Investigating Intraspecific Variation of *Ralstonia solanacearum* strains in West Malaysia using Whole Cell Fatty Acid Analysis

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Abstract

Surveys were conducted between the years of 2005-2006 at several locations in the northern, central and southern parts of West Malaysia to study the polymorphism of *R. solanacearum* strains. These sites included vegetable and farms with known hosts of the pathogen, such as banana, tomato, eggplant, chili and tobacco. Sample collection was conducted by randomly collecting the suspected plants and weeds, including soil and water samples, from selected areas. The bacterium was detected in all samples by Nested-PCR. The bacteria were isolated from positive samples by using semi-selective media and were further confirmed by biochemical and BIOLOG test. FAME (Fatty Acid Methyl Esters) profiling? was performed for the detection of polymorphism among bacterial isolates. The results showed that the fatty acid composition varies for all *R. solanacearum* isolates. Grouping of *R. solanacearum* isolates by fatty acid composition suggested the existence of eight groups namely group A, B, C, D, E, F, G and H.

Introduction

R. solanacearum is a complex taxonomic unit in which the strains display an important diversity at different levels (physiological, serological, genetic characteristics, and host range). In order to describe this intra specific variability, several systems of classification have been proposed. Thus, the species was subdivided into five races according to its host range and into 6 biovars based on utilization of three disaccharides and three hexose alcohols. Races and biovars usually do not correspond. Moreover, there is considerable genetic variation among strains within each race or Biovar. This genetic and pathogenic variation makes development of diagnostic, detection, and control measures of *R. solanacearum* more difficult. Several molecular methods have been developed to identify diversity and subdivide races or biovars of *R. solanacearum* for strain characterization. This study was conducted for comparison of fatty acid profiles for characterizing of *R. solanacearum* strains in West Malaysia.

Materials and Methods

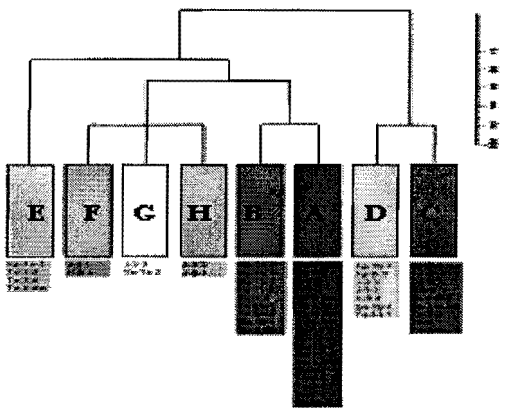
During the years of 2005-2006, several locations were selected within the Northern, Central and Southern parts of Peninsular Malaysia to study the polymorphism of strains of *R. solanacearum*. These sites included vegetable farms and other production areas planted with known hosts of the pathogen, such as Banana and tobacco. Sample collection was conducted by collecting suspected crop plants and weeds. Soil and water samples were randomly collected from selected areas. The bacterium was detected in all samples by nested-PCR. Using semi-selective media the bacterium was isolated from positive samples and was confirmed by biochemical tests and the BIOLOG identification system. FAME (fatty Acid Methyl Esters) test, was performed for

detection of polymorphism among bacterial isolates. To do so, bacteria were grown for 24h at 28^0C on TSBA Agar. Fatty acids were obtained from samples as described by Miron Sasser (1990). The extracts were analyzed with the Gas Chromatography System (HP model 5898). GC included with a 25 m x 0.2 mm silica capillary column (He2 as carrier gas) and a flame-ionization detector (FID).

Results and Discussion

The results of FAME showed that fatty acid composition varies for all *R. solanacearum* isolates. Totally 8 types of fatty acid were found among *R. solanacearum* strains. Four Fatty acids [(16:0); (16:1); (14:0) and (14:1)] were identified in all strains. Grouping of *R. solanacearum* isolates by fatty acid composition using PCA (Principal Components Analysis) suggested that the existence of eight groups namely group A, B, ...G and H (Fig.1). These groups usually were affected by host means that the strains from same hosts have been grouped in same FAME groups. Biovar type and sampling site were not so effective in FAME profiles.

Fig: 1



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References

Agrios, R. E. F. 2005. Plant Pathology. 5th edition. Academic Press. Pp. 920.

Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Ann. Rev. of Phytopathol. 29: 67-79.

Khan, A. A., Furuya, N., and Matsuyama, M. 1999. Identification of Ralstonia solanacearum isolated from wilted tobacco plant by fatty acid profiles and PCR-RFLP analysis. J. *Fac. Agr., Kyushu Univ,* 44(12): 59-65.

Myron Sasser, 1990. Bacterial Identification by Gas Chromatographic Analysis of Fatty Acids Methyl Esters (GC-FAME). MIDI Sherlock Microbial Identification System References.