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Phenotypic and genotypic diversity of blood disease bacterium and 
*Ralstonia solanacearum* phylotype IV strains

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Banana blood disease is one of the most important horticultural diseases in Indonesia and is a threat to Australia and surrounding countries. Previous studies have confirmed that the blood disease bacterium (BDB), the causal organism of blood disease of banana, is phylogenetically closely related to members of the *R. solanacearum* species complex (Taghavi et al., 1996). *R. solanacearum* is a heterogenous species with considerable phenotypic and genotypic variation within the species *R. solanacearum* and its close relatives which comprise the species complex. Within this species complex strains of the BDB belong to Phylotype IV, one of the four genetic groups described within the species complex. Phylotype IV contains the most phenotypically diverse range of strains within the species complex and includes *Ralstonia syzygii*, the BDB, and *R. solanacearum* strains (Fegan and Prior, 2006).

This study aims to clarify the taxonomic relationship of the BDB to other members of the *R. solanacearum* species complex by employing phenotypic and genotypic methods, with a view to facilitating the unambiguous identification of the BDB and thereby aiding diagnosis and management of banana blood disease. Phenotypic characterisation of 33 strains of *R. solanacearum*, 25 strains of the BDB, and 6 strains of *R. syzygii* was accomplished using classical phenotypic tests (46 physiological and biochemical tests) and BIOLOG metabolic fingerprinting. Genotypically the diversity of 27 strains of *R. solanacearum* phylotype IV, 25 strains of BDB, and 6 strains of *R. syzygii* was assessed by phylogenetic analysis of partial endoglucanase gene sequences and the diversity of 20 strains of *R. solanacearum* phylotype IV, 7 strains of BDB, and one strain of *R. syzygii* was assessed by phylogenetic analysis of 16S-23S rRNA gene internal transcribed spacer (ITS) region sequences.

Phylogenetic analysis of the 16S-23S rRNA gene ITS region of *R. solanacearum* phylotype IV strains showed that all *R. solanacearum* phylotype IV, BDB, and *R. syzygii* strains grouped in one cluster that was distinct from sequences from *R. solanacearum* strains within the other phylotypes. Phylogenetic analysis of partial endoglucanase gene sequences showed that all *R. solanacearum* phylotype IV strains formed a monophyletic group that was distinct from sequences from *R. solanacearum* strains within other phylotypes. Within phylotype IV partial endoglucanase gene sequences revealed two clusters, the first cluster contains Indonesian *R. solanacearum* strains, one Australian *R. solanacearum* strain, one tomato *R. solanacearum* strain isolated from tomato and all BDB strains. The second cluster contains Indonesian *R. solanacearum* strains from clove, one Indonesian *R. solanacearum* strain from tomato, one Japanese *R. solanacearum* strain isolated from potato, and all *R. syzygii* strains. Endoglucanase gene sequences of BDB and certain *R. solanacearum* phylotype IV strains isolated from tomato and potato in Indonesia were very closely related sharing 100% similarity. Endoglucanase gene sequences were superior to 16S-23S rRNA ITS region sequences for revealing strain diversity, however, BDB cannot be distinguished from other *R. solanacearum* phylotype IV strains based on endoglucanase gene or 16S-23S rRNA gene ITS region sequences.

Although BDB strains are genotypically closely related to *R. solanacearum* phylotype IV strains, our work has shown that phenotypically, *R. solanacearum* and BDB strains differ greatly from each another, with all BDB strains clustering separately from *R. solanacearum* phylotype IV strains and *R. syzygii* strains. Several phenotypic characteristics such as nitrate reduction, starch hydrolysis, citrate utilization, and acid production from some sugars, differentiate BDB strains from *R. solanacearum* strains. BIOLOG™ plates were used for metabolic fingerprinting of BDB and *R. solanacearum* phylotype IV strains. The metabolic patterns generated by BIOLOG™ showed that BDB strains have a more restricted ability to use carbon sources for growth than *R. solanacearum* strains but were able to use more substrates than *R. syzygii* strains.

In conclusion although BDB strains are phylogenetically closely related to *R. solanacearum* phylotype IV strains and *R. syzygii* they are phenotypically distinct.

References:

Fegan, M & Prior, P. 2006. Australasian Plant Pathol. 35: 93-101


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