

ABSTRACT

In this study the relationship between the intrinsic levels of the extracellular enzymes protease, amylase, phosphatases, and extracellular polysaccharides (EPS) in *Xanthomonas campestris* pv. *phaseoli* (*X. c.* pv. *phaseoli*) strains and pathogenesis on three cultivars of *Phaseolus vulgaris* were examined. Results failed to show a definite correlation between symptom development and the levels of extracellular enzyme activity in *X. c.* pv. *phaseoli* strains. For example *X. c.* pv. *phaseoli fuscans* strain EK-11 showed very good virulence on kidney bean cultivars and also high activities for extracellular enzymes, nonpathogenic epiphytic *X. c.* pv. *phaseoli* strain 395-86 produced comparable levels of extracellular enzymes to those of pathogenic *X. c.* pv. *phaseoli* strains despite being avirulent. There was also no direct correlation between EPS production and virulence. The strain Xp dry which produced a relatively low level of EPS was very virulent, while epiphytic strains 395-86 and RCR-32 despite being avirulent produced large amounts of EPS. There was no correlation between phosphatase levels and virulence.

Protease and amylase from two pathogenic strains, *X. c.* pv. *phaseoli* strain V4S1 and *X. c.* pv. *phaseoli fuscans* strain EK-11, and an epiphytic strain 395-86 were partially purified and characterized. The fraction showing protease activity was purified using ammonium sulphate precipitation, ion exchange chromatography and gel permeation chromatography to yield a product purified by a factor of 8.25. Proteolytic enzyme was characterized by its β -casein digestion pattern. Amylase enzyme was purified using

ammonium sulphate precipitation and ion exchange chromatography to yield a product purified by a factor 2.1. Amylolytic activity was characterized by its iodine blue value, reducing sugar curves, carbohydrate substrate utilization and molecular weight.

Inhibitor studies indicated that the proteolytic enzymes from the three different *X. c. pv. phaseoli* strains were different. While they were all metalloenzymes, their different reactions to metal chelators indicated that these were different. Proteolytic activity of strain V4S1 was a zinc metalloprotein with a β -casein digestion pattern similar to PRT3 described for *X. c. pv campestris* by Dow *et al.* (1993). Its β -casein digestion pattern was distinct from PRT1 and PRT2 described for *X. c. pv campestris* by Dow *et al.* (1990). Digestion of β -Casein digestion resulted in products of molecular weights of ~24 kd, ~17 kd, ~11 kd, ~7 kd. Amylolytic activities from all three *X. c. pv. phaseoli* strains showed differences in their reactions to enzyme inhibitors indicating that they were different enzymes. The molecular weight of purified amylolytic activity from *X. c. pv.* strain V4S1 was ~45 kd. HPLC of the starch digestion products along with the iodine blue value and reducing sugar curves indicate that the amylase is an α -amylase. The temperature optimum for protease activity from *X. c. pv. phaseoli* strains V4S1, *X. c. pv. phaseoli fuscaus* strain EK-11, and epiphytic strain 395-86 were 37°C, 37°C, and 42°C, respectively. The optimum temperature for all three amylolytic activities was 42°C. The pH optimum for proteolytic activity was pH 6.0 while that for amylolytic activity was 6.5.

Chemical and transposon Tn5 mutagenesis were done to isolate protease, amylase

and EPS mutants from *X. c. pv. phaseoli* strain V4S1 to study their role in pathogenicity. Chemical mutagenesis using NTG and EMS produced two types of EPS⁻ mutants, three Amy⁻ Prot⁻ mutants (lacking both amylolytic and proteolytic activities) and one Amy⁺⁺ Prot⁺⁺ mutants (high levels of amylase and protease). No mutants were obtained using Tn5 mutagenesis. One EPS⁻ and all the Amy⁻ Prot⁻ mutants were avirulent. Lysis of Amy⁻ Prot⁻ mutants with chloroform failed to release any intracellular amylolytic and proteolytic activity indicating that there was no synthesis of these enzymes. The other EPS⁻ mutant and the Amy⁺⁺ Prot⁺⁺ mutants were virulent. Spontaneous Amy⁻ Prot⁻ revertants produced lower amylolytic and proteolytic activity than the wild type and were avirulent. The growth rates of the wild type and *X. c. pv. phaseoli* mutants were evaluated in broth culture and on the leaf surface. The growth rates of all strains in broth culture were similar, however the avirulent *X. c. pv. phaseoli* strains failed to multiply on the leaf surface.

This is the first study done to examine the role of extracellular enzymes and EPS in the virulence of *X. c. pv phaseoli*. These results show that while there was no correlation between the wild type levels of extracellular enzymes and virulence, mutants lacking in both amylase and protease were virulent. Similarly there was no correlation between wild type levels of EPS and virulence but one type of EPS⁻ mutant was avirulent while another type showed virulence. The proteolytic activities from *X. c. pv. phaseoli*, *X. c. pv. phaseoli fuscans* and epiphytic *X. c. pv. phaseoli* when characterized were shown to be different enzymes. Revertants of Amy⁻ Prot⁻ which had only a fraction of the amylase and protease

activity were avirulent indicating that both the type and quantity of extracellular enzymes are important for pathogenicity. Avirulent Amy⁻ Prot⁻ and EPS⁻ mutants of *X. c. pv. phaseoli* were unable to multiply on the leaves of *P. vulgaris* but only survived.