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A variety of Xanthomonas campestris pathogenic to Zantedeschia aethiopica

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Abstract

A new bacterial pathogen of *Zantedeschia aethiopica* is described and a brief description is given of the disease symptoms. According to its physiological and biochemical reactions, and on the basis of its DNA base composition and pathogenic reactions, the new organism is tentatively designated as *Xanthomonas campestris* var. *zantedeschiae* Joubert et Truter var. nov.

Introduction

Diseased leaves of cultivated and wild arums, *Zantedeschia aethiopica* (Linn.) Spreng., were collected during the 1966/67 summer in the Transvaal Province, Republic of South Africa. Subsequently wild and cultivated arums showing similar symptoms were found in the Province of Natal.

Initially the lesions have a watersoaked appearance. During wet weather a soft rot usually sets in which involves large areas of the lamina and midrib. Under dry conditions the lesions become necrotic, turn brown and, depending on their position on the leaf, vary in shape and size. The adjoining leaf tissue becomes chlorotic. The necrotic tissue may become torn or drop out partly or almost completely. In severely infected leaves the lesions coalesce, affecting extensive leaf areas (Fig. I).

This paper is a report on the incitant of the disease.

Materials and methods

Typical watersoaked lesions on arum leaves collected in six different localities in the Transvaal were microscopically examined. In all lesions bacteria were present in great numbers. Tissue from six representative lesions was used to prepare dilution plates on a medium consisting of meat extract, 0.3%; peptone, 0.5%; glucose, 2%; yeast extract, 1%; agar (Difco), 2%. This medium, which is henceforth referred to as the NAGY-medium, has previously been found to stimulate growth and pigment production in a number of xanthomonads tested by the authors. The isolates were subsequently transferred to tryptone-glucose-extract-medium (Difco). Stock cultures were maintained on YDC-medium (Corey and Starr, 1957).

After purification all six isolates were used in morphological and physiological

Fig. 1. Leaves of *Z. aethiopica* infected with *X. campestris* var. *zantedeschiae* var. nov.

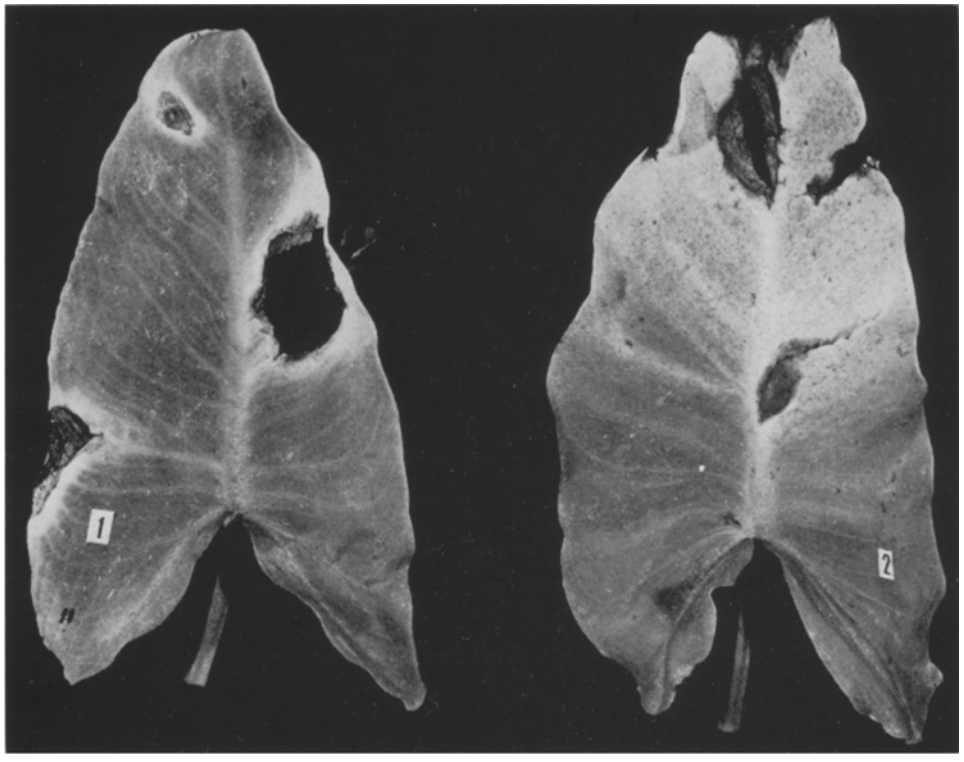

Fig. 1. Bladeren van Z. aethiopica geïnfecteerd met X. campestris var. zantedeschiae var. nov.

studies.

In identifying the organism the procedures recommended by Dye (1962) were followed. These were supplemented by tests described by Cowan and Steel (1965) and Gibbs and Skinner (1966). A yeast extract medium containing 2-deoxy-D-ribose, $6 \times 10^{-2}M$, yeast extract 0.5%; agar 2% (Hochster, 1963) was used as a differential medium.

Leifson's method was applied to stain the flagella (Leifson, 1930) and flagellation was also studied in the electron microscope using negative staining.

Test plants were inoculated by pricking a suspension of freshly isolated bacteria into young healthy leaves (Dowson, 1949). Leaves were also inoculated by spraying with a suspension of bacteria, with and without rubbing the surface with carborundum, and by injecting the bacterial suspension into the leaf by means of a syringe. All inoculated plants were kept for 5 days in a moisture saturated atmosphere at 27 °C.

After having established that all the isolates were morphologically and physiologically identical the DNA base composition of one of the isolates was determined by Prof. J. De Ley, Ghent, Belgium.

Cultures of *X. dieffenbachiae* and *X. translucens* were used in all control tests. These cultures were obtained from Prof. M. P. Starr, Davis, California and Dr D. W. Dye, Auckland, New Zealand, respectively.

Results

All the arum isolates tested were found to have identical morphological, cultural and physiological characteristics.

Morphology: Cells from 24 hour old slant cultures on the NAGY, YDC, and tryptone-glucose-extract media were short, gram negative, non-sporing rods occurring singly or in short chains. The organism was motile by means of a single polar flagellum (Fig. 2).

Cultural characteristics: Colonies on NAGY and YDC medium were bright yellow, smooth, round, entire, slimy and became macroscopically visible after approximately 36 hours incubation at 28 °C. Diffusible pigments were not produced on any of the media used. The yellow pigment of the colonies was insoluble in water or ethanol but

Fig. 2. Negatively stained preparation of *X. campestris* var. *zantedeschiae* var. nov. with polar flagellum. (× 25,000).

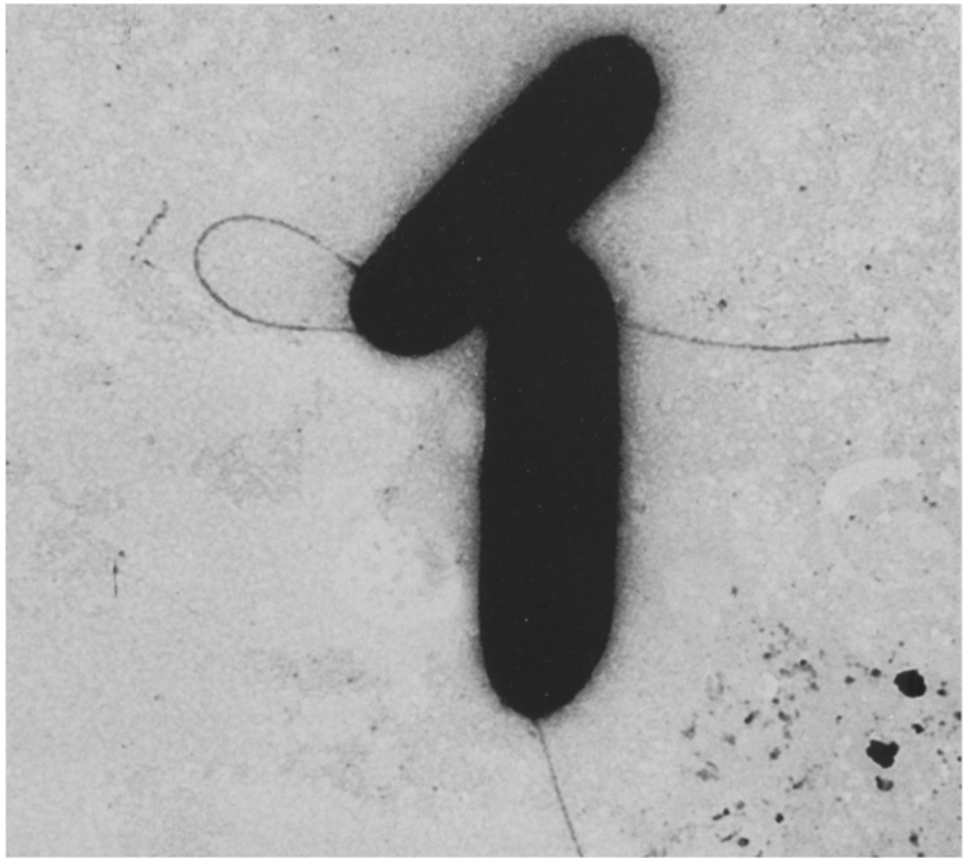

Fig. 2. Negatief gekleurd preparaat van X. campestris var. zantedeschiae var. nov. met polaire zweep haar. (25.000 ×).

soluble in boiling methanol. Broth became turbid and a slight sediment was formed, but no pellicle. The organism was strictly aerobic and grew optimally at 28 °C. At 40 °C there was no growth.

DNA base composition: The bacterium was found to have a guanine-cytosine ratio of 66.0% GC and the melting point, T_m was 96.4 °C.

Physiological characteristics: The cultures exhibited a strictly oxidative metabolism of glucose. In a weakly buffered medium small amounts of acid were produced from sucrose, glucose and arabinose but not from dulcitol, mannitol, inulin, rhamnose, lactose, salicin, sorbitol and inositol. The cultures were catalase positive and oxidase weakly positive. Starch was hydrolyzed and gelatine liquified. Hydrogen sulphide was produced from cysteine. Asparagine was not sufficient as a sole source of carbon and nitrogen. Nitrates were not reduced. Propionate and succinate were utilized, but not benzoate, tartrate and gluconate. Aesculin was split. Phenylalanine was not deaminated and there was no production of indole, acetoin and urease.

The isolated organism as well as *X. translucens*, *X. phaseoli*, *X. campestris* and a *Sarcina* species failed to grow on Hochster's medium (Hochster, 1963); while *Escherichia coli*, *Erwinia carotovora* and *Bacillus cereus* var. *mycoides* grew well.

The organism was sensitive to polymyxin B, neomycin, terramycin, novobiocin, aureomycin, oleandomycin, streptomycin and kanamycin but resistant to cloxacillin, penicillin and bacitracin.

Pathogenicity tests: When healthy leaves were inoculated with freshly isolated cultures of the organism by pricking with a fine needle, small necrotic spots developed (Fig. 3). Large necrotic lesions were produced when a suspension of the organism was injected

Fig. 3. Symptoms on *Z. aethiopica* leaves 10 days after artificial infection with *X. campestris* var. *zantedeschiae* var. nov.

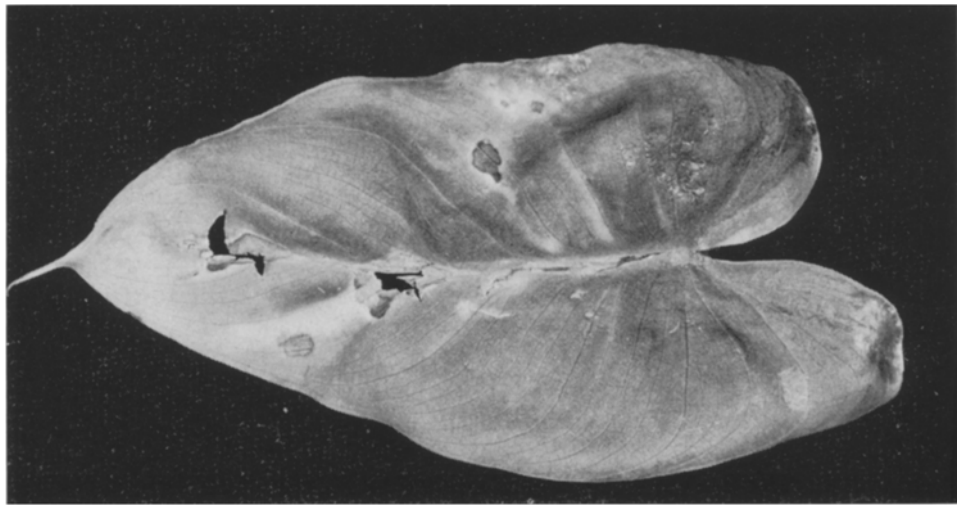

Fig. 3. Symptomen op Z. aethiopica bladeren 10 dagen na kunstmatige infectie met X. campestris var. zantedeschiae var. nov.

into the leaves by means of a syringe. Organisms identical to the inoculum were re-isolated from both types of lesions. However, lesions identical to those found in nature could not be produced as the conditions under which natural infection occurs had not been investigated and could therefore not be simulated. All other methods of inoculation gave negative results. A hypersensitive reaction resulted when a suspension of the organism was injected into healthy tobacco (*Nicotiana tabacum*) leaves.

Inoculation of *Dieffenbachia picta*, *Phormium tenax*, *Saccharum officinarum*, *Brassica oleracea* f. *capitata*, *B. oleracea* f. *botrytis*, and *Begonia semperflorens* produced no symptoms.

No symptoms developed on *Z. aethiopica* leaves inoculated with *X. dieffenbachiae*.

Discussion

On the basis of the morphological, cultural and physiological characteristics and the phytopathogenic nature of the isolated organism it should be classified as a species of *Xanthomonas*. Furthermore the DNA base composition of the bacterium is similar to that of several other species of *Xanthomonas* determined by Colwell and Mandel (1964) and De Ley and van Muylem (1963).

De Ley and co-workers (1966) and Lelliott (personal communication) concluded that there is sufficient similarity between the different species of *Xanthomonas* to regard them as a single 'genospecies'. On the basis of DNA homology with different *Pseudomonas* spp. it was proposed by De Ley et al. (1966) that the members of this 'genospecies' be incorporated in the genus *Pseudomonas* as *P. campestris*. However, Lelliott (personal communication) is of the opinion that more comparative work is needed before the inclusion of *Xanthomonas* into *Pseudomonas* can be accepted.

Two xanthomonads have been reported to be pathogenic on members of the Araceae (Breed et al., 1957; Buchanan et al., 1966) viz. *X. dieffenbachiae* on *Dieffenbachia picta* and *X. conjac* on *Amorphophallus konjac*. Cultures of the latter pathogen were not available. Judging from the description of the latter bacterium as given by Breed et al. (1957), Dye (personal communication) considers that it is not a xanthomonad.

In view of the fact that *X. dieffenbachiae* and the isolated organism could not be cross-inoculated, they must be considered as different host adapted forms. It is suggested that the new organism be considered as a hitherto unreported xanthomonad adapted to a parasitic existence on *Z. aethiopica*. Following the nomenclature proposed by De Ley and Friedman (1965) and pending the decision regarding the classification of *Xanthomonas* to be taken by the Board of Trustees of Bergey's Manual, the name *X. campestris* var. *zantedeschiae* Joubert et Truter var. nov. is suggested.

Type cultures of the pathogen have been deposited in the National Collection of Plant Pathogenic Bacteria, Harpenden, England and the Collection of Plant Pathogenic Bacteria, Auckland, New Zealand.

Samenvatting

Een variëteit van Xanthomonas campestris pathogeen voor Zantedeschia aethiopica

Een bacterieziekte van de aronskelk (*Zantedeschia aethiopica*) begint met waterdoor-

drenkte plekken, welke gedurende de vochtige warme zomermaanden overgaan in een zachtrot en, gedurende droge perioden, in bruine necrotische plekken (Fig. 1). De ziekte is tot dusver gevonden in de provincies Transvaal en Natal van de Republiek van Zuid Afrika. Een gele bacterie, pathogeen voor aronskelken, werd uit het aangetaste weefsel geïsoleerd. Op grond van morfologische, fysiologische en pathologische eigenschappen, zowel als van de DNA-base verhouding werd de bacterie voorlopig beschreven als *Xanthomonas campestris* var. *zantedeschiae* Joubert et Truter var. nov.

Acknowledgements

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References

Breed, R. S., Murray, E. G. D. & Smith, N. R., 1957. Bergey's Manual of Determinative Bacteriology, 7th ed. Williams & Wilkins, Baltimore.

Buchanan, R. E., Holt, J. G. & Lessel, E. G., 1966. Index Bergeyana. Williams & Wilkins, Baltimore.

Colwell, R. R. & Mandel, M., 1964. Adansonian analysis of deoxyribonucleic acid base composition of some gram-negative bacteria. J. Bacteriol. 82: 913–919.

Corey, R. R. & Starr, M. P., 1957. Colony types of *Xanthomonas phaseoli*. J. Bacteriol. 74: 137–140.

Cowan, S. T. & Steel, K. J., 1965. Manual for the identification of medical bacteria. University Press, Cambridge.

Ley, J. De & Friedman, S., 1965. Similarity of *Xanthomonas* and *Pseudomonas* deoxyribonucleic acid. J. Bacteriol. 89: 1306–1309.

Ley, J. De, Park, J. W., Tijtgat, R. & Ermengem, J. van, 1966. DNA homology and taxonomy of *Pseudomonas* and *Xanthomonas*. J. gen. Microbiol. 42: 43–56.

Ley, J. De & Muylem, J. van, 1963. Some applications of deoxyribonucleic acid base composition in bacterial taxonomy. Antonie van Leeuwenhoek 29: 344–358.

Dowson, W. J., 1949. Manual of bacterial plant diseases. Adam & Charles Black, London.

Dye, D. W., 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N.Z. Jl. Sci. 5: 393–416.

Gibbs. B. M. & Skinner, F. A., 1966. Identification methods for microbiologists. Academic Press, London/New York.

Hochster, R. M., 1973. In: Hochster, R. M. & Quastel, J. H. (Ed.), Metabolic inhibitors. Vol. I. Academic Press, London/New York.

Leifson, E., 1930. A method of staining bacterial flagella and capsules together with a study of the origin of flagella. J. Bacteriol. 20: 203–211.