COMPREHENSIVE REPORT FOR 1993

Infection of potato plants by fungal pathogens results in the induction of several proteins (Pierpoint *et al.*, 1990; Woloshuk *et al.*, 1991). A group of proteins known as pathogenesis related proteins are secreted into intracellular spaces. One of the pathogenesis related protein with strong sequence similarity to thaumatin has been shown to inhibit the growth of fungal pathogens *in vitro* (Woloshuk *et al.*, 1991). This protein has been purified from potato, tomato, tobacco and a number of other plants (Vigers *et al.*, 1991; Huynh *et al.*, 1992). The amino terminal sequence of the potato and tomato thaumatin-like protein has been reported recently. An immediate objective of our research project is aimed at cloning a complementary DNA (cDNA) that codes for an antifungal protein (thaumatin-like protein) from potato. The isolated cDNA will be used to generate transgenic plants that are overproducing the antifungal protein to confer resistance to fungal pathogens (Cornelissen and Melchers, 1993).

We have been taking two different approaches to isolate the cDNA. In one approach we are using polymerase chain reaction to amplify the sequences corresponding to thaumatin-like proteins from potato (Pierpoint *et al.*, 1990). In another strategy we are using a heterologous probe (a partial cDNA for thaumatin-like protein from *Arabidopsis*) to screen a potato cDNA library. Both these strategies are yielding positive results as described below.

Polymerase chain reaction (PCR): We are using a cDNA library as a template for PCR amplification. The cDNA library was prepared from RNA isolated from stolon tips and was cloned into a λZAP II vector. Amino terminal amino acid sequence for potato thaumatin-like protein was used in designing a degenerate oligo primer (Fig. 1) which is called AFP (antifungal protein primer). The primers that corresponds to the sequence on either side of the multiple cloning site in the vector are SK and KS. The primers were synthesized in the Macromolecular Resource Facility on the campus. As shown in Figure 2., one of the PCR conditions (Day and Reddy, 1994) used yielded a band the size of which is closer to the expected size product. This amplified product was isolated from the gel and cloned into a plasmid vector. The dsDNA template of plasmid DNA from the clones has been isolated and being used as a template for sequencing. We intend to complete the sequence analysis of these clones within a couple of months.

Screening of a potato cDNA library with a heterologus probe: While our PCR work is in progress, a partial cDNA from *Arabidopsis* that codes for thaumatin-like protein was identified by the investigators involved in *Arabidopsis* genome sequencing project. The 5' end sequence of the clone indicated that it codes for putative thaumatin like protein. We have obtained this clone and sequenced the 3' end (Sanger *et al.*, 1977) and confirmed that it does code for antifungal protein. The cDNA clone is currently being used to screen the potato cDNA library under low stringent condition. We are screening about 10^6 pfu of potato library with *Arabidopsis* cDNA that is labeled with non-radioactive digoxegenin labeled probe as described (Reddy, et al., 1990; Day and Reddy, 1994). First round of screening showed a few positives. These putative positives are currently being plaque purified by second and third round of screening. Once the clones are isolated the cDNAs will be rescued in a plasmid form using a helper phage and used for sequencing. Within next two to three months we hope to have the sequences isolated by either of these methods. Characterization of the cDNAs at the sequence level will enable us to start the next phase of the work which is to construct the plasmid vector with desired promoters for transforming plants. A new postdoc to work exclusively on this project will be joining our group in the first week of March. The binary vectors, promoter and technical expertise needed is being routinely used in our laboratory in our other research projects.

Amino acid sequence	A	T	F	D	I	T	N
Primer sequence	5' GCI	ACI	TTT	GAT	ATA	ACI	AAC
			C	C	T		T
					C		

FIG. 1 : Sequence of the degenerate primer (AFP) used for PCR amplification. The amino acid sequence is shown a single letter code. "I" in the primer sequence denotes Inosine.

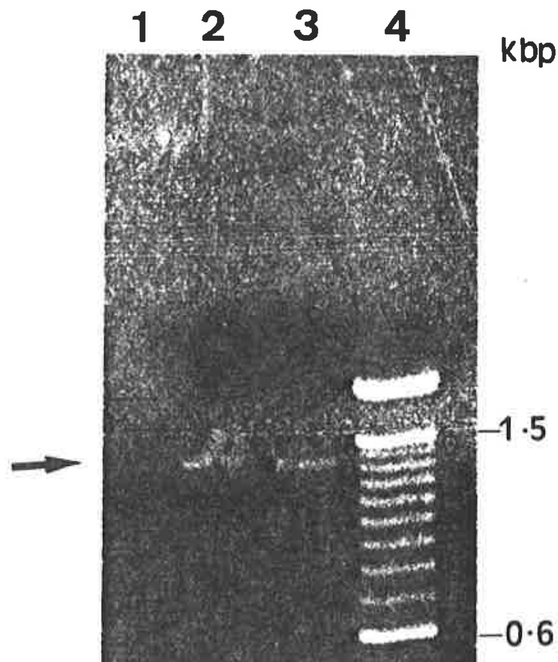

FIG. 2: Agarose gel electrophoresis of PCR amplified product obtained by using the degenerate primer and the vector primers (SK or KS) corresponding to the flanking sequence of cloning site in the vector. one (lanes 1 and 2) or three (lanes 3) microlitres of potato cDNA library was used as a template. Lanes 1: PCR product obtained using AFP and SK primers ; Lanes 2 and 3: PCR product obtained by AFP and KS primers; Lane 4: Molecular weight markers. The amplified DNA from the area indicated by an arrow was electroeluted, subcloned into a plasmid. Thirty five cycles (94°C for 1 min, 42°C for 1 min and 72°C for 2 min) of amplification were performed in a 100 µl reaction mixture (Day and Reddy, 1994). The PCR product was separated in a 2% agarose gel. Arrow indicates the PCR product.

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