

**B-88: Preparation of a specific DNA probe for *Pseudomonas solanacearum* by subtractive hybridization**

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Bacterial wilt caused by *Pseudomonas solanacearum* E F Smith is one of the most important diseases of the world. This pathogen is grouped into 3 races based on host range. Race 2 infects banana, heliconias and plantains. Race 3 only infects potato. In Sri Lanka occurrence of race 1 biovar 3, race 1 biovar 4, race 3 biovar 2 has been reported. However, we are yet lucky in not having race 2 that infects banana and plantains.

Success of effective quarantine measures against *Pseudomonas solanacearum* depends on use of quick and sensitive detection techniques. In order to devise a technique for eventual preparation of DNA probe for race 2, we prepared a DNA probe sensitive for detection of race 3 biovar 2 that infects potato only, using subtractive hybridization.

DNA sequences present in target strain of race 3 biovar 2 but not present in driver strain race 1 biovar 3 were enriched by the removal of common DNA. Total genomic DNA (75 g) of driver strain was sheared by ultrasonification. The genomic DNA of target strain race 3 biovar 2 (0.075 g) was digested with ECORI, extracted with phenol chloroform and precipitated with ethanol. The race 3 biovar 2 DNA (0.075 g) was labelled with digoxigenin-11-dUTP by random primer labelling. Both DNA for race 3 and race 1 were denatured at 100°C for 5 min and were allowed to re-anneal at 42°C for 6 h, in hybridization buffer.

This preparation was used directly as a probe by adding it to southern blots of race 1 and race 3, genomic DNA's. Probe produced substantial hybridization to the race 3 biovar 2 genomic DNA providing a quick detecting tool for race 3.