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Proteolytic and deaminative activity in bacterial speck of tomato

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Pseudomonas syringae pv. *tomato* (PST) (Okabe) Alstatt (Alstatt, 1944) causes damage to tomatoes (Goode & Sasser, 1980; Yunis *et al.*, 1980). The pathogen is specific to tomato. Symptoms on the leaves are dark brown to black specks with a distinct yellow halo (Bashan *et al.*, 1981). The primary infection sites are the stomata and the bases of leaf trichomes (Bashan *et al.*, 1981). Necrosis can be observed microscopically 100 h after inoculation. In the susceptible cultivar, VF-198, bacterial counts of the pathogen increased to 10^6 colony forming units per cm^2 of leaf 72 h after inoculation. Bacterial counts decreased in the leaves of the resistant tomato cultivar Rehovot 13 (Bashan *et al.*, 1981).

Culture filtrates of PST and extracts from homogenized diseased leaves caused necrosis and chlorosis when applied to healthy tomato leaves. Considerable amounts of gaseous ammonia were produced during development of bacterial speck of tomato in susceptible plants (Bashan *et al.*, 1980). The pH level of the diseased leaves increased to 7.5 and above, coinciding with the production of ammonia. Electrolyte leakage and symptom formation was preceded by the production of toxic quantities of ammonia (Bashan *et al.*, 1980).

In culture, protease production by PST occurs mainly during the logarithmic

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growth phase. Optimum protease activity was at pH 7.0 and at 30°C. Addition of proteinaceous compounds such as peptone, proteose peptone No. 3, casein hydrolyzate, casein, bovine serum albumin, egg albumin and soluble tomato leaf proteins to the growth medium enhanced enzyme production by PST only slightly. No correlation was found between pathogenicity and proteolytic activity in culture. When compared with PST, several saprophytic pseudomonads had even higher proteolytic activity.

Proteolytic activity was higher in leaves of inoculated susceptible plants than in resistant ones, and reached a maximum at advanced stages of infection (120 h after inoculation). Of the seven proteolytic isozymes found in extracts from diseased plants, four originated from the pathogen, two were from the host, and one new isozyme was produced by the pathogenic interaction. Increasing inoculum concentration did not cause an increase in proteolytic activity. This might be due to the fact that activity occurred mainly at later stages of disease development.

There was a direct correlation between disease severity and proteolytic activity in infected tissue in 21 tomato cultivars and lines with varying susceptibility to the disease. Proteolytic activity occurred mainly around developing necrotic zones, was highest in younger leaves, and decreased with leaf age.

The nitrogen content of infected plants decreased slightly during later stages of disease development. During the first 100 h following inoculation there was a decrease in soluble proteins with a parallel increase in free amino acids, mainly asparagine and glutamine. Only small increases in free amino acid content could be detected in inoculated resistant plants.

The pathogen had the ability to utilize asparagine, glutamine, serine, threonine and glycine as sole nitrogen sources in culture. These amino acids were also degraded in the diseased susceptible plant in that order. PST produced several deaminating enzymes in culture, including asparaginase, glutaminase, threonine dehydratase, glycine synthase and serine dehydratase. However, only asparaginase and glutaminase were markedly more active in leaves of diseased plants than in healthy ones.

Proteolytic and deaminating activities of several saprophytic pseudomonads were similar to or higher than those of PST grown in culture, but they failed to cause disease in tomato plants. The amino acids asparagine, glutamine, serine, threonine and glycine were applied separately to leaves at a concentration of 10^{-3}M before inoculation with PST. Their presence resulted in greater disease development, necrosis and ammonia production.

We suggest that necrosis formation in bacterial speck of tomato develops as follows: PST produces and induces proteolytic activity in the susceptible plant. Free amino-acids, especially asparagine and glutamine, are produced and apparently degraded by the pathogen to ammonium and carbon skeleton in diseased tissue by the deaminating activity of asparaginase and glutaminase.

The ammonium ion liberated accumulates in the tissue, and when the pH reaches or exceeds 7.5 the ion is converted into gaseous ammonia. It causes irreversible

damage to membranes in the surrounding area, resulting in electrolyte leakage from the tissue and formation of speck symptoms.

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Mechanisms of antagonism toward *Pseudomonas syringae* by non-ice nucleation active bacteria on leaves

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Three species of epiphytic bacteria incite frost injury to the plants on which they reside by a process known as ice nucleation. The two most important species include many pathotypes of *Pseudomonas syringae*, and *Erwinia herbicola* (Lindow, 1982d, 1983 b).

These species are very active in ice nucleation and catalyze ice formation in water, which would otherwise supercool, at temperatures only slightly below 0 degrees C (Lindow, 1983b). Because of their ubiquity on leaf surfaces, ice formation can be detrimental to frost sensitive plants such as corn. We have also previously shown that the frost sensitivity of corn and other plants treated with certain antagonistic non-ice nucleation active bacteria is lower than the same plants challenge inoculated only with ice nucleation active strains of either *P. syringae* or *E. herbicola* (Lindow, 1982, a,c,d, 1983α; Lindow *et al.*, 1983 α,b). This form of biological control of frost injury has been shown to be effective on several different crop species and appears to be due to the limitation of epiphytic populations of *P. syringae* and *E. herbicola* in the presence of non-ice nucleation active bacteria (Lindow 1982c, 1983α).

A collection of eighty-eight non-ice nucleation active bacteria have been identified from the surfaces of six plant species to be highly effective antagonistic bacteria to