Serine Proteases in the Extracellular Preparations of *Phytophthora infestans*: Does their Presence Relate to the Aggressiveness of the Pathogen?

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Abstract

In this study the aggressiveness of nine isolates of *P. infestans* was determined using detached leaflets from cultivars Bintje and Stirling. The growth of the isolates on the leaflets was recorded on a daily basis, for seven days, and an assessment of their aggressiveness could then be made. Extracellular preparations (ECPs) from the zoospore suspension of each isolate were used as a source of proteolytic activity. The ECPs were found to contain a level of serine protease activity using BTEE (N-Benzoyl-L-Tyrosine Ethyl Ester) as a substrate and recording the absorbance at 256 nm. The possible relationship between the serine protease activity and the aggressiveness of the isolate is discussed.

Keywords: *Phytophthora infestans;* aggressiveness; serine protease

INTRODUCTION

The potato crop in Northern Ireland (NI) provides a substantial income to many farmers. Unfortunately the weather conditions in NI favour the development of the oomycete pathogen, *Phytophthora infestans*, responsible for potato late blight, which is estimated to cost ca. 8% of the crop value. In general, fungicides are applied in an attempt to control the disease. However, because of problems, including concerns with environmental pollution and pathogen resistance, an alternative disease control method is required (KREGAR & STRUKELJ 1999; LORITO *et al.* 1994), particularly breeding potato cultivars with durable resistance to late blight.

Studing the pathogenicity of *P. infestans* may contribute to development of a blight resistant potato cultivar. There are many substances which are produced by plant pathogens to aid them to colonise the host. These include a variety of enzymes which degrade cell wall substances such as pectin, cellulose and proteins. The proteins in plant cells are important as constituents of the cell membrane, regulatory enzymes and structural components of the cell wall. The group of enzymes responsible for protein degradation are known as proteases or proteinases. There are four mechanistic classes of these, based on the active amino acid, known as serine, metallo, cysteine and aspartic (LASKAWSKI & KATO 1980).

PARIS and LAMATTINA (1999) showed the presence of proteolytic activity in extracellular preparations (ECPs) of *P. infestans*. In this study, the activity of serine proteases in ECPs of *P. infestans* and its possible association with pathogen aggressiveness has been assessed.

MATERIALS AND METHODS

Nine isolates of *P. infestans* were selected from the APSD/DARD (Applied Plant Science Division, Department of Agriculture & Rural Development) culture collection. Single spore isolates were made from each of the original cultures (CATEN & JINKS 1968). The majority of isolates were from Northern Ireland potato crops of 1996–2000, although one isolate from USA (MT95-RED), and one from Mexico (PINN-A) were included for comparative reasons. The isolates were maintained initially on pea agar plates (HOLLOMAN

1965) and then on detached healthy leaflets of cv. Up-to-Date prior to experimentation.

Aggressiveness test

The aggressiveness of the isolates was assessed by measuring their growth on detached healthy leaflets of cvs. Bintje and Stirling (CARLISLE *et al.* 2002). The cultivars differed in race non-specific resistance to foliage blight, as indicated by their NIAB (National Institute of Agricultural Botany) ratings. On the 1–9 scale, cv. Bintje is rated as 2 (extremely susceptible) and cv. Stirling is rated as 8 (highly resistant). Lesion sizes were recorded daily for seven days after inoculation.

Serine protease activity

Extracellular preparations (ECPs) of each isolate were obtained and used as a source of proteolytic activity (PARIS & LAMATTINA 1999). Zoospore suspensions were prepared from isolates growing on detached lea ets, and standardised to 10^4 zoospores per ml. The serine protease activity was measured using BTEE (N-Benzoyl-L-Tyrosine Ethyl Ester) as the substrate and recording the absorbance at 256 nm (HUMMEL 1959).

RESULTS AND DISCUSSION

In the aggressiveness test each isolate was able to grow on the susceptible cv. Bintje, but more variation was noted in growth on cv. Stirling (Figure 1). The Mexican isolate PINN-A infected very few leaves of cv. Stirling, whereas the NI isolate 16/99 produced large lesions on this cultivar, and would therefore appear to be the most aggressive of the isolates studied.

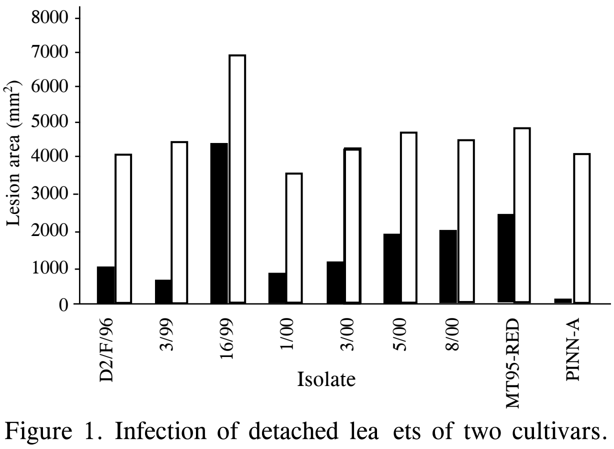

Figure 1. Infection of detached lea ets of two cultivars. Area under lesion expansion curve (AULEC), seven days after inoculation. L.S.D. ($P < 0.05$) = 6.389

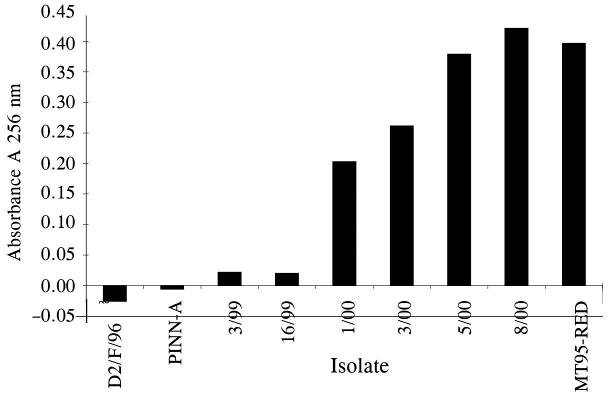

Figure 2. Serine protease activity in the extracellular preparations of *Phytophthora infestans* isolates

Serine protease activity varied markedly between isolates (Figure 2), being greatest with 5/00, 8/00 and MT95-RED. The proteolytic activity did not appear to be related to the aggressiveness of isolates, as indicated by lesion growth rate, since 16/99 which was most aggressive had low serine protease activity.

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