

Monoclonal antibodies against bacterial canker of avocado

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SYNOPSIS

Monoclonal antibodies were prepared against a Pseudomonas syringae isolated from cankerous lesions on avocado with the purpose of determining the etiology and pathogenicity of the disease.

Bacterial canker is a new disease of avocado (*Persea americana* Mill), characterised by cankerous lesions on the trunk and branches with watery pockets underneath the bark (Myburgh & Kotzé, 1982). A *Pseudomonas syringae* was isolated from the lesions, although not consistently so (Myburgh & Kotzé, 1983). The *Pseudomonas* from avocado produced a pronounced virulent reaction in pathogenicity tests and complied with the requirements for phytopathogenic pseudomonads (Korsten & Kotzé, 1984). However, Koch's postulates could not be fully fulfilled, since typical field symptoms had not developed under greenhouse conditions (Korsten, 1984). A comparison with three closely related *P. syringae* isolates indicated that the *Pseudomonas* from avocado is probably a new pathovar. This paper reports on the production of monoclonal antibodies (ma) against the *Pseudomonas* isolate, for determining the pathogenicity of the organism and differentiating it from other closely related species and pathovars.

A log phase culture of the *Pseudomonas* was harvested from a King's B slant and suspended in 10 ml phosphate-buffer saline (PBS) (pH 7,4). Black mice (C57) were injected intraperitoneally with 1 ml of a suspension containing 10^7 cells/ml. The mice received booster injections after two and six weeks and again three days before fusion. Fusion was done with murine myeloma cell line Sp 2 (obtained from the Department of Biochemistry, University of Pretoria), cultured in Dulbecco's modified Eagles medium (DMEM), supplemented with 2 mM L-glutamine and 12 per cent heat activated horse serum. Harvested spleen cells were mixed in a ratio of 5:1 with the myeloma cells and then centrifuged. Fusion was achieved with the addition of 41 per cent (W/v) polyethylene glycol 1500 (PEG) (MA Bioproducts) over a period of one min. The PEG suspension was diluted over a period of five min with DMEM medium, to a final volume of 40 ml. Cells were washed and seeded in HAT medium (supplemented DMEM, 12 per cent HS and hypoxanthine-aminopterin-thymidine selective agents) [Serevac Chemicals] in 96-well culture plates. The medium was changed every three to four days.

The supernatant was screened during a 10-day period from microscopically visible clones using the enzyme-linked immunosorbent assay (ELISA) technique. Bacteria adhered directly to the micro test plates and were incubated overnight at 4°C. The antigen was fixed with two per cent gluteraldehyde for one hour at 37°C before the hybridoma culture fluid was added to the wells. Enzyme horseradish peroxidase and substrate p-phenylene diamine were used as a detection system. Plates were read at 450 nm using a Titertek Multiskan MC. The same procedure was used for all subsequent screening. Selected positives were cloned in soft agar and stable clones were grown for ascite production.

Clones which gave the strongest reaction with ELISA tests against the *Pseudomonas*, were used to raise ascitic fluid. C 57 Black & Balb/c mice were primed with Freund's incomplete adjuvant three days prior to injection with 10⁶ hybridoma cells. The ascitic fluid was harvested after 10 days by tapping the peritoneum and then frozen at -75°C. A very strong reaction was obtained with the ascitic fluid using the ELISA test. The antibody is currently being purified and characterised.

With the availability of a very specific monoclonal antibody against the *Pseudomonas* isolated from avocado bark canker, the following can be investigated: (1) The association of this organism with the disease; (2) spreading of the organism in and on the tree, as well as in the orchard; (3) alternative hosts; (4) grouping of this isolate as a new pathovar; (5) rapid identification of potentially infected plants; (6) screening of nursery plants with the ELISA test.

REFERENCES

- 1 Korsten, L., 1984. Bacteria associated with bark canker of avocado. *MSc Thesis*. University of Pretoria. 118 pp
- 2 Korsten, L & Kotzé. J M, 1984. Bacterial canker of avocado *S Afr Avocado Growers Assoc Yrb.* **7**. 88-89
- 3 Myburgh. L & Kotzé. JM 1982 Bacterial disease of avocado. *S Afr Avocado Growers Assoc Yrb.* **5**, 105-106
- 4 Myburgh. L & Kotzé. JIM. 1983. Bacterial canker of avocado. *S Afr Avocado Growers Assoc Yrb.* **6**, 88-89