Molecular Characterization of *Pseudomonas syringae* pv. *tomato* Isolates from Tanzania

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Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* is an emerging disease of tomato in Tanzania. Following reports of outbreaks of the disease in many locations in Tanzania, 56 isolates of *P. syringae* pv. *tomato* were collected from four tomato-producing areas and characterized using pathogenicity assays on tomato, carbon source utilization by the Biolog Microplate system, polymerase chain reaction and restriction fragment length polymorphism (RFLP) analysis. All the *P. syringae* pv. *tomato* isolates produced bacterial speck symptoms on susceptible tomato (cv. 'Tanya') seedlings. Metabolic fingerprinting profiles revealed diversity among the isolates, forming several clusters. Some geographic differentiation was observed in principal component analysis, with isolates from Arusha region being more diverse than those from Iringa and Morogoro regions. The Biolog system was efficient in the identification of the isolates to the species level, as 53 of the 56 (94.6%) isolates of *P. syringae* pv. *tomato* were identified as *Pseudomonas syringae*. However, only 23 isolates out of the 56 (41.1%) were identified as *Pseudomonas syringae* pv. *tomato*. The results of this work indicate the existence of *P. syringae* pv. *tomato* isolates in Tanzania that differ significantly from those used to create the Biolog database. RFLP analysis showed that the isolates were highly conserved in their *hrpZ* gene. The low level of genomic diversity within the pathogen in Tanzania shows that there is a possibility to use resistant tomato varieties as part of an effective integrated bacterial speck management plan.

KEY WORDS: Bacterial speck; Biolog system; polymerase chain reaction (PCR); restriction fragment length polymorphism (RFLP); tomato.

INTRODUCTION

*Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie is the causal organism of bacterial speck of tomato (*Solanum lycopersicon* Mill.), now recognized as a significant source of economic loss in tomato production (8, 22, 46). Bacterial speck has been reported in many tomato production areas in Europe, North America, and Australia. In Africa, the disease has been reported in Morocco, South Africa (8), Zimbabwe (38), and recently in Tanzania (35). Earlier surveys conducted from tomato seeds and field plants showing leaf spot symptoms in Tanzania did not reveal the presence of the bacterial speck pathogen (20). Yield reductions result from the reduced photosynthetic capacity of infected...
foliage and from lesions on the fruit that render them unsuitable for the fresh market (43). In the field, yield losses vary from 75% in plants infected at an early stage of growth to 5% in plants infected later in the season (14,45). Disease management measures include the use of resistant tomato varieties, field sanitation, pesticide application and use of pathogen-free seed (8).

The most common methods of identification and classification of plant pathogenic pseudomonads have been based on phenotypic and biochemical characteristics (15,25,44). Although these approaches have been of great value they are laborious, time-consuming and not very suitable for seed and plant certification or quarantine programs where accuracy, speed and specificity are of utmost importance. Until recently, DNA-DNA hybridization techniques employing coronatine synthesis genes as probes (11), coupled with the use of serological techniques such as indirect fluorescent antibody staining and enzyme-linked immunosorbent assay (ELISA), have been used in an attempt to increase precision and speed of identification, but without much success (46). To overcome the shortcomings of those attempts, Zaccardelli et al. (46) employed polymerase chain reaction (PCR) amplification of an internal fragment of the hrpZpst, a chromosomal gene located in the hrp/hrc pathogenicity island, which is essential for disease induction in host plants and hypersensitive response in non-hosts (3). This approach represented a rapid and sensitive diagnostic tool for high precision screening of infected tomato plants. Another diagnostic tool that has been reported to be reliable and fast is Biolog (Biolog Inc., Hayward, CA, USA) metabolic profiling (4,18).

The use of molecular techniques in plant pathology has greatly increased our understanding of host–pathogen interactions over the last decade (2). This understanding has in turn enabled us to develop better strategies for managing plant diseases. Race/strain differentiation has enabled researchers to understand why the use of speck-resistant tomato varieties has not been consistently effective in the management of this disease: because there are no known tomato varieties with resistance to all the races of the pathogen (13,28-30). However, since genetic resistance still constitutes one of the most sustainable approaches to bacterial speck management, the search for tomato varieties with horizontal resistance to the disease is an ongoing endeavor (13,43). Ultimately, the success of such varieties in any given location will depend on sound knowledge of existing variation within populations of P. syringae pv. tomato. In view of the fundamental need for such information, the present study aimed at identifying and characterizing isolates of P. syringae pv. tomato from different tomato ecosystems in Tanzania by pathogenicity tests on tomato, Biolog, PCR and restriction fragment analysis (RFLP).

MATERIALS AND METHODS

Leaves and fruits showing bacterial speck symptoms were collected from 49 fields in four tomato-growing regions in Tanzania (Arusha, Iringa, Morogoro and Dodoma). From each location, infected tomato fruits and leaves were collected, placed in paper bags and brought to the laboratory for processing.

**Bacterial isolation** Tissue segments of ~2 mm² were excised from advancing lesion margins on symptomatic tomato leaves or fruits. The tissue segments were macerated in a few drops of sterile distilled water in a sterile petri dish, and allowed to stand for 10–15 min in a laminar airflow chamber. A loopful of the resulting suspension was streaked onto plates of King’s medium B and incubated for 72 h at 25–28°C to obtain putative colonies of P.