ISSN 0853-8212

JURNAL PENELITIAN TANAMAN INDUSTRI

(INDUSTRIAL CROPS RESEARCH JOURNAL)

Volume III No. 1 Mei, 1997

DAFTAR ISI

Badan Penelitian dan Pengembangan Pertanian
Agency for Agriculture Research and Development
PUSAT PENELITIAN DAN PENGEMBANGAN TANAMAN INDUSTRI
Central Research Institute for Industrial Crops
BOGOR - INDONESIA

JURNAL PENELITIAN TANAMAN INDUSTRI : merupakan publikasi ilmiah primer yang memuat hasil penelitian primer komoditi tanaman industri yang belum pernah dimuat pada media apapun, diterbitkan oleh Pusat Penelitian dan Pengembangan Tanaman Industri. Terbit enam kali setahun.

Alamat Redaksi :

Pusat Penelitian dan Pengembangan Tanaman Industri

Jl. Tentara Pelajar No. 1, Bogor 16111

Telp. (0251) 336194, 313083. Faks. (0251) 336194

Untuk keperluan tukar menukar dan sebagainya, agar menghubungi alamat redaksi.

Biaya cetak dari APBN T.A. 1997/1998, Bagian Proyek Penelitian dan Pengembangan Tanaman Industri

ANALYSIS OF WHOLE CELL PROTEIN PROFILES OF ISOLATES OF *Pseudomonas solanacearum* AND RELATED SPECIES FROM INDONESIA BY SDS-PAGE

SUPRIADI[1)], J. G. ELPHINSTONE[2)], J. HENNESSY[2)] and A. ROBINSON-SMITH[3)]

[1)] Research Institute for Spice and Medicinal Crops
[2)] Central Science Laboratory
[3)] Department of Crop and Disease Management

ABSTRACT

Pseudomonas solanacearum, P. syzygii, and blood disease bacterium (bdb) are endemic in Indonesia. Despite they have different host plants, these pathogens show similarity on several biochemical and physiological aspects. Study on sodium dodecyl sulphate polycrylamide gel electrophoresis (SDS-PAGE) of the whole protein patterns of isolates of the pathogens was aimed to help in determining the pathogens. Whole protein profile of 30 isolates of *P. solanacearum* comprising biovar 1-3 from different hosts, 5 isolates of *P. syzygii* from clove plants and 3 isolates of bdb from banana plants were studied in 1994 at the Departement of Crop and Disease Management, IACR-Rothamsted, U.K. and the patterns were numerically analyzed using a computerized program (GelCompar Version 2.1). The result clustered all isolates of *P. solanacearum* biovar 3 at 78% similarity, separated from biovar 1,2 and N2. The grouping obtained did not correspond with the pathogenicity, host and geographical origin of the isolates. Isolates of *P. syzygii* and BDB formed distinctive cluster from *P. solanacearum*, however, they jointed at 52% similarity. Protein profiles of isolates of bdb and *P. solanacearum* race 2 (banana strain) from the Philippines showed closer relationship (72% similarity), but differed from race 2 from Colombia and Grenada. Ginger isolates of *P. solanacearum* from Indonesia, Australia and China formed different electrophoretic patterns. This results supported earlier studies that isolates of *P. solanacearum, P. syzygii* and bdb of banana from Indonesia are closely related.

Key words : *Pseudomonas solanacearum , P. syzygii,* protein profile, electrophoresis, Indonesia

RINGKASAN

Analisis pola protein isolat Pseudomonas solanacearum dan spesies sekerabatnya dari Indonesia dengan SDS-PAGE

Pseudomonas solanacearum, P. syzygii, dan bakteri penyakit darah (bdb) adalah patogen yang endemik di Indonesia. Walaupun ketiga patogen itu mempunyai tanaman inang yang berbeda, tetapi ketiganya memiliki beberapa sifat fisiologi dan biokimia yang sama. Penelitian pola protein ketiga macam patogen itu dimaksudkan untuk membantu determinasi sifat-sifat patogen. Penelitian menggunakan 30 isolat *P. solanacearum* dari berbagai tanaman inang, lima isolat *P. syzygii* dari tanaman cengkeh, dan tiga isolat bdb dari tanaman pisang yang dilakukan pada tahun 1994 di Department of Crop and Disease Management, IACR- Rothamsted, Inggris, kemudian hasilnya dianalisis secara numerik dengan komputer menggunakan program GelCompar Version 2.1. Hasil analisis menunjukkan, bahwa isolat-isolat *P. solanacearum* biovar 3 berada dalam satu kelompok (clustered) dengan derajat kesamaan 78%, terpisah dari biovar 1, 2 dan N2. Pola protein isolat-isolat bakteri yang digunakan tidak berkorelasi dengan sifat patogenisitas, inang asal, dan lokasi asal dari isolat-isolat bakteri. Pola protein *P. solanacearum* berbeda dengan pola yang ditunjukkan oleh *P. syzygii* dan patogen penyebab penyakit darah (bdb) pada tanaman pisang, tetapi pola-polanya mempunyai kemiripan satu dengan lainnya, dengan derajat kesamaan 52%. Pola protein isolat BDB mempunyai kemiripan dengan isolat *P. solanacearum* ras 2 yang berasal dari tanaman pisang (strain pisang) dari Filipina (derajat kesamaan 72%), tetapi berbeda sekali dengan strain pisang dari Kolombia dan Grenada. Isolat *P. solanacearum* yang berasal dari tanaman jahe dari Indonesia menunjukkan pola protein berbeda dengan yang dihasilkan oleh isolat *P. solanacearum* dari Australia dan Cina. Hasil penelitian ini memperkuat penelitian sebelumnya, bahwa di antara isolat-isolat *P. solanacearum, P. syzygii* dan BDB yang berasal dari Indonesia mempunyai kesamaan satu sama lainnya.

Kata kunci : *Pseudomonas solanacearum , P. syzygii,* pola protein, elektrophoresi, Indonesia

INTRODUCTION

Pseudomonas solanacearum is a heterogeneous species which cause wilt on many hosts in tropical and subtropical countries. Traditionally, they are grouped into five races and five biovars (HAYWARD, 1991). The race and biovar classifications are not always related, except for those race 2 (banana strains) is biovar 1, and race 3 (potato strains) is biovar 2.

Evidence from DNA studies of strains of *P. solanacearum* using nine *P. solanacearum*-specific probes that specified for virulence or the hypersensitive response (COOK *et al.*, 1989), and the DNA fingerprinting patterns obtained after restriction enzyme analysis (GILLING and FAHY, 1993) suggested that *P. solanacearum* may be divided into at least two subspecies which reflected of separate evolutionary origin (HAYWARD, 1991). Subspecies one is primarily of Asian origin (consisted of strains of biovars 3, 4 and 5), whereas the other is Americas (consisted of strains of biovar 1, 2 and N2) (COOK *et al.*, 1991).

Variation amongst isolates of *P. solanacearum* in Indonesia with respect to pathogenicity and physiological characters has been recognized since 1912, and HAYWARD (1991) found that predominant strains belonged to race 1 (biovar 3) which attacks many host plants and is widely distributed, and race 3 (biovar 2) which mainly attacks potato grown at higher altitudes.

Two other plant pathogens which are closely related to *P. solanacearum* are endemic in Indonesia. These pathogens are *P. syzygii*, the cause of Sumatra disease of clove (BENNETT *et al.*, 1985; ROBERTS *et al.*, 1990) and the bacterium that

causes blood disease of bananas (BDB) was previously identified as *P. celebensis* by Gaumann in 1923, and reinstated by EDEN-GREEN and SASTRAATMADJA (1990). These three pathogens have different ecosystems. *P. syzygii*, is transmitted by the tube-building cercopoids (Machaerotidae), *Hindola fulva* (in Sumatra) and *H. striata* (in Java) (EDEN-GREEN *et al.*, 1992), hosts limited mainly to cloves and is not pathogenic to Solanaceous. BDB infects both dessert (AAA group, pisang Ambon and pisang Nangka; AA group, pisang Mas) and cooking bananas (AAB group, pisang Raja; ABB group, pisang Kepok and pisang Siam). Mechanical inoculation of solanaceous plants with BDB did not produce symptoms (EDEN-GREEN and SASTRAATMADJA, 1990). These pathogens could be differentiated on the basis of their pathogenicity by artificial inoculation into host plants such as clove, banana and Solanaceae, and physiological properties (EDEN- GREEN and SASTRAATMADJA, 1990). BDB and *P. syzygii* isolates differ physiologically from other *P. solanacearum* races 1-4 from other countries (EDEN-GREEN, 1993).

Despite their differential characteristics, close relationships between *P. solanacearum*, *P. syzygii* and the BDB three pathogens have been determined from the studies of their DNA/ DNA hybridization (ROBERTS *et al.*, 1990), serology (EDEN-GREEN *et al.*, 1988) and fatty acid profiles (ROBERTS *et al.*, 1990). However, COOK *et al.* (1991) showed that restriction fragment length polymorphism (RFLP) of BDB isolates differed from those of *P. solanacearum* race 2 from Latin America. Furthermore, sequence data for a 292 bp segment of DNA encoding 16S sRNA, has indicated isolates of BDB are more closely related to *P. solanacearum* than to *P. syzygii* or *P. pickettii* (SEAL *et al.*, 1993).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins with computerized analysis of patterns has been used to determine pathovars of *Xanthomonas campestris* (VAUTERIN *et al.*, 1991) and human pathogens such as *Providencia rettgeri* (formerly *Proteous rettgeri*; COSTAS *et al.*, 1989). This method is therefore used to determine protein patterns of isolates of *P. solanacearum* and closely related species from Indonesia.

MATERIAL AND METHODS

Isolates of *P. solanacearum*, *P. syzygii* and blood disease bacterium (BDB) were obtained either by direct isolation from diseased plants or from culture collection of bacteria held at IACR-Rothamsted, UK (Appendix 1). The study was conducted at the Department of Crop and Disease Management, IACR-Rothamsted, U.K. Cultures of *P. solanacearum* and BDB were maintained in sterile distilled water stored at room temperature whereas *P. syzygii* were maintained on slope of periwinkle (PW) agar medium (DAVIS *et al.*, 1981) at 4 °C.

Whole cell protein analysis of strains of *P. solanacearum*, *P. syzygii* and BDB was performed by SDS-PAGE following a method modified from that described by STEAD (1992) using Hoefer Scientific Instrument, Model SE 600 electrophoresis apparatus. Cultures of *P. solanacearum* and BDB were grown on sucrose peptone agar (SPA) medium (HAYWARD, 1964) for 2 days, whereas *P. syzygii* were grown on PW medium for 5-7 days at 29 °C. The reference strain, *Psychrobacter immobilis* (used for normalizing gels), was grown for 2 days at 29° C on blood agar medium (JUNI and HEYM, 1986).

Colonies were suspended in sterile phosphate-buffered saline (PBS) at pH 7.2, in a preweighed centrifuge tube. Cells were washed by centrifuging at 10 000 rpm for 10 minutes, discarding the supernatant, resuspending the pellet in PBS and repeating the process three times. The tube and pellet were reweighed and the cells were extracted by suspending them in sample treatment buffer (STB) at pH 6.7 containing 0.75 g Tris, 5 ml mercaptoethanol, 10 ml glycerol, 35 ml bidistilled water, 4.3 ml 1N HCl, 1.149 g EDTA, made up to 100 ml with distilled water. For every 1 mg of cells, 0.018 ml STB and 2 l of 20 % sodium dodecyl sulphate (SDS) were mixed in a clean eppendorf tube. The mixture was then boiled for 10 minutes, cooled on ice and centrifuged for 10 minutes at 10 000 rpm. The supernatant containing the dissolved whole bacterial protein fraction was collected and stored at -80 °C.

Electrophoresis was performed in a 12% (w/v) gel slab, run vertically (30 mA constant current, 4 °C) until the bromophenol blue tracking dye (0.005% w/v in STB) added into samples had migrated near the bottom of the gels (3 h). Gel was stained for 1 h with Coomassie Blue (R250) solution (125 ml 2% aqueous stock Coomassie Blue, 500 ml methanol, 100 ml acetic acid and 375 ml distilled water), and destained overnight with several changes of destaining solution (25% v/v methanol and 10% v/v acetic acid in distilled water). Finally, gel was soaked in destaining solution containing 2% glycerol for 1 h. Gels were then dried at room temperature overnight in a drying frame (Camlab) between two cellophane sheets which were previously soaked in 10% glycerol for 1 h.

The protein profiles on the dried gels were scanned by a densitometer (Ultroscan XL, LKB 2222-020, Pharmacia LKB Biotechnology, LKB Produkter AB, Box 305, S-161 26 Bromma, Sweden) and the patterns were converted into densitometer records. Numerical analysis was performed following the methods described by VAUTERIN and VAUTERIN (1992) using Gel Compar Version 2.1 software (Applied Maths, Risquons-Toutstraat 38, B-8511 Kortrijk, Belgium) at the Central Science Laboratory, Hatching Green, Harpenden, UK. The program calculated the similarity between traces by the Pearson product moment correlation coefficient (*R*. SOKAL and SNEATH, 1963). Cluster analysis was performed on the matrix of correlation values by the unweighted pair group method using arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

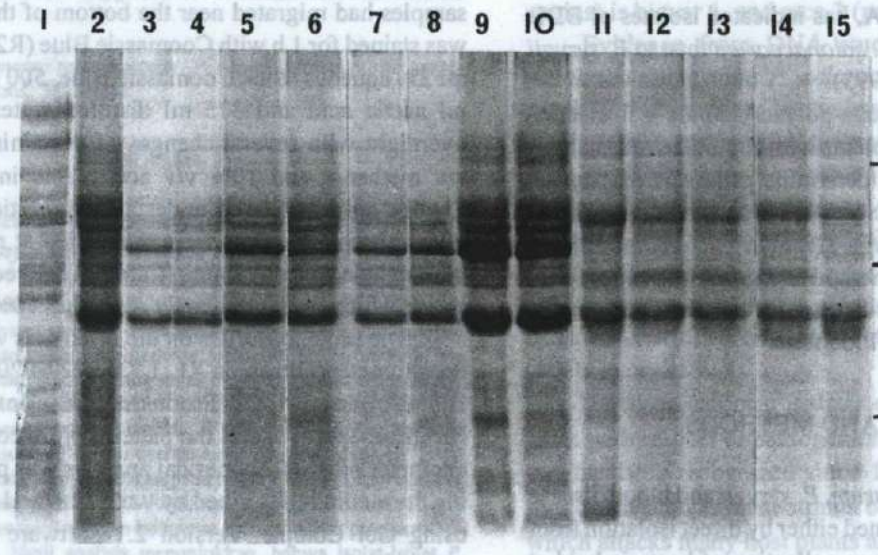
SDS-PAGE of whole cell protein extracts of *P. solanacearum* and related species produced many discrete bands with molecular weights of 14-100 kDa (Figure 1). Differences between strains were obvious in the major protein bands with molecular weights in the range of 20-45 kDa. For example, isolates of biovar 3 were characterised with the presence of a major band of proteins with approximately 38 kDa, which was lacking from biovar 1, 2 and N2. The protein profiles were reproducible both within and between gels as shown by comparing the profiles of the reference strain of *Psychrobacter immobilis* (three samples per gel) which showed 90% similarity. Similarly, the protein profiles of several clones of *P. solanacearum* (isolate R484) on different gels also showed 90% similarity.

Based on their SDS-PAGE protein profiles, Indonesian isolates of *P. solanacearum* representing biovars 1, 2 and 3, and *P. syzygii* and BDB could be separated into two major groups with only 52% similarity between the groups (Figure 2). Group I (65% similarity) contained isolates of *P. solanacearum*, whereas group II (58% similarity) was made up of isolates of both *P. syzygii*, *P. solanacearum* race 2 (banana strain) from the Philippines and Colombia, and BDB. Biovars 1, 2 and N2 (lowland strain biovar 2) of *P. solanacearum* formed a sub-group (I.2; 82% similarity), whereas all biovar 3 strains could be grouped into sub-group I.1 (78% similarity).

Isolates of *P. syzygii* formed a single homogeneous group, as did isolates of BDB. The protein profiles of one strain of *P.cepacia* differed significantly from those of *P. solanacearum*, *P. syzygii* and BDB showing only 27% similarity. Closer electrophoretic profiles were shown between isolates of BDB and *P. solanacearum* race 2 from the Philippines (R484 and R633) and single isolate from Colombia (R367a). However, single isolate of *P. solanacearum* race 2 from Grenada (R155a) formed a different group. The protein profiles of single isolate of *P. cepacia* differed from those of *P. solanacearum*, *P. syzygii* and BDB showing only 24 similarity.

Comparison of protein profiles of *P. solanacearum* ginger isolates showed that ginger strains from Indonesia differed from those of Australian and China origin with a 75% similarity level (Figure 3).

The results of the protein profile analysis of *P. solanacearum* from different hosts in Indonesia showed electrophoretic variation amongst the isolates tested. This variation, however, was not corresponded with their pathogenicity in host plants. Studies on their pathogenicity on tomato and ginger (SUPRIADI, 1994) showed that ginger isolates were



Lane 1 = *Psychrobacter immobillis* (reference strain), lanes 2 - 20 = *Pseudomonas solanacearum* biovar 3(2 = R791, tomato; 3 = S823, clove; 4 = R439, chili, 5 = R427, potato; 6 = R661, eggplant; 7 = R433, peanut; 8 = R850, weed; 9 = R810, ginger; 10 = R812, ginger), lanes 11 - 14 biovar 2 (11 = R792, chili; 12 = R 784, potato; 13 = R780, potato; 14 = R452, clove), and lane 15 = biovar 1 (R221, clove).

Figure 1. SDS-PAGE whole cell protein profiles of strains of *P. solanacearum* biovars 1 - 3 from various hosts in Indonesia

Gambar 1. Pola protein isolat P. solanacearum biovar 1-3 dari berbagai tanaman inang di Indonesia SDS-PAGE

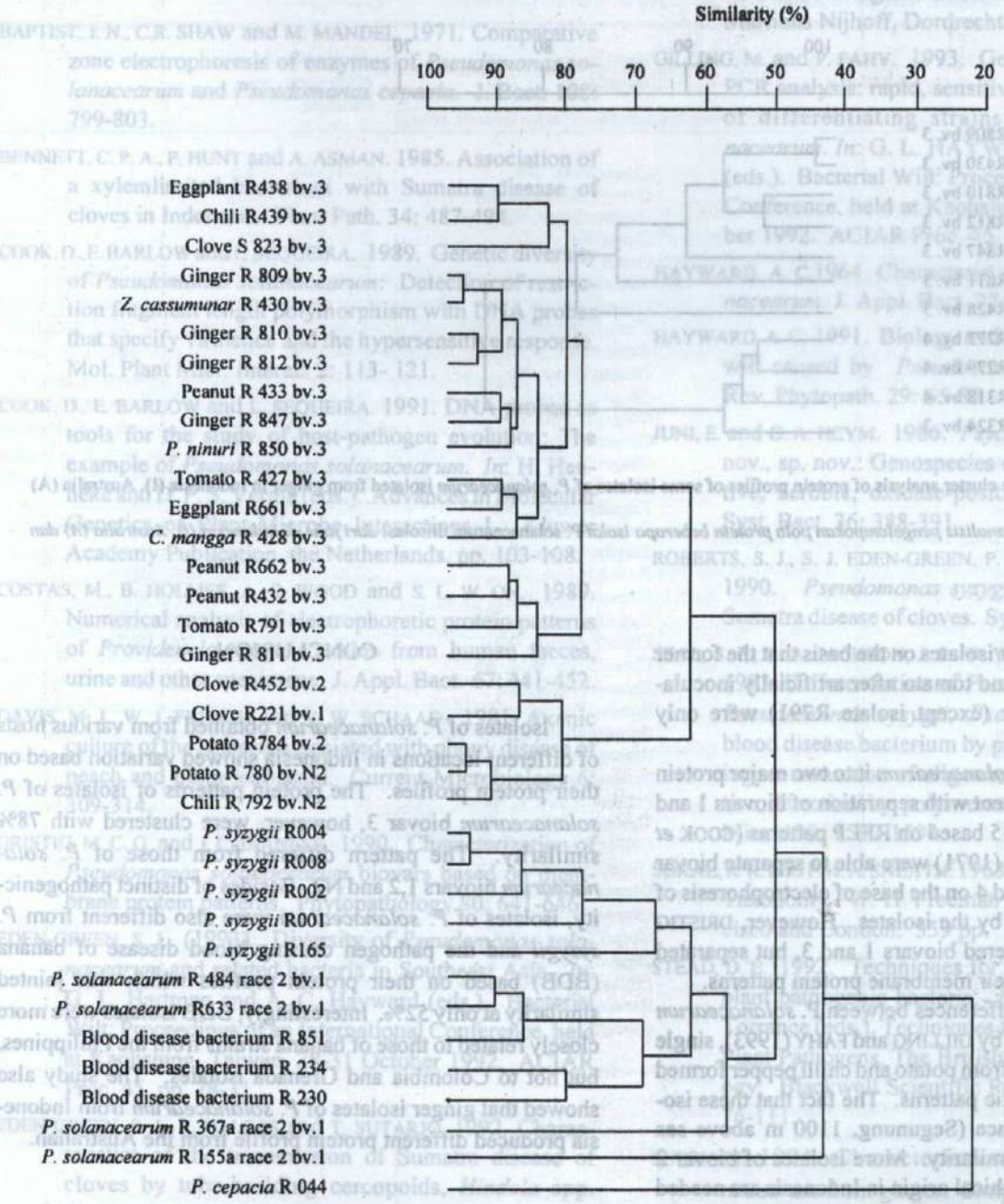


Figure 2. Dendrogram of the cluster analysis of protein profiles of some isolates of *P. solanacearum, P. syzygii* and blood disease bacterium (BDB) from Indonesia

Gambar 2. Dendrogram dari analisis pengelompokan pola protein beberapa isolat P. solanacearum, P. syzygii *dan bakteri penyakit darah (BDB) dari Indonesia*

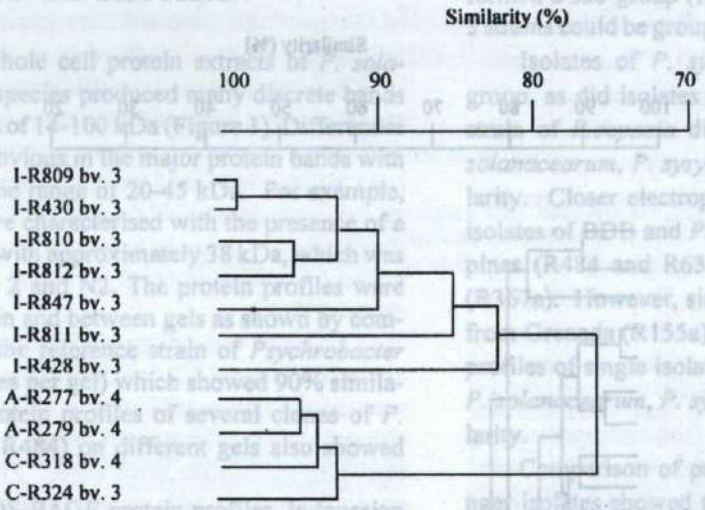


Figure 3. Dendrogram of the cluster analysis of protein profiles of some isolates of *P. solanacearum* isolated from ginger in Indonesia (I), Australia (A) and China (C)

Gambar 3. Dendrogram dari analisis pengelompokan pola protein beberapa isolat P. solanacearum diisolasi *dari jahe di Indonesia (I), Australia (A) dan China (C)*

separated from non-ginger isolates on the basis that the former were able to wilt ginger and tomato after artificially inoculation, whereas non-ginger (except isolate R791) were only killed tomato.

The grouping of *P. solanacearum* into two major protein profiles may be in agreement with separation of biovars 1 and 2 from those of biovars 3-5 based on RFLP patterns (COOK *et al.*, 1991). BAPTIST *et al.*, (1971) were able to separate biovar 1 and 2 from those of 3 and 4 on the base of electrophoresis of seven enzymes produced by the isolates. However, DRISTIG and DIANESE (1990) clustered biovars 1 and 3, but separated from biovar 2 based on their membrane protein patterns.

Despite genotypic differences between P. *solanacearum* biovars 2 and N2 reported by GILLING and FAHY (1993), single isolate of biovar 2 and N2 from potato and chilli pepper formed very similar electrophoretic patterns. The fact that these isolates were from same place (Segunung, 1100 m above sea level) may explain this similarity. More isolates of biovar 2 or N2 from wider geographical origin in Indonesia are needed to be tested to verify this preliminary study.

Close relationship of isolates of *P. syzygii* and BDB was found here from their protein profiles, supported earlier finding on their relationships based on DNA/ DNA studies and fatty acid profiles (ROBERTS *et al.*, 1990; STEAD, 1992; SEAL *et al.*, 1993). In addition, the protein profile of biovar 1 (race 2) from the Philippines was placed in the same group with BDB and *P. syzygii*. Studies with indirect ELISA using two monoclonal antibodies apparently specific to *P. solanacearum* showed that these isolates were not bound to the sera (SUPRIADI, unpublished data).

CONCLUSION

Isolates of *P. solanacearum* obtained from various hosts of different locations in Indonesia showed variation based on their protein profiles. The protein patterns of isolates of *P. solanacearum* biovar 3, however, were clustered with 78% similarity. The pattern differed from those of *P. solanacearum* biovars 1,2 and N2. Besides of distinct pathogenicity, isolates of *P. solanacearum* were also different from *P. syzygii* and the pathogen causing blood disease of banana (BDB) based on their protein profiles which was jointed similarity at only 52%. Interestingly, BDB isolates were more closely related to those of banana strains from the Philippines, but not to Colombia and Grenada isolates. The study also showed that ginger isolates of *P. solanacearum* from Indonesia produced different protein profile from the Australian.

ACKNOWLEDGEMENTS

The first author is indebted to the British Council, UK and the Agency for Agricultural Research and Development (AARD), Indonesia for their financial support during the study. We are indebted to the Plant Pathology Department, Rothamsted Experimental Station (RES) and the Central Science Laboratory (CSL), Harpenden, UK for providing facilities to perform this study.

REFERENCES

BAPTIST, J. N., C.R. SHAW and M. MANDEL. 1971. Comparative zone electrophoresis of enzymes of *Pseudomonas solanacearum* and *Pseudomonas cepacia*. J. Bact. 108: 799-803.

BENNETT, C. P. A., P. HUNT and A. ASMAN. 1985. Association of a xylemlimited bacterium with Sumatra disease of cloves in Indonesia. Plant Path. 34: 487-494.

COOK, D., E. BARLOW and L. SEQUEIRA. 1989. Genetic diversity of *Pseudomonas solanacearum*: Detection of restriction fragment length polymorphism with DNA probes that specify virulence and the hypersensitive response. Mol. Plant Micr. Interac. 2: 113- 121.

COOK, D., E. BARLOW and L. SEQUEIRA. 1991. DNA probes as tools for the study of host-pathogen evolution: The example of *Pseudomonas solanacearum*. *In*: H. Henneke and D. P. S. Verma (eds.). Advances in Molecular Genetics of Plant-Microbe Interactions I. Kluwer Academy Publication, the Netherlands. pp. 103-108.

COSTAS, M., B. HOLMES, A. C. WOOD and S. L. W. ON. 1989. Numerical analysis of electrophoretic protein patterns of *Providencia rettgeri* strains from human faeces, urine and other specimens. J. Appl. Bact. 67: 441-452.

DAVIS, M. J., W. J. FRENCH and N. W. SCHAAD. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Current Microbiology 6: 309-314.

DRISTIG, M. C. G. and J. C. DIANESE. 1990. Characterization of *Pseudomonas solanacearum* biovars based on membrane protein patterns. Phytopathology 80: 641-646.

EDEN-GREEN, S. J. (1993). Diversity of *Pseudomonas solanacearum* and related bacteria in Southeast Asia. *In*: G. L. Hartman and A. C. Hayward (eds.). Bacterial Wilt: Proceedings of an International Conference, held at Kaohsiung, Taiwan, 28-31 October 1992. ACIAR Proc. 45. pp. 28-31.

EDEN-GREEN, S. J., R. BALFAS and T. SUTARJO. 1992. Characteristics of the transmission of Sumatra disease of cloves by tube-building cercopoids, *Hindola* spp. Plant Path. 41: 702- 712.

EDEN-GREEN, S. J. and H. SASTRAATMADJA. 1990. Blood disease of banana present in Java. FAO Plant Prot. Bull. 38: 49-50.

EDEN-GREEN, S. J., SUPRIADI, N. HASNAM and P. HUNT. 1988. Serological relationship between the xylem-limited bacterium causing Sumatra disease of cloves in Indonesia and *Pseudomonas solanacearum*. *In*: E. L. Civerolo, A. Collmer, R. E. Davis and A. G. Gillaspie (eds.). Proceeding of the 6th International Conference on Plant Pathogenic Bacteria, Maryland, USA, 1985. Martinus Nijhoff, Dordrecht. pp 357-363.

GILLING, M. and P. FAHY. 1993. Genomic fingerprinting and PCR analysis: rapid, sensitive and inexpensive means of differentiating strains of *Pseudomonas solanacearum*. *In*: G. L. HAYWARD and A. C. Hayward (eds.). Bacterial Wilt: Proceedings of an International Conference, held at Kaohsiung, Taiwan, 28-31 October 1992. ACIAR Proc. 45. pp. 85-92.

HAYWARD, A. C.1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bact. 27: 265-277.

HAYWARD, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Ann. Rev. Phytopath. 29: 65-87.

JUNI, E. and G. A. HEYM. 1986. *Psychrobacter immobilis* gen. nov., sp. nov.: Genospecies composed of Gram-negative, aerobic, oxidase-positive coccobacilli. Int. J. Syst. Bact. 36: 388-391.

ROBERTS, S. J., S. J. EDEN-GREEN, P. JONES and D. J. AMBLER. 1990. *Pseudomonas syzygii*, sp. nov., the cause of Sumatra disease of cloves. Syst. Appl. Micr. 13: 34-43.

SEAL, S. E., L. A. JACKSON, J. P. W. YOUNG and M. J. DANIELS. 1993. Differentiation of *Pseudomonas solanacearum, Pseudomonas syzygii, Pseudomonas pickettii* and blood disease bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. J. Gen. Micr. 139: 1587-1594.

SOKAL, R. R. and P. H. A. SNEATH. 1963. Principles of Numerical Taxonomy. W. H. Freeman and Company, San Francisco and London. 359 pp.

STEAD, D. E. 1992. Techniques for detecting and identifying plant pathogenic bacteria. *In*: J. M. Duncan and L. Torrance (eds.). Techniques for the Rapid Detection of Plant Pathogens. The British Society for Plant Pathology. Blackwell Scientific Publications. Oxford. pp. 76-111.

SUPRIADI. 1994. Characteristics of *Pseudomonas solanacearum* from ginger. Simposium Tanaman Industri II. Cipayung, 21-23 Nopember 1994: 7 pp.

VAUTERIN, L., J. SWINGS and K. KERSTERS. 1991. Grouping of *Xanthomonas campestris* pathovars by SDS-PAGE of proteins. J. Gen. Micr. 137: 1677-1687.

VAUTERIN, L. and P. VAUTERIN. (1992). Computeraided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. Europ. Micr., Nov./Dec.: 37-41.

Appendix 1. List of isolates of *P. solanacearum*, *P. syzygii* and blood disease bacterium (BDB) used in this study.
Lampiran 1. Daftar isolat P. solanacearum, P. syzygii dan bakteri penyakit darah (BDB) yang digunakan dalam studi ini

Code *Kode*	Identity *Identitas*	Biovar *Biovar*	Host/Origin[1] *Inang/Asal*	Contributor[2] (Orig.code, year *Sumber)*
R001	*P. syzygii*	-	Clove/Solok, WS	E-G (S444, 1980)
R002	*P. syzygii*	-	Clove/Sukamantri, WJ	E-G (S442, 1983)
R004	*P. syzygii*	-	Hindola/Solok, WS	E-G (S627, 1985)
R008	*P. syzygii*	-	Clove/WS	E-G (S504, 1985)
R004	*P. cepacia*	-	Onion	BUR (NCP2993,?)
R155a	*P. solanacearum*	I	Banana/Grenada	E-G (M107A,)
R165	*P. syzigii*	-	Clove/Ciawi, WJ	E-G (T298, 1987)
R221	*P. solanacearum*	I	Clove/Solok, WS	BEN (CMIb9043,1980)
R230	BDB	-	Banana/Jonggol, WJ	E-G (T334, 1987)
R234	BDB	-	Banana/Sinjai, SS	E-G (T391, 1988)
R277	*P. solanacearum*	4	Ginger/Australia	HAY (HAY007A,UW141,?)
R279	*P. solanacearum*	4	Ginger/Australia	HAY (HAY092) R318
	P. solanacearum	4	Ginger/China	HE (CIP281, 1987)
R324	*P. solanacearum*	3	Ginger/China	HE (CIP282, 1987)
R367a	*P. solanacearum*	I	Plantain/Colombia	KEL (CIP32)
R427	*P. solanacearum*	3	Tomato/Subang, WJ	E-G (TomSuban1,1990)
R428	*P. solanacearum*	3	Curcuma/Cimanggu, WJ	SUP (T447, 1988)
R430	*P. solanacearum*	3	Zingiber/Cimanggu, WJ	SUP (T451, 1988)
R432	*P. solanacearum*	3	Peanut/Maros, SS	SUB (SUB1105MR,T494, 1990)
R433	*P. solanacearum*	3	Peanut/Bogor, WJ	SUB (SUB1105B,T494, 1990)
R438	*P. solanacearum*	3	Eggplant/Pasaman, WS	SUB (SUB1505WS,T499, 1990)
R439	*P. solanacearum*	3	Chilli/Pasaman, WS	SUB (SUB1805WS,T500, 1990)
R452	*P. solanacearum*	2	Clove/Sukamantri, WJ	E-G (S710, 1985)
R484	*P. solanacearum*	I	Banana/Philippines	E-G (P14)
R633	*P. solanacearum*	I	Banana/Philippines	JE (JE-Mok2)
R661	*P. solanacearum*	3	Eggplant/Subang, WJ	JE (JE-Sub5,1991)
R662	*P. solanacearum*	3	Peanut/Malang, EJ	MM (JE-Mah7, 1991)
R780	*P. solanacearum*	N2	Potato/Segunung, WJ	JE (IPotstem1,1992)
R784	*P. solanacearum*	2	Potato/Segunung, WJ	JE (IPottub3, 1992)
R791	*P. solanacearum*	3	Tomato/Segunung, WJ.	JE (Itomstem4, 1992
R792	*P. solanacearum*	N2	Chilli/Segunung, WJ	JE (IPepem1, 1992)
R809	*P. solanacearum*	3	Ginger/Sukamulya, WJ	SUP (T594, 1992)
R810	*P. solanacearum*	3	Ginger/Sukamulya, WJ	SUP (T598, 1992)
R811	*P. solanacearum*	3	Ginger/Sukamulya, WJ	SUP (T600, 1992)
R812	*P. solanacearum*	3	Ginger/Cicurug, WJ	SUP (T603, 1992)
R847	*P. solanacearum*	3	Ginger/Cimanggu, WJ	SUP (T585, 1992)
R850	*P. solanacearum*	3	*Phylanthus*/Cimanggu,WJ	SUP (T727, 1993)
R851	BDB	-	Banana/Sumompo, SS	SUP (T636, 1992)
S823	*P. solanacearum*	3	Clove/Bogor, WJ	E-G (S823, 1986)

[1] EJ = East Java; WJ = West Java; SS = South Sulawesi (Indonesia)
[2] BEN = C. P. A. BENNETT; BUR = BURKHOLDER; E-G = S. J. EDEN- GREEN ; JE = J. G. ELPHINSTONE; HE = He ; KEL = A. KELMAN ; MM = M. MACHMUD; SUB = S. SUBANDIAH; SUP = SUPRIADI