Note

Optimal Constitution of
Pectinolytic Enzymes from *Erwinia*
carotovora for Enzymatic
Maceration of Mitsumata
(*Edgeworthia papyrifera*
Sieb. et Zucc) Bast[†]

Hiroyuki TANABE, Yoshinari KOBAYASHI,
Yutaka FUSHIMI,* Hirosugu KANASAKI*
and Suguru TADA*

Government Industrial Research Institute,
Shikoku, 2-3-3, Hananomiya-cho,
Takamatsu 761, Japan

*Fushimi Pharmaceutical Co., Ltd.,
1676, Nakatsu-cho,
Marugame 763, Japan

Received August 8, 1986

In a series of investigations on the biochemical pulping of bast fibers, it was demonstrated that the enzymatic maceration of mitsumata (*Edgeworthia papyrifera* Sieb. et Zucc) bast proceeded through the concerted action of *endo*-pectate lyase (*endo*-PATE; EC 4.2.2.2) and *endo*-pectin lyase (*endo*-PNTE; EC 4.2.2.10) at an activity ratio of 34 to 1.¹⁾ But the data do not always mean the most

efficient constitution ratio of the two enzymes for the pulping. This paper deals with the determination of the optimal constitution.

The pectins of various origins listed in Table I were obtained as follows: as to citrus sudachi, the fruit was cut in half, and after the juice had been squeezed out, it was dried. Pectin contained in the dried material was extracted with dil. HCl, the pH being adjusted to 2.0, and after filtration, it was precipitated with isopropanol. The other pectins were prepared through extraction from the ground epicarp and bark, the pH being adjusted to 2.0 with dil. HCl followed by neutralization to pH 6.2 with dil. NaOH, and subsequent precipitation with isopropanol. Pectins of different esterification degrees were obtained by esterifying the pectin from satsuma mandarin with methanol under bubbling with anhydrous HCl was according to the modified directions of Manabe.²⁾ As a carbon source for bacterial cultures, 0.5% of each pectin or glycerol was used and the composition of other medium constituents was the same as described previously.³⁾ Thirty-five ml of this medium was aseptically introduced into a 200-ml Erlenmeyer flask. A culture of *E. carotovora* FERM P-7576³⁾ was inoculated into this medium and grown at 27°C with rotary shaking at 180 rpm for 40 hr. The activities of both *endo*-PATE and *endo*-PNTE in the supernatant of each culture liquid were assayed by the procedures described previously,^{1,3)} Both enzymes were found to be always induced in a mutually accompanying manner by these pectins and the activity ratio of PATE to PNTE showed a very narrow range of 20.7 to 33.8 (Table I). Even constitutive enzyme production using glycerol was not exceptional as to this aspect, showing a ratio of 23.0.

Enzymatic maceration activity toward mitsumata bast

TABLE I. DEPENDENCE OF ENZYME PRODUCTION ON PECTIN AND INFLUENCE OF MC TREATMENT ON IT

Pectin	PATE productivity (units/ml)	PNTE productivity (units/ml)	PATE/PNTE
Satsuma mandarin (<i>Citrus unshiu</i> Markov.)	80.1	3.7	21.6
Sudachi (<i>Citrus sudachi</i> Hort.)	69.0	2.4	28.8
Lemon (<i>Citrus limon</i>)	86.8	4.2	20.7
Apple (<i>Malus pumila</i> Mill.)	80.9	3.1	26.1
Mitsumata bast (<i>Edgeworthia papyrifera</i> Sieb. et Zucc.)	74.8	3.2	23.4
Kozo (<i>Broussonetia kazinoki</i> Sieb.) bast	65.5	3.0	21.8
Satsuma mandarin (100% esterified)	91.4	3.2	28.6
(70% esterified)	79.2	2.9	27.3
(40% esterified)	75.0	2.6	28.8
(7% esterified)	58.5	2.1	27.9
Citrus pectin (Sunkist Growers, Inc.) - MC	94.6	2.8	33.8
+ MC	30.8	35.0	0.88
(Glycerol)	23.0	1.0	23.0

[†] Biochemical Pulping. Part XIX. Previous paper: *Agric. Biol. Chem.*, 50, 2779 (1986).

FIG. 1. Optimal Constitution of PATE and PNTE for Maceration of Caustic Soda-pres soaked Mitsumata Bast.

Caustic soda (1% NaOH)-pres soaked mitsumata bast (20 g, dry weight) was pulped with the crude enzyme produced by *E. carotovora* FERM P-7576 with (□, Δ, ● and ▲) or without (■, ▲, ● and ▼) the addition of mitomycin C. Simultaneously the same procedure was performed with the purified enzyme composite of *endo*-PATE and *endo*-PNTE at an appropriate mixing ratio (□, Δ, ○ and ▽). The total enzyme activity of *endo*-PATE and *endo*-PNTE in each enzyme preparation was adjusted to 800 units. The enzyme reaction was performed in 200 ml of 0.1 M $\text{NH}_4\text{OH-NH}_4\text{Cl}$ buffer (pH 9.5) with rotary shaking at 30°C for 6 hr.⁴⁾

was assayed according to the method stated earlier.⁴⁾ Crude enzyme⁴⁾ and purified enzyme composites of *endo*-PATE⁴⁾ and *endo*-PNTE¹⁾ with different constitution ratios were prepared so that the sum of the two enzyme activities was 800 units. The reaction time of 6 hr was chosen because the macerating reaction had not reached its plateau at this time. As shown in Fig. 1, the highest efficiency was achieved at a mixing ratio of 30 to 1. This value was rather close to the constitution ratio for the crude enzyme preparation.

Treatment of strain FERM P-7576 with mitomycin C (MC), a kind of DNA-synthesis inhibitor,⁵⁻¹⁰⁾ was carried out in medium (pH 6.8) consisting of 0.5% citrus pectin (Sunkist Growers, Inc.), 0.5% polypeptone, 0.5% yeast extract and 0.5% NaCl. At the mid-exponential phase of growth, MC was added to a final concentration of 0.5 μg/ml. Further cultivation was performed until cell lysis occurred. With this treatment PNTE production increased dramatically with the simultaneous depression of PATE production, which led to an extremely low PATE to PNTE activity ratio value (Table I). The maceration behavior of the MC-induced crude enzyme preparation is presented in Fig. 1. The maceration degree compared poorly with that of the spontaneously secreted one. Purification of the PNTE from this crude enzyme preparation was then performed.¹⁾ On comparison of physicochemical and enzymological properties of the resultant

purified MC-induced PNTE with spontaneously obtained PNTE,¹⁾ no significant differences were observed (data not shown). This suggests, together with the mutually similar macerating behaviors of the two enzymes (data at PATE/PNTE of 0.88 in Fig. 1), that the two are identical, that is, the low maceration activity of the MC-induced crude enzyme is likely due not to the properties of the PNTE contained but to the considerably low constitution ratio of PATE to PNTE because the maceration proceeds through the combined actions of PATE and PNTE.¹⁾

These results demonstrate that *endo*-PATE is the dominant maceration factor with the supplementary aid of *endo*-PNTE. This conclusion is partially coincident with the previous reports that *endo*-PATE plays an essential role in plant-tissue maceration,¹¹⁻¹⁶⁾ but the mechanism of the present indispensability of *endo*-PNTE, or the high resistance of pectic moieties in the bast to deesterification must be elucidated in the future.

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