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단 보

Plant Cell Wall as an Inducer of Pectate Lyase of *Erwinia rhapontici*

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*Erwinia rhapontici*의 Pectate Lyase를 유도하는 식물 세포벽

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ABSTRACT : Pectate lyase (Pel) depolymerizes pectin and polygalacturonate and is thought to play a role in bacterial invasion into host plants. Effects of plant extracts and cell walls on the induction of Pel from *Erwinia rhapontici* strain 1 were examined to know host-parasite relationships associated with the pathogenicity of *E. rhapontici*. Pel activity was detected neither in minimal salts glycerol (MSG) medium nor in minimal salts polygalacturonate (MSP) medium containing plant extracts. Pel activity, however, was detected in MSP media amended with cell walls of Chinese cabbage, lettuce leaves, potato tubers, celery petioles, onion bulbs and carrot roots at the late stage of the bacterial culture. The Pel-inducing plant factors were water-insoluble and heat-labile.

***Key words* : *Erwinia rhapontici*, cell wall, pectate lyase.**

Since *Erwinia rhapontici* was first described from rhubarb by Millard (10), it has been reported from onion (9, 11), garlic (4), hyacinth (16) and wheat (15). *E. rhapontici* rotted Chinese cabbage and lettuce leaves, radish and carrot roots, potato tubers and celery petioles by artificial inoculation (4).

Soft rot *Erwinia* produces cell wall degrading enzymes such as pectate lyase (Pel), polygalacturonase, cellulase and pectin lyase. One of the most important enzymes in cell wall degradation is Pel which has been shown to be an important virulence determinant of the soft rot *Erwinia* (2, 6). Pel production by the bacteria is inducible by the degradation products of the pectin in the culture medium without plant tissues (14), and is also induced in planta (3, 7, 12, 13) and by plant extracts (17). Pel is also induced upon the contact of the bacteria with the appropriate plant tissues and plant cell walls (5, 18). Zuker *et al.* (20) and Zuker and Hankin (18, 19) reported that potato tissue contributes a factor which makes a large synergistic effect on induction of Pel synthesis in *E. carotovora* and *Pseudomonas fluorescens*. Pel production in *E. chrysanthemi* was also induced by isolated plant cell walls (5).

In contrast, *E. rhapontici* did not produce Pel in culture media containing glycerol, pectin and polygalacturonate as a carbon source (3). However, Pel, which caused electrolyte loss, tissue maceration and cell death of potato tuber tissue, was detected in the macerated plant tissues after inoculation of *E. rhapontici*. Therefore, Pel is probably an important factor in the pathogenicity of *E. rhapontici* (3). In this study, experiments were designed and conducted to understand the significance of Pel induction in *E. rhapontici* by cell walls of various host plants in relation to its pathogenicity.

E. rhapontici strain 1 (Er 1) (3) was grown in minimum salts (MS)-glycerol (MSG) medium which was composed of MS (2) supplemented with 0.5% (v/v) glycerol. Plant extracts and cell walls were prepared from Chinese cabbage, lettuce leaves, potato tubers, carrot roots, onion bulbs and celery petioles by the method of Hahn *et al.* (8). MS media supplemented with 0.5% (w/v) polygalacturonate (MSP media) were added with 0.5% (w/v) extracts of Chinese cabbage, let-

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tuce leaves, potato tubers, celery petioles, onion bulbs and carrot roots, 0.5% (w/v) cell walls of the plant parts that were sterilized with diethyl ether, or autoclaved plant cell wall of Chinese cabbage. Each medium in a 250 ml flask was inoculated with Er 1, and cultured at 28°C on a shaker (140 rpm). A soft rot *Erwinia* species, *E. chrysanthemi* Ec 16, was also tested in its capacity to produce Pel as mentioned above using the extract and the cell wall of only Chinese cabbage.

To determine the activity of Pel present in the medium, bacterial cultures were centrifuged at 10,000 rpm for 20 min (4°C), and the supernatants were used for Pel assay. Pel assay conditions were described previously (3). One unit of Pel activity was defined as the amount of enzyme that caused an increase of 1.0 absorbance (235 nm) at 30°C per min.

Er I produced Pel only when it was grown in MSP medium supplemented with the cell walls of Chinese cabbage, lettuce leaves, celery petioles, carrot roots, and onion bulbs (Table 1). Pel activity was not detected in the bacterial cultures amended with glycerol, pectin, polygalacturonate and extracts of Chinese cabbage, lettuce leaves, celery petioles, carrot roots and onion bulbs. Pel was also not induced by the autoclaved cell wall of Chinese cabbage. These results suggest that Pel inducer(s) may be heat-sensitive and present in cell walls as non-diffusible forms (8). However, the nature of the inducing substance from the host plant and the bacterial gene that governs the response to plants are not known.

In contrast to *E. rapontici*, *E. chrysanthemi* Ec 16 induced Pel in the media with or without plant cell wall. Pel was induced in MS medium with glycerol or pectin, and in MSP medium added with Chinese cabbage extract as well as plant cell wall. The differences in Pel induction between *E. rapondici* and *E. chrysansemi* have not been well documented yet. Pectin is degraded to PGA and galacturonate which can be utilized as nutrients for the production of Pel by *E. chrysanthemi* strains. However, *E. rhapontici* could not utilize PGA to produce Pel (data not shown), suggesting that the system of Pel induction in *E. rhapontici* may be considerably different from that in *E. chrysanthemi*, al-

Table 1. Effect of various supplements on pectate lyase production from *E. rhapontici* 1 (Er 1) and *E. chrysanthemi* 16 (Ec 16) in minimal salts medium

Bacterial strain	Media[a]	Activity (units/ml)[b]		
		1	3	5 day
Er 1	MS+glycerol	0	0	0
	MS+PGA	0	0	0
	MS+PGA+Chinese cabbage CW	0	1.5	2.9
	MS+PGA+lettuce CW	0	0.9	2.3
	MS+PGA+celery CW	0	1.6	4.9
	MS+PGA+carrot CW	0	1.8	3.8
	MS+PGA+potato CW	0	0	0.4
	MS+PGA+onion CW	0	0.6	1.7
	MS+PGA+Chinese cabbage extract	0	0	0
	MS+PGA+lettuce extract	0	0	0
	MS+PGA+celery extract	0	0	0
	MS+PGA+carrot extract	0	0	0
	MS+PGA+potato extract	0	0	0
	MS+PGA+onion extract	0	0	0
	MS+PGA+autoclaved Chinese cabbage CW	0	0	0
Ec 16	MS+glycerol	0.4	0.6	0.3
	MS+PGA	1.5	8.7	6.1
	MS+PGA+Chinese cabbage CW	2.0	5.4	4.9
	MS+PGA+Chinese cabbage extract	3.7	5.9	4.4
	MS+PGA+autoclaved Chinese cabbage CW	1.6	1.9	2.4

[a] MS : Minimal salts, PGA : Polygalacturonic acid, CW : Cell wall. Carbon sources, plants cell walls and plant extracts were added to make 0.5% (w/v) final concentrations.

[b] Enzyme activity in the supernatant of bacterial filtrate was measured.

though soft rot symptoms caused by the two organisms are similar.

요 약

Pectate lyase(Pel)는 펙틴과 펙틴산을 분해하며, 기주식물의 감염에 관여한다. *Erwinia rhapontici*에 있어서 기주와 병원균의 병원성과의 상호관계를 구명하기 위하여 pectate lyase(Pel) 활성에 미치는 식물체 추출물과 세포벽의 효과를 검토하였다. 본 균은 glycerol이 포함된 minimal salts(MSG) 배지와 식물체 추출물이 첨가된 MSP 배지에서는 Pel 활성이 검출되지 않았다. 그러나 배추, 상추 잎, 감자 괴경, 셀러리 잎자루, 양파 인경, 당근 뿌리의 세포벽이 첨가된 MSP 배지에서는 Pel의 활성이 검출되었다. Pel을 유도하는 식물 인자는 불용성이고, 열처리에 불안전하였다.

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