Note

Plant-tissue Maceration Caused by Pectinolytic Enzymes from *Erwinia* spp. under Alkaline Conditions†

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Among many proposals as to the mechanism of plant-tissue maceration,[1] pectiolytic enzymes have been demonstrated to significantly influence its inception and development,[2~5] but a thorough investigation of this mechanism, especially quantitative analysis, has not yet been conducted so far. The authors' previous study established that the enzymatic maceration of mitsumata (*Edgeworthia papyrifera* Sieb. et Zucc.) bast in the process of biochemical pulping under alkaline conditions proceeds through the concerted actions of *endo*-pectate lyase (*endo*-PATE, EC 4.2.2.2) and *endo*-pectin lyase (*endo*-PNTE, EC 4.2.2.10) from soft-rot *Erwinia carotovora* FERM P-7576,[6] where *endo*-PATE is the primary agent for the maceration and *endo*-PNTE plays a supplementary but indispensable role.[7,8] The purpose of the present study is to clarify whether or not this maceration mechanism under alkaline conditions is applicable to other pulping systems comprising a variety of plant tissues and *Erwinia* strains.

The defibration activities[9] of extracellular crude enzymes[10] from several *Erwinia* isolates toward mitsumata bast were compared on the basis of the total productivity of *endo*-PATE[10] and *endo*-PNTE[6] from each strain (Table I). Althouth both the defibration activity and the total enzyme activity varied from strain to strain, a clear parallelism was recognized between the two: greater defibration occurred with higher enzyme activity. It has already been reported that other pectinases, pectic hydrolases and pectin esterase, do not contribute to this maceration at pH 9.5.[6]

E. chrysanthemi GIR 2002, the *endo*-PNTE of which was proved to malfunction toward mitsumata bast fibers,[8] was examined as to the defibration activity of its extracellular crude enzyme toward several plant tissues, kôzo (*Broussonetia kazinoki* Sieb.) bast, gampi (*Wikstroemia sikokiana* Fr. et Sav.) bast, flax (*Linum usitatissiumum* L.) bast, kenaf (*Hibiscus sabdariffa* var. *altissima*) and sugarcane (*Saccharum officinarum* L.) bagasse, as in the above-mentioned manner. Table II presents the data in comparison with the corresponding data for the enzyme from *E. carotovora* FERM P-7576. Although the productivity of pectinolytic enzyme by strain GIR 2002 (96.2 units/ml for PATE and 3.0 units/ml for PNTE) was comparable to that by strain FERM P-7576 (see Table I in ref. 7), the crude enzyme from GIR 2002 was significantly inferior to the other, as to the defibration degree for any pulping material.

On the other hand, the defibration activity of the crude enzyme from FERM P-7576 after mitomycin-C treatment[7] was also assayed as to the above materials, and was

Table I. Defibration Activity toward Bast and Pectinolytic-enzyme Activity of the *Erwinia* Extracellular Crude Enzyme

Bacteria	Source[a]	PATE activity (units/ml)	PNTE activity (units/ml)	Defibration activity[b] (%)
E. carotóvora				
GIR 1026	YU, 5(E)	21.4	0.9	43.3
GIR 1027	YU, 7(E)	60.6	1.7	67.9
GIR 1028	YU, 40	20.1	0.8	38.4
GIR 1031	SU, —	71.0	1.6	80.5
GIR 1032	NU, E7112	4.5	0.2	20.3
GIR 1035	NU, ES7929	4.5	0.1	18.1
GIR 1036	NU, KC13	3.8	0.4	22.0
GIR 1043	TU, OED-1	19.7	0.8	48.8
E. chrysanthemi				
GIR 2003	NU, E7725	1.9	0.1	12.4
GIR 2004	NU, E7732	5.5	0.3	19.6

[a] YU: Faculty of Agriculture, Yamagata University, Yamagata, Japan.
SU: Faculty of Agriculture, Shizuoka University, Shizuoka, Japan.
NU: Faculty of Agriculture, Niigata University, Niigata, Japan.
TU: Faculty of Agriculture, The University of Tokyo, Tokyo, Japan.

[b] Expressed as (100−shives content).

Table II. Defibration Activity of Three Crude Enzymes Showing Typical PATE/PNTE Ratios toward Several Plant Tissues

Plant tissue	*E. chrysanthemi* GIR 2002	*E. carotovora* FERM P-7576	MC-treated FERM P-7576
Kôzo	21.3 (%)	40.5 (%)	13.8 (%)
Gampi	50.4	81.1	27.1
Flax	17.9	37.5	2.4
Kenaf	18.5	44.3	7.5
Sugarcane	20.8	36.8	7.1

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found to be remarkably lower than that of the corresponding one from the untreated strain (Table II). As reported earlier,[7] through this treatment with a kind of DNA-synthesis inhibitor,[11~15] PNTE productivity is enhanced while PATE productivity is depressed approximately to the level of the PNTE, though the total productivity remains almost constant. Tsuyumu *et al.* have proposed the presence of DNA-synthesis inhibitors in plant tissues, which may cause plant disease due to the induction of PNTE production.[16,17] It is probable that the production ratio of PATE to PNTE shifts to the optimal value for the pathogenicity in the presence of an appropriate amount of the PNTE-inducing agents in plant tissues, but their excessive presence depresses PATE production, which culminates in the non-appearance of the disease symptoms.

In this study, it was found that the same maceration behavior was widely observed as in the case of mitsumata bast and the enzyme from *E. carotovoa* FERM P-7576. This finding suggests that the previously proposed mechanism for the maceration of mitsumata bast by the enzyme from FERM P-7576, the concerted actions of *endo*-PATE as the primary agent and *endo*-PNTE as a supplementary but indispensable one, is generally acceptable for our enzymatic-pulping process under alkaline conditions.

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