J. Life Science. (2001), 11(2), 92~93

JOURNAL OF
Life Science

Thin Layer Chromatogram by an Extracellular β-Amylase of *Bacillus* sp. KYJ 963 and its Amino Acid Composition

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Received: September 6, 2001

Abstract *Bacillus* sp. KYJ 963, which was isolated from Korean salt-fermented anchovy (anchovy-*jeot*), produces an extracellular β-amylase. The analysis of the digestion products of substrates by thin layer chromatography from the purified protein revealed that the enzyme could not hydrolyze maltose or α-cyclodextrin. In the amino acid composition analysis, the major characteristic of the β-amylase was the high proportion of amino acids that possess short side chain such as glycine and alanine.

Key words: *Bacillus* sp. KYJ 963, extracellular β-amylase, TLC profile, amino acid composition

Introduction

Starch and its partially hydrolyzed products can be hydrolyzed by a variety of amylases such as α-amylase, β-amylase, and glucoamylase. Among these enzymes, food and beverage industries employ β-amylase to convert starch into maltose solutions. β-Amylase (1,4-α-D-glucan maltohydrolase, EC 3.2.1.2) is an exo-type enzyme that hydrolyzes the α-1,4 glucosidic linkages and successively liberates β-maltose from the nonreducing end of starch, glycogen, and maltooligosaccharides. β-Amylases are known to be produced by plants and some gram-positive spore-forming bacteria such as *Bacillus cereus* [1], *Bacillus polymyxa* [2], *Bacillus circulans* [3], and *Clostridium thermosulfurogenes* [4-6].
Bacillus sp. KYJ 963, which was isolated during the fermentation process of anchovy-*jeot*, produces an extracellular β-amylase with a molecular mass of approximately 59,000 [7]. The β-amylase was purified and its enzymatic properties were reported [7]. This paper describes the thin layer chromatogram of the hydrolysates of substrates by an extracellular β-amylase of the *Bacillus* sp. KYJ 963 and its amino acid composition.

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MATERIALS AND METHODS

Bacterial strain and growth condition

The bacterial strain used in this study was *Bacillus* sp. KYJ 963 [8] isolated from anchovy-*jeot*. Unless otherwise stated, *Bacillus* sp. KYJ 963 was grown in a liquid medium containing 0.5% polypeptone, 0.5% yeast extract in 50 mM Tris-HCl (pH 7.5) at 37°C.

Purification of the extracellular β-amylase

The purification of the extracellular β-amylase was performed as described previously [7].

Thin Layer Chromatography (TLC)

Hydrolysis of 0.5 ml of soluble starch, maltose, or cyclodextrine (1% in 50 mM Tris-HCl, pH 7.5) was carried out in a mixture containing 0.4 ml of 50 mM Tris-HCl (pH 7.5) and 0.1 ml of enzyme solution (50 units) at 45°C. The reaction was stopped by boiling for 2 min at 100°C. The sugars released by the enzymatic hydrolysis of amylase were separated by ascending TLC aluminium sheet (20 × 20 cm, silica gel 60F254, Merk Co., Germany) with a solvent system of n-butanol-ethanol-water (5 : 3 : 2). Spots on the sheet were detected with a silver nitrate/sodium hydroxide solution [solution A (0.5% silver nitrate in acetone)/solution B (0.5 M sodium hydroxide in ethanol)] and dried at room temperature for 30 min.

Amino acid composition of the extracellular β-amylase

The analysis of amino acid composition, the purified amylase was hydrolyzed in 6 N HCl at 110°C for 24 h. The amino acid composition of the enzyme was analyzed with HPLC (Waters Co., Milford, U.S.A.) equipped with a Pico-Tag column (3.9×300 mm) after phenylisocyanate derivatization. To determine the tryptophan content, the enzyme was directly digested with 4 M methanesulfonic acid and analyzed. Cysteine residues were oxidized to cysteic acid with a mixture of formic acid and hydrogen peroxide (19 : 1, v/v)

and analyzed.

RESULTS AND DISCUSSION

Thin layer chromatography analysis of the digesting products of substrates by an extracellular β-amylase

The hydrolysis of substrates (soluble starch, maltose, and alpha-cyclodextrine) was carried out at 45°C and pH 7.5. Samples were removed at intervals during incubation and analyzed (Fig. 1). The final end products of soluble starch hydrolysis by an extracellular β-amylase were maltose and limit dextrin, and the enzyme could not hydrolyze maltose (lane 4) and α-cyclodextrine (lane 5), indicating that th enzyme was an β-amylase.

Amino acid composition

Amino acid composition of the extracellular β-amylase is given in Table1. A major characteristics is the high proportion of amino acids that possess short side chain such as glycine and alanine.

ACKNOWLEDGMENT

This work was financially supported by Changwon National University in 2001.

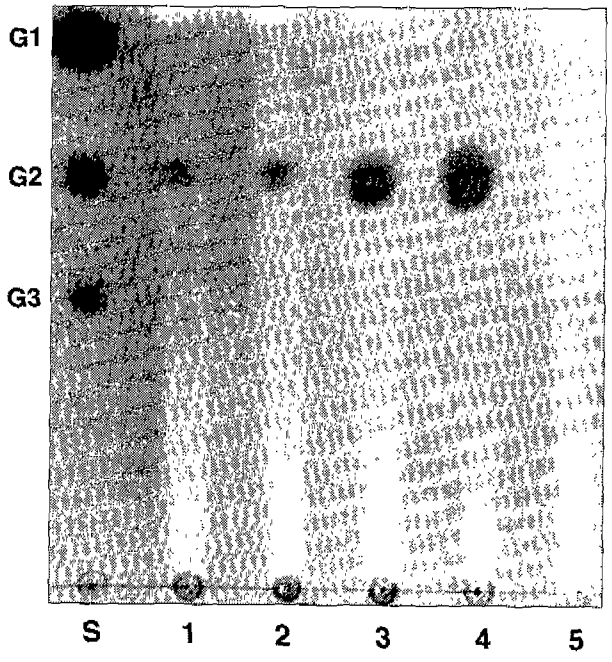

Fig. 1. Thin layer chromatogram of the hydrolysates of substrates by an extracellular amylase of *Bacillus* sp. KYJ 963. Enzyme reaction was carried out as described in Materials and Methods. The hydrolysis products of soluble starch were analyzed at different reaction time. Lane 1, 0.1h; lane 2, 0.5h; lane 3, 12h. The hydrolysis products of maltose (lane 4) and a-cyclodextrin (lane 5) were also analyzed at 12h. As standards (S), glucose (G1), maltose (G2), and maltotriose (G3) were used.

Table 1. Amino acid compositions of the extracellular β-amylas

Amino acid	Mole %
Gly	17.19
Ala	12.33
Glx	11.91
Val	7.82
Lys	7.52
Ser	7.16
Leu	6.19
Pro	5.96
Asx	5.43
Thr	5.27
Ile	3.87
Phe	2.48
Trp	2.09
Arg	1.92
His	1.86
Met	0.52
Tyr	0.21
Cys	0.18
Cys	20.08

Asx, Asp+Asn; Glx, Glu+Gln; Cys2, Cys+Cys.

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