Pseudomonas syringae pv.maculicolaの病原性におけるコロナチン産生の役割

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Roles of Coronatine Production by *Pseudomonas syringae* pv. *maculicola* for Pathogenicity

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Pseudomonas syringae pv. *maculicola* (MacCulloch) Young *et al.*, a pathogen of bacterial leaf spot of crucifers, causes necrotic lesions surrounded by chlorotic halos on infected host leaves. This bacterium produces a chlorosis-inducing phytotoxin coronatine[8], which is also known to be produced by several other *P. syringae* pathovars including : pv. *atropurpurea* (Reddy & Godkin) Young *et al.*[12], pv. *glycinea* (Coerper) Young *et al.*[10], pv. *tomato* (Okabe) Young *et al.*[9], and pv. *morsprunorum* (Wormald) Young *et al.*[8]. Generally, phytotoxin production appears to be an important component in plant pathogenesis for most pathovars of *P. syringae*[6]. Coronatine production by *P. s.* pv. *atropurpurea* was reported to contribute to the formation of necrotic lesions with chlorotic halo but not to interfere with the growth of the bacteria in Italian ryegrass leaves[13,14]. On the other hand, it was reported that coronatine synthesis is important in the virulence of *P. s.* pv. *tomato* and contributes significantly to both lesion expansion and multiplication of the bacterium in tomato leaves[2]. These facts apparently suggest that coronatine does not always play uniform roles in pathogenesis among different groups of coronatine-producing bacteria. In contrast to those two bacteria, little is known about the roles of coronatine production for *P. s.* pv. *maculicola*. Hence this paper describes an evaluative study on its role(s) for the pathogenicity in the bacterium.

Coronatine production by *P. s.* pv. *maculicola* strain H3-6 is mediated by an 83 kb plasmid pMAC1 carrying coronatine biosynthesis genes[17], analogous to pCOR1 in *P. s.* pv. *atropurpurea*[15], p4180A in pv. *glycinea*[3], and pPT23A in pv. *tomato*[1]. A coronatine non-producing derivative (Cor^-) of H3-6 designated 6-1-3 was previously generated by curing of pMAC1[17]. The strain 6-1-3 was indistinguishable from H3-6 ; growth rate on King's B medium[7], colony morphology and some physiological properties were not measurably different (data not shown). Pathogenicity of 6-1-3 was compared to H3-6 using 5 week old Chinese cabbage plants (*Brassica campestris* var. *pekinensis*, cv. Akihuku) that were grown in greenhouse at 25°C. Five milliliter of each bacterial suspension (*ca.* 1×10^6 cfu/ml) was sprayed on the back side of a leaf blade ; each sample was done in triplicate. After which the inoculated plants were incubated in a humid chamber for 12 hr, and then transferred to greenhouse at 25°C. Three days after inoculation, H3-6 and 6-1-3 produced similar water-soaked lesions that changed into black necrotic lesions at 2-3 mm in diameter 2 weeks after inoculation. Strain 6-1-3 produced no chlorotic halo around the necrotic lesions in contrast to H3-6 which produced narrow yellowish halos around them (Fig. 1A, B). The number of lesions per leaf and size of lesions were basically similar between H3-6 and 6-1-3.

The effect of coronatine on lesion expansion on leaf midrib was also examined. Puncture-inoculation was performed by pricking the surface of leaf midrib with a single needle (22G) through a drop (5 μl) of each bacterial suspension (*ca.* 1×10^6 cfu/ml) placed on it. Water-soaked stripe lesions caused by H3-6 gradually elongated along the vascular system whereas black necrotic lesions caused by 6-1-3 did not elongate and were limited to the inoculated sites (Fig. 1C, D).

To compare the growth rate of wild type versus Cor^- derivative in host leaf tissues, the bacterial populations were monitored at timed intervals after inoculation. Five microliter of each bacterial suspension (*ca.* 1×10^6 cfu/ml) was dropped on the surface of leaf blade, and then the leaf was pricked with a single needle through the drop of suspension. Three pieces of leaf discs (10 mm in diameter) including the inoculated point were cut off with a cork borer imediately, 1, 2, 4, 7, 10 and 14 days

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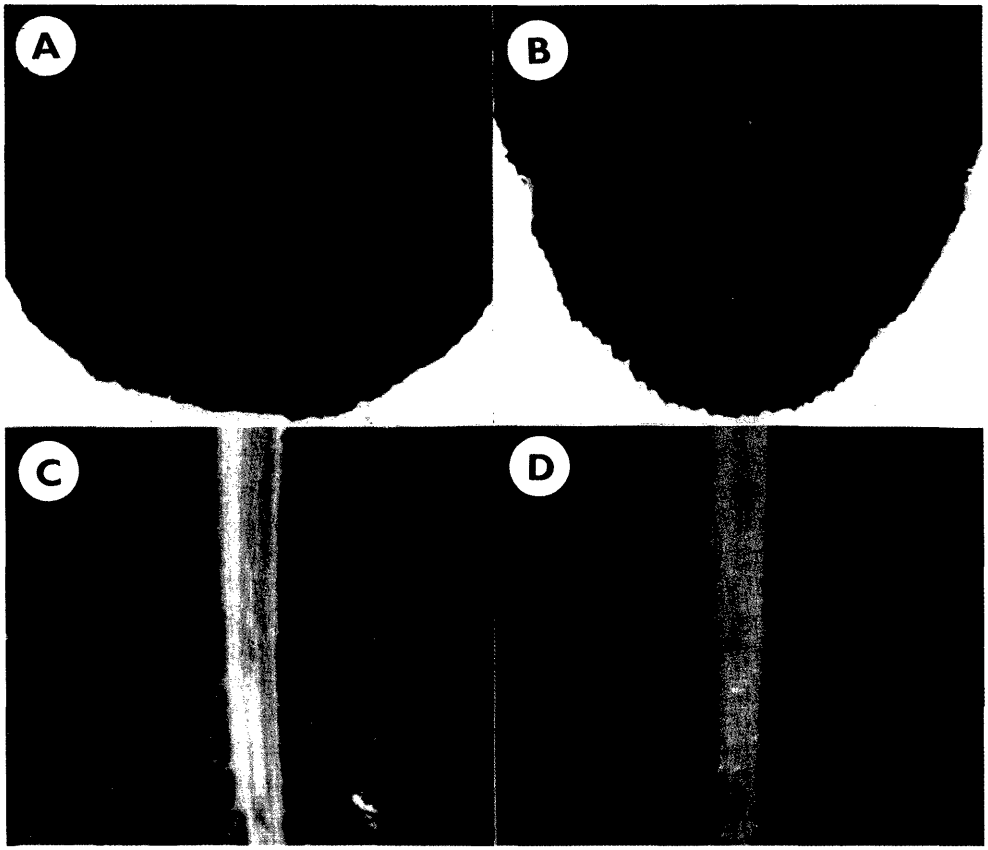

Fig. 1. Disease symptoms on leaf blades (A, B) and midribs (C, D) of Chinese cabbage inoculated with *P. syringae* pv. *maculicola* H3-6 (A, C) and 6-1-3 (B, D). Leaf blades and midribs were inoculated by spray and stab method, respectively.

after inoculation, and each leaf disc was homogenized in sterile water with a mortar and pestle. Viable bacteria in the homogenates were counted by the serial dilution plate method on King's B plate. Two independent experiments were conducted. Time course analysis revealed that strain 6-1-3 grew at the same rate as H3-6 ; bacterial populations of both 6-1-3 and H3-6 rapidly increased to about 10^5 cfu per leaf disc within 4 days and peaked over 10^6 cfu per leaf disc 10 days after inoculation (Fig. 2).

Although *P. s.* pv. *maculicola* has been thought to produce coronatine as one of virulence factors, role(s) of coronatine in pathogenesis have not been evaluated in this bacterium so far. Inoculation tests of Cor^- derivative of *P. s.* pv. *maculicola* H3-6 revealed that coronatine is not required for some parts of symptom development such as necrotic lesion formation, but does contribute to development of chlorotic halo symptom. These observations on symptom expression well coincided with those in the cases of *P. s.* pv. *tomato*[2].

It was also found that coronatine might assist the expansion of lesions especially on leaf midribs, though, it is unclear whether coronatine functions directly in lesion expansion or in allowing the pathogen to spread its population in leaf midrib tissues. Since the occurrence of lesion development on leaf midribs is mostly

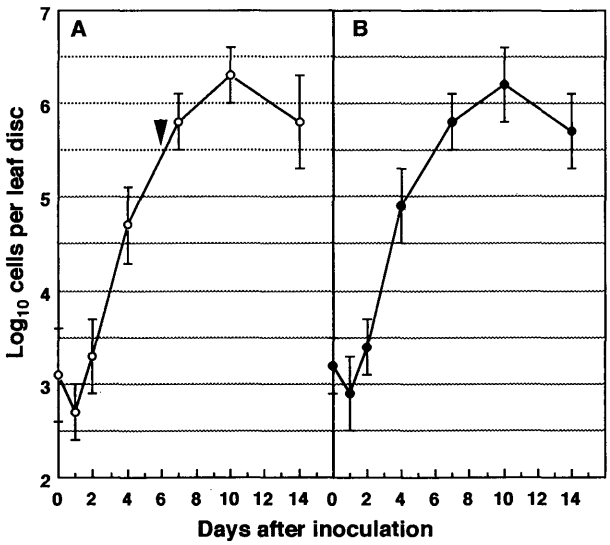

Fig. 2. Time course of bacterial population in leaf tissues of Chinese cabbage inoculated with *P. syringae* pv. *maculicola* H3-6 (A) and 6-1-3 (B). Each value represents an average of the numbers of viable bacteria in 6 leaf discs, and the vertical bars mean standard deviations. The arrow head indicates the point of time at which the chlorotic halo symptom appeared.

uncommon under natural conditions, this test does not completely mimic the natural infections. Nevertheless, it is possible that wounding may facilitate the lesion expansion by coronatine. The rate of bacterial growth in leaf tissues was similar between wild type and Cor$^-$ derivative, suggesting that coronatine did not affect the bacterial growth in host tissues. This observation contrasts that of *P. s.* pv. *tomato*, in which coronatine enhances bacterial growth rate in tomato leaf tissues[2]. In addition, for the infection of *P. s.* pv. *tomato* strain DC3000 on *Arabidopsis*, roles of coronatine were reported to differ depending on the inoculation conditions[11]. It was demonstrated that coronatine production is required under more natural inoculation conditions for the successful infection on *Arabidopsis* by the bacterium, and that coronatine may play a critical role during the early stages of infection. In this study we used the spray inoculation method, which may possibly cause stomatal infection that is close to natural infection conditions. Thus, the roles of coronatine for *P. s.* pv. *maculicola* appear to be slightly different from those for *P. s.* pv. *tomato*.

Strains of *P. s.* pv. *maculicola* have been reported to be classified into four distinct groups on the basis of several bacteriological properties and symptoms on cauliflower, turnip, radish and tomato[16]. Among the four groups, one group did not produce coronatine as a result of protrusion assay of potato tuber tissues. On the other hand, *P. s.* pv. *tomato* was reported to include different strains based on the production of different symptoms and toxins[9]. Moreover, *P. s.* pv. *maculicola* and pv. *tomato* are thought to be closely related[4,5], and both pathotype strains were reported to be settled into one of the four groups, leading a conclusion that they are proposed to be identified as pv. *maculicola*[16]. These suggest that the strains of *P. s.* pv. *maculicola* and pv. *tomato* can be relatively heterogenic in taxonomic terms. Therefore, we think that the roles of coronatine production for *P. s.* pv. *maculicola* must be evaluated dependent upon each combination of bacterial strains and host plants.

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和 文 摘 要

田村勝徳・朱 亜峰・佐藤 守・寺岡 徹・細川大二郎・渡辺実：*Pseudomonas syringae* pv. *maculicola* の病原性におけるコロナチン産生の役割

Pseudomonas syringae pv. *maculicola* の病原性に果たすコロナチンの役割について検討した。菌株はハクサイ分離株 H3-6 と，コロナチン産生遺伝子を保有するプラスミド pMAC1 を除去したコロナチン非産生変異株 6-1-3 を用いた。約 10^6 cfu/ml の細菌懸濁液をハクサイ葉身に噴霧接種したところ，いずれも接種約1週間後に直径2～3 mm の黒色壊死斑を形成した。6-1-3 株と H3-6 株との間で病斑数に有意差はなかったが，前者では壊死斑の周囲に黄色ハローは認められなかった。一方，中肋部に穿刺接種した場合，H3-6 株では維管束に沿って水浸状条斑が伸長したのに対して，6-1-3 株では黒色病斑の拡大が顕著に抑制された。また，6-1-3 株はハクサイ葉組織内で H3-6 株とほぼ同様に増殖した。以上の結果から，pv. *maculicola* のコロナチン産生性は細菌の宿主組織内での増殖と黒色壊死斑の形成には影響しないが，黄色ハロー症状の発現と葉中肋部での病斑の拡大に寄与することが明らかとなった。

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