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Soft Rot of Radish Caused by *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi*

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ABSTRACT

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Bacterial soft rot of radish frequently occurs in Taiwan and was reported to be caused by *Erwinia carotovora* subsp. *carotovora* (Ecc). In 1988, a total of fifty strains of soft-rotting *Erwinia* was isolated from diseased radish tissues collected from several fields in the central and southern parts of Taiwan, among them, forty-six strains were identified as Ecc, while the other four strains were *Erwinia chrysanthemi* (Ech) with phenotypic characteristics similar to the strains in subdivision II according to Dickey's classification. Strains of both Ecc and Ech induced soft rot extensively on a wide variety of vegetable tissues. However, Ech strains were more virulent to bulb scales of onion than most Ecc strains. The rotting ability of Ech on radish taproot tissues was also stronger than that of Ecc especially at higher temperature (32°C). In addition, when radish taproot tissues were inoculated with cell suspensions of both Ecc and Ech in a 1:1 ratio, Ecc was dominant in the rotted tissues at 28°C or below, but Ech became dominant at higher temperature (32°C).

(Key words: radish, bacterial soft rot, *Raphanus sativus, Erwinia carotovora* subsp. *carotovora, Erwinia chrysanthemi).*

INTRODUCTION

Bacterial soft rot has been observed on a wide variety of crops in Taiwan[8,16]. It is a very destructive disease and often causes a great loss of crops. Radish (*Raphanus sativus* L.) is an important vegetable grown commonly in Taiwan. It is mainly used as cooked vegetable and in pickle. Bacterial soft rot of this crop has frequently occurred in the fields especially when the growning season is wet and warm. The di-

sease was reported to be caused by *Erwinia caropovora* subsp. *carotovora* (Ecc) [8,16], but has not yet been sufficiently investigated, however. In 1988, a strain of *Erwinia chrysanthemi* (Ech) was incidentally isolated from soft rot affected radish tissues collected from a field. It brought our attention because there was no report relating Ech to the etiology of radish soft rot in field conditions. The objectives of this study were to determine the species of soft rot erwinias associated with the bacterial soft rot of radish in Taiwan and to examine the effect of temperature on the virulence and population of Ecc and Ech to and in radish taproot tissues.

MATERIALS AND METHODS

Samples of bacterial soft rot affected radish tissues were collected from fields mostly located in central and southern parts of Taiwan. Isolations were made on nutrient agar (NA) or CVP plates[2,17]. *Erwinia*-like colonies formed on the plates were selected and purified for further studies. The bacterial strains obtained were maintained in steril distilled water in screw cap tubes at room temperature. Before being used they were streaked on NA or CPG plates [2] and incubated at 30°C for 48 hr. A single colony from each strain was then transferred to NA or YDC medium [5] and incubated for 2 days before it was used for various tests. Methods to determine physiological and biochemical characteristics of the cultures were according to methodology recommended by Dye[5,6], Dickey[3], Graham [7] and Schaad[15].

The ability of the bacterial strains to induce soft rot was tested in the laboratory on the detached plant parts of unknown cultivars from markets. Slices or sections of storage organs or fruits and detached petioles of plants were inoculated by a toothpick inserting into tissues with a small amount of inoculum taken from a 24-48 hr YDC slant culture of each strain. They were then placed in a sealed plastic bag with a high moisture and incubated at 30°C. Observations on rotting of tissues were made 48 hr after inoculation.

In the experiment to examine the effect of temperature on the virulence of Ecc and Ech to radish taproot tissues, taproots were washed and surface disinfested by immersion in 10% Clorox (final concentration NaOCL 0.5%) for 20 min, then rinsed with distilled water and allowed to air dry. A 0.1 ml of bacterial suspension containing $3x10^7$ CFU/ml was inoculated into each of the holes (9 mm in diam. and 5 mm in depth) made on taproots. They were then placed in a sealed plastic bag with a high moisture and incubated at various temperatures. The wet weight of macerated tissues was measured 48 hr after inoculation.

To determine the effect of temperature on the population of Ecc and Ech in rotted radish taproot tissues, a 50 μl of bacterial suspension containing 3×10^7 CFU / ml of Ecc RSC32 was added with equal volume of Ech RSC4 at the same concentration into the hole (9mm in diam. and 5mm in depth) made on radish taproot. They were then placed in a sealed plastic bag with a high moisture and incubated at various temperatures. Portions of fluid from rotted juicy tissues were removed 48 hr after inoculation. Serial dilutions were made and plated on eggyolk agar [14]. On eggyolk agar, Ech RSC4 produced lecithinase and formed colonies with opaque zones around them, while Ecc RSC32 did not produce lecithinase, and formed colonies without opaque zones. The different growth characteristics of Ecc RSC32 and Ech RSC4 on eggyolk

agar allow differentiation of these two bacteria when they were mixed together. The ratio of populations of these two bacteria in the fluid of rotted juicy tissues could therefore be determined.

RESULTS AND DISCUSSION

In 1988 fifty strains of *Erwinia*-like bacteria were isolated from rotted radish petioles and taproot tissues(Fig. 1) collected from fields at various localities mostly in central and southern parts of Taiwan. These strains induced soft rot on slices of potato tubers, and were Gram-negative, oxidase negative, facultatively anaerobic rods, motile with peritrichous flagella and fermented glucose. All these strains were therefore considered as soft rot erwinias [15]. These strains were fruther characterized into species or subspecies according to Garham[7] and Schaad[15]. Of these, forty-six strains were identified as Ecc and the other four

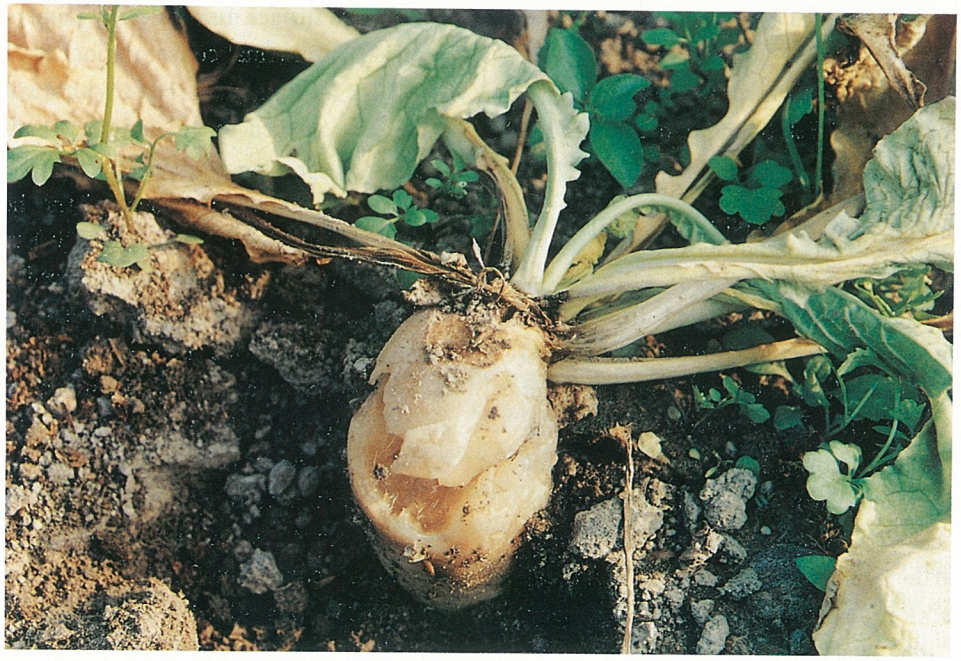


Fig. 1. Symptoms caused by soft-rotting *Erwinia* on radish.

strains were Ech (Table 1). Strains of Ecc were isolated from samples collected from fields in eight counties whereas strains of Ech were only isolated from one field at Taichung county. Thus, bacterial soft rot of radish occurred in central and southern parts of Taiwan, was mainly caused by Ecc. However, Ech was the sole soft-rot bacterium isolated from a few diseased samples, it could also be the primary pathogen of the disease in certain fields. This appears to be the first report to associate Ech with bacterial soft rot of radish in field conditions. Certain physiological variations among Ecc strains were observed in this study. According to Graham [7] and Dye [6], most Ecc strains do nto produce indole. However, about 76% of Ecc strains from radish in this study produced it (Table 1). Our previous study also revealed that above half of Ecc strains from various hosts from Taiwan produced indloe[17]. In addition, although Ecc radish strains produced acid from trehalose, there were two

strains did not (Table 1). Both Ecc and Ech have a wide host range. Ech infects plants which include especially tropical and subtropical crops[1,3]. Strains of Ech had been isolated from celery, corn, potato, Welsh onion, *Rhycostylis gigantea* and *Phalaenopsis* sp. from the field conditions in Taiwan [8,9]. Dickey[3] and Dickey

Table 1. Characteristics of soft-rotting *Erwinia* from radish in Taiwan

Characteristics	Reaction [1]	
	E. carotovora subsp. *carotovora* (40 strains)	*E. chrysanthemi* (4 strains)
Acid from		
lactose	+	−
maltose	−	−
trehalose	+(44)	−
α-methylglucoside	−	−
Indole	+(35)	+
Growth in 5% NaCl	+	−
Lecithinase	−	+
Phosphatase	−	+
Reducing substances from sucrose	−	−
Sensitivity to erythromycin (50 μg/ml)	−	+

1) +, all strains positive; −, all strains negative;
+ ( ), number in parenthesis indicated number of strains showed positive reaction.

and Victoria [4] divided strains of Ech into six subdivisions based on physiological and biochemical properties. Two phenotypic subdivisions, II and IV, were identified in Taiwan[8]. Ech strains from corn, Welsh onion, *Rhyncostylis gigantea* and *Phalaenopsis* sp. and most strains from potato were in subdivision IV. Ech strains in subdivision II had only been isolated from celery and potato[8,18]. The four Ech radish strains isolated in this study, liquefied gelatin, utilized sodium tartrate, were sensitive to penicillin G, grew at 39°C, produced acid from melibiose, raffinose, but not from D-arabinose and inulin. Their characteristics were therefore similar to those of the strains in Dickey's phenotypic subdivision II [3,4]

To examine the ability of the bacterial strains to induce soft rot on plant tissues,

ten randomly selected strains of Ecc and four strains of Ech were tested. Strains of Ecc and Ech usually induced soft rot extensively on storage tissue slices of radish and potato, petioles of cabbage and Chinese cabbage, and fruit sections of cucumber, eggplant and sweet pepper. However, difference in maceration ability was observed when detached bulb scales of onion were inoculated. All four Ech strains showed strong rotting ability on the bulb scales of onion, while most Ecc strains were very weak.

Temperature has a great effect on the relative virulence of soft rot bacteria[10,11]. In the induction of decay in potato tubers, *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica* (Eca) and pectolytic clostridia were equally virulent at 22°C, but there were marked differences at 16°C [12]. In the rotted tubers, Eca was dominant at 16°C whereas the clostridia were dominant at 20°C [12]. Our experiments conducted on the radish taproot tissues showed that Ech RSC4 was more virulent than Ecc RSC32 especially at higher temperature (32°C). The rotting ability of both Ecc RSC32 and Ech RSC4 decreased greatly when incubation temperature was lowered to 20°C (Table 2). When Ecc RSC32 was inoculated with Ech RSC4 into radish taproot tissues, Ecc RSC32 was dominant at the temperature in the range of 20-28°C, while Ech RSC4 became dominant in the decayed tissues when temperature was increased to 32°C (Table 3). This seemed to reflect their growth temperature characteristics because Ech has a higher optimum temperature than Ecc [13]. The differential effects of temperature on the relative virulence of different soft rot bacteria may be attributed to the growth rate and the level of enzyme production at various temperatures[10]. Although only four strains of Ech were isolated from one field in this study, it seemed that Ech might be dominant and become the main causal agent of the disease in certain circumstances.

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Table 2. Effect of temperature on decay of radish taproot tissues 48 hr after inoculation with *Erwinia carotovora* subsp.*carotovora* RSC32 and *Erwinia chrysanthemi* RSC4 [1)]

Temperature (°C)	Tissue decayed (g fresh weight) [2)]	
	E. *carotovora* subsp. *carotovora* RSC 32	*E. chrysanthemi* RSC32
20	1.8 e	0.7 e
24	7.9 d	16.4 c
28	11.2 d	39.5 b
32	12.7 cd	48.1 a

1) A 0.1 ml of bacterial suspension containing $3x10^7$ cells/ml of each strain was inoculated into the holes (9 mm in diam. and 5 mm in depth) made on taproots.
2) Each value was mean of 9 replicates. Values followed by the same letter were not significantly different (P = 0.01) by Duncan's multiple range test.

Table 3. Effect of temperature on populations of *Erwinia carotovora* subsp. *carotovora* RSC 32 (Ecc RSC32) and *Erwinia chrysanthemi* RSC 4 (Ech RSC4) in decayed taproot tissues 48 hr after inoculation with a mixture of both bacteria [1)]

Temperature (°C)	Ratio of populations (%) [2)]		
	Ecc RSC 32	:	Ech RSC 4
20	80.8	:	19.2
24	73.0	:	27.0
28	78.9	:	21.1
32	1.8	:	98.2

1) Cell suspensions of both Ecc RSC32 and Ech RSC4 were inoculated into the taproot tissues in a 1:1 ratio with each containing $1.5x10^6$ cells.
2) Populations of Ecc RSC32 and Ech RSC4 in the decayed tissues were determined on egg-yolk agar. On which colonies of Ech RSC4 could be differentiated from those of Ecc RSC32.

Erwinia carotovora subsp. *carotovora*與*Erwinia chrysanthemi*引起蘿蔔細菌性軟腐病

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摘 要

莊明富、曾國欽、徐世典 1989 ***Erwinia carotovora*** subsp. ***carotovora*與*Erwinia chrysanthemi*引起蘿蔔細菌性軟腐病** 植保會刊31：358—365。

蘿蔔細菌性軟腐病於台灣田間經常可見，然對其病原細菌之特性，未曾有較深入之探討。民國77年間筆者等自台灣中南部不同地區之蘿蔔田，採回罹軟腐病之蘿蔔塊根與葉柄組織，共分離了50個*Erwinia*屬軟腐細菌菌株，經生理生化特性測定結果，其中46個菌株為*Erwinia carotovora* subsp. *carotovora*（Ecc），另4個菌株為*Erwinia chrysanthemi*（Ech），此4個Ech菌株皆屬於Dickey分類體系之第Ⅱ型。Ecc與Ech菌株對許多蔬菜組織常具有強之致腐能力，然而Ecc與Ech菌株在洋蔥鱗片上之致腐能力，則有很大差異，許多Ecc菌株對洋蔥鱗片之致腐能力很弱，而Ech菌株則很強。又溫度可影響此兩種軟腐細菌之致腐能力，於蘿蔔塊根組織上，Ech菌株常較Ecc菌株之致腐能為強，尤其於較高的溫度下（32℃）。以等量之Ecc與Ech細菌同時接種於蘿蔔塊根組織，在較高的溫度下（32℃），於軟腐組織中Ech之族羣較Ecc爲優勢。

（關鍵字：蘿蔔，細菌性軟腐病）。