled and concentrated using an ultrafiltration system were loaded onto a Sephadex G-75 column .

Protein determination - Dye binding assay(Bradford 1976) was used for quantitative determination of protein using a bovine serum albumin(Sigma) as a standard.

Gel electrophoresis - 12% SDS-PAGE.

Protease activity - Protease activity were performed at 25℃ by azocasein method.

Characteristic of protease - This paper was investigated properties of protease as effect of temperature on enzyme activity, temperature stability, effect of pH on enzyme activity, pH stability, effect of NaCl concentration, effect of inhibitor on enzyme activity and kinetic constants.

RESULT AND DISCUSSION

Salt tolerance protease was purified from anchovy sauce. Protease of ammonium sulfate fraction was hightest enzyme activity in 40~60% fraction. Salt-tolerant protease of *Bacillus subtilis* was shown two - peak on the ion - exchange chromatography. Molecular weight of protease was 14,000 daltons by SDS-electrophoresis. Even though protease activity decreased as NaCl content increased, protease activity of enzyme was still actuvity up to 30% of NaCl content. Temperature stability and optimum temperature of Salt tolerance protease was each 30 and 50℃. pH stability and optium pH was each 5.5 and 5.0. Azocasein was best substrate for this salt-tolerant protease. Protease was inhibited greatly by NEM, which was known as a representative of serine protease like trypsine type. Therefore, Protease was serine protease like trypsin type.

References

1. Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248~254