Note

Arabinan-degrading Enzymes from *Streptomyces diastatochromogenes* 065

Kazuaki HIGASHI,[†] Isao KUSAKABE,
Tsuneo YASUI, Tadayuki ISHIYAMA*
and Yoichiro OKIMOTO*

*Institute of Applied Biochemistry,
The University of Tsukuba, Ibaraki 305, Japan*

**Department of Agricultural Chemistry,
Faculty of Agriculture,
Tamagawa University,
Machida 194, Japan*

Received December 3, 1982

There are many reports dealing with α -L-arabinofuranosidases (EC 3.2.1.55, AFase) produced by microorganisms, especially by fungi.^{1~4)} Recently, some AFases from *Streptomyces* have been reported.

Kaji *et al.*⁵⁾ reported that the purified AFase from *Streptomyces* sp. 17-1 could liberate arabinose from arabinan, arabinoxylan, and *p*-nitrophenyl α -L-arabinofuranoside. The reaction velocity of hydrolysis of arabinan was rapid up to 29%, and stopped at 38%. From these results, they speculated that side chains in beet arabinan were hydrolyzed first and the main chain was hydrolyzed slowly. However, data were not shown.

Komae *et al.*⁶⁾ purified an AFase from *Streptomyces purpurascens* IFO 3389, and they noted that this enzyme acted on *p*-nitrophenyl α -L-arabinofuranoside but not on arabinan.

Kaji *et al.*⁷⁾ purified an AFase from *Streptomyces massaporeus* IFO 3841, and reported that a low molecular weight arabinoside (*p*-nitrophenyl α -L-arabinofuranoside) is the best substrate for this enzyme.

Kusakabe *et al.*⁸⁾ studied some properties of AFase from *Rhodotorula flava*, *Streptomyces* sp., and *Aspergillus niger*. They found that the extent of hydrolysis of arabinan by these three enzymes were 68, 50 and 97%, respectively.

We were interested because these results indicate a difference of the mode of action on arabinan or the substrate specificity of AFase from *Streptomyces* sp. and that from *A. niger*.

A strain of *Streptomyces*, *Streptomyces* sp. No. 065, which produced potent arabinan-degrading enzymes, was isolated from soil. This strain was identified as *Streptomyces diastatochromogenes* following the method

of Shirling and Gottlieb.⁹⁾

Production of extracellular enzyme was observed with arabinan, which was prepared following the method of

TABLE I. EFFECTS OF CARBON SOURCES ON PRODUCTION OF EXTRACELLULAR AND INTRACELLULAR ARABINAN-DEGRADING ENZYMES

	Growth	Specific activity	
		Extracellular enzyme	Intracellular enzyme
Arabinan	+++	0.60	0.57
Carboxymethyl cellulose	++	0	0
Soluble starch	+++	0	0
Pectin	++	0.09	0.04
L-Arabinose	++	0.12	0.14
D-Glucose	++	0	0
D-Galactose	++	0	0

Growth: +++, abundant; ++, medium; +, poor.

Basal medium: carbon source, 2.0%; polypeptone, 0.6%; yeast extract, 0.1%; KH_2PO_4 , 1.0%; NH_4NO_3 , 0.3%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05%, pH 5.5.

Produced enzyme activity was expressed as specific activity (U/mg protein).

TABLE II. SUBSTRATE SPECIFICITIES OF EXTRACELLULAR AND INTRACELLULAR ARABINAN-DEGRADING ENZYMES

Substrate	Concentration (%)	Relative activity (%)	
		Extracellular enzyme	Intracellular enzyme
Arabinan	1	100	100
Arabinoxylan ^a			
X/A = 2.6	1	8	8
X/A = 17.5	1	7	8
Gum arabic	1	8	3
Soluble xylan	1	15	4
Arabinoxylobiose	0.63	5	52
Phenyl α -L-arabinofuranoside	2.66	25	70

^a Xylose/arabinose ratio.

Arabinoxylan was dissolved in 0.5N NaOH, neutralized with 0.5N HCl, and prepared to 2.5% with water. The reaction mixture, which containing 1 ml of substrate, 1 ml of McIlvaine buffer (pH 6.0) and 0.5 ml of the enzyme (1 U as arabinan degrading activity), was incubated at 50°C for 30 min. The amount of reducing sugar in the reaction mixture was determined by the Somogyi method.¹³⁾

[†] Present address: Department of Agricultural Chemistry, Faculty of Agriculture, Tamagawa University, Machida 194, Japan.

Abbreviations: AFase, α -L-arabinofuranosidase; and P α LAF, phenyl α -L-arabinofuranoside.

FIG. 1. Paper Chromatogram of the Reaction Products of Extracellular and Intracellular Arabinan-degrading Enzymes from *S. diastatochromogenes* 065.

The high molecular weight fraction in the reaction mixture was removed by precipitation with ethanol.

(A), extracellular; (B), intracellular arabinan-degrading enzymes.

Kusakabe *et al.*⁸⁾ from sugar beet pulp, and with L-arabinose; but it not produced with other carbon sources (Table I). These results agreed with that of Kaji *et al.*^{5,10)} Intracellular enzyme was also produced with arabinan and L-arabinose (Table I).

S. diastatochromogenes 065 was cultivated in shaking flasks (500 ml) containing 100 ml of medium (arabinan 2.0% as arabinose, polypeptone 0.6%, yeast extract 0.1%, NH_4NO_3 0.3%, KH_2PO_4 1.0% and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05%, pH 5.5) at 27°C for 4 days on a reciprocal shaker (125 oscill/min, 7 cm stroke).

As shown in Table II, culture filtrate of this strain could hydrolyze arabinan and phenyl α -L-arabinofuranoside (P α LAF), which was synthesized from L-arabinose.¹¹⁾

In addition to the culture filtrate, the supernatant of mycelia of this strain destructured by french press (1 kg/cm², at -10~0°C) also hydrolyzed arabinan and P α LAF (Table II). This is the first report of the existence of an intracellular arabinan-degrading enzyme in *Streptomyces*, and only one intracellular AFase, the enzyme from *Sclerotinia fructigena*, was known.¹²⁾

If the culture filtrate contained only *endo*-type enzymes, oligosaccharides ought to be detected on paper chromatograms, and if it was a mixture of *exo*- and *endo*-types, only arabinose or arabinose with oligosaccharides should be detected. But as shown in Fig. 1, only arabinose was detected as the hydrolysis product from arabinan. Furthermore, when oligosaccharides are hydrolyzed by an *exo*-type enzyme, it seems that the extent of hydrolysis of arabinan does not reach 40% (Fig. 2).

The supernatant seemed to contain an *exo*-type enzyme.

FIG. 2. Courses of Hydrolysis of Arabinan by Extracellular and Intracellular Arabinan-degrading Enzymes from *S. diastatochromogenes* 065.

The reaction mixture containing 4 ml of 2.5% arabinan, 4 ml of McIlvaine buffer (pH 6.0) and 2 ml of enzyme (2 U) was incubated at 50°C. One ml of the reaction mixture was pipetted out at each time and the amount of reducing sugar was determined.

(○), extracellular; (●), intracellular arabinan-degrading enzymes.

Indeed, the extent of hydrolysis of arabinan was about 90% (Fig. 2), and only arabinose was detected on paper chromatograms (Fig. 1). But it is not obvious whether *endo*-type enzymes exist or not.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- 1) A. Kaji and K. Tagawa, *Nippon Nôgeikagaku Kaishi*, **38**, 580 (1961).
- 2) A. Fuchs, J. A. Jobsen and W. H. Wouts, *Nature*, **206**, 714 (1965).
- 3) A. Kaji and K. Tagawa, *Nippon Nôgeikagaku Kaishi*, **40**, 325 (1966).
- 4) A. L. J. Cole and R. K. S. Wood, *Phytochemistry*, **9**, 695 (1970).
- 5) A. Kaji, M. Sato and Y. Tsutsui, *Agric. Biol. Chem.*, **45**, 925 (1981).
- 6) K. Komae, A. Kaji and M. Sato, *Agric. Biol. Chem.*, **46**, 1899 (1982).
- 7) A. Kaji, M. Sato, O. Yoshihara and A. Adachi, *Tech. Bull. Fac. Agr., Kagawa Univ.*, **34**, 79 (1982).
- 8) I. Kusakabe, T. Yasui and T. Kobayashi, *Nippon Nôgeikagaku Kaishi*, **49**, 295 (1975).
- 9) E. B. Shirling and D. Gottlieb, *Int. J. Syst. Bacteriol.*, **16**, 313 (1966).
- 10) K. Tagawa and A. Kaji, *J. Ferment. Technol.*, **46**, 693 (1968).
- 11) K. Higashi, H. Hori, T. Ishiyama, Y. Okimoto, I. Kusakabe and T. Yasui, *Agric. Biol. Chem.*, **47**, 1123

- (1983). *Gen. Microbiol.* **79**, 321 (1973).
- 12) F. Laborda, A. H. Fielding and R. J. W. Byrde, *J.* 13) M. Somogyi, *J. Biol. Chem.*, **161**, 61 (1945).
-