FINGERPRINT ANALYSIS OF DNA FRAGMENTS OF *Pseudomonas solanacearum* E.F. SMITH ISOLATES

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Ringkasan

Studi terhadap sidikjari pola susunan potongan-potongan DNA isolat-isolat bakteri *Pseudomonas solanacearum* telah dilakukan untuk perbandingan langsung isolat khususnya antara isolat-isolat kacang tanah dan bukan kacang tanah. Isolat-isolat yang berasal dari tanaman inang dan lokasi yang berbeda digunakan dalam penelitian tersebut.

DNA kromosom dari isolat-isolat tersebut diekstraksi menggunakan modifikasi metoda yang digunakan oleh Samadpour et al. (1988). Sampel-sampel DNA dipotong-potong sempurna menggunakan enzim-enzim pemotong DNA Eco RI, Hae III, Bam HI, Not I, atau Sal I. Potongan-potongan sampel DNA dielektroporesis pada 0.7% gel agarose menggunakan tegangan 30 volt selama 14 – 16 jam. Ethidium bromide 0.5 μg/ml digunakan untuk pengecatan potongan-potongan DAN sampel pada gel agarose. Pengamatan terhadap pola susunan potongan-potongan DNA sampel pada gel agarose menggunakan UV trasilluminator dan hasilnya difoto dengan kamera polaroid. Koefisient persamaan antara isolat berdasarkan pola susunan potongan-potongan DNA-nya dihitung dengan program TAXAN version 4.0.

Hasil penelitian tersebut menunjukkan bahwa isolat-isolat yang berbeda mempunyai pola susunan potongan-potongan DNA yang berbeda, walaupun isolat-isolat dari biovar 3 mempunyai pola susunan dasar yang serupa. Koefisien persamaan antara isolat-isolat dari biovar 3 berkisar antara 81.3 – 96.9%. Isolat-isolat dari biovar 3 dan isolat dari biovar 2 atau aberrant biova 2 mempunyai koefisien persamaan yang rendah. Ditemukan adanya korelasi antara pola susunan potongan-potongan DNA dengan lokasi atau geografi asal dari isolat-isolat tersebut daripada dengan patogenisitasnya.

Abstract

A study on the DNA fragment fingerprints of some isolates of *Pseudomonas solanacearum* was done for a direct comparison between isolates especially between peanut and non peanut isolates. Several isolates originating from different host plants and different localities in Australia and Indonesia were used in the experiment.

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The chromosomal DNA of the isolates were extracted using the modification method used by Samadpour *et al.* (1988). The DNA samples were completely digested using restriction enzymes of *Eco* RI, *Hae* III, *Not* I, *Bam* HI or *Sal* I. The DNA fragments were electrophoresed on 0.7% agarose gel at 30 volt for 14 – 16 h. The DNA fragments on the gel were stained using 0.5 μg/ml ethidium bromide for 20 minutes and then visualized on a UV transilluminator and photographed. The similarity between the isolates on the DNA banding patterns were analyzed using Taxan version 4.0 computer program. Different isolates gave different DNA banding patterns. The similarity coefficients between the isolates of biovar 3 ranged from 81.3 to 96.9%, whereas the similarity coefficients between the isolates of biovar 3 and biovar 2 isolate or aberrant biovar 2 isolate were low. Furthermore, the results suggested that the DNA banding patterns reflected more geographical origin of the isolates rather than their host plant origin.

Introduction

Bacterial wilt caused by *P. solanacearum* is common on many crops throughout the tropics and subtropics, but the disease on peanut occurs only in isolated areas (Mehan *et al.*, 1986). The disease on peanut is the only important bacterial disease and causes considerable losses in major peanut production areas of Indonesia, China, and Uganda (Mehan *et al.*, 1986, Machmud, 1986, He *et al.*, 1983, Opio and Busolo-Bulafu, 1990). There was no report of peanut bacterial wilt in Australia.

P. solanacearum is a heterogeneous species (Hayward, 1964, Buddenhagen and Kelman, 1964). As a plant pathogen the bacterium has a very wide range of host plants and based on the pathogenicity, the bacterium was divided into four races (Buddenhagen and Kelman, 1964, He *et al*, 1983). Race 1 consists of strains with very wide range of host plants including solanaceae and leguminoceae. Therefore, peanut isolates belong to race 1.

Based on the biochemical characteristics, the bacterium was divided into five biovars (Hayward, 1964, He *et al*, 1983). Peanut isolates originating from Indonesia commonly belong to biovar 3 (Machmud, pers. comm.), whereas peanut isolates originating from China were biovar 3 or biovar 4.

The distribution of distinct pathotypes in nature in relation to ecology and etiology of bacterial wilt of peanut needs to be investigated. It is not known whether the sporadic occurrence of bacterial wilt of peanut is reflection of the restricted distribution of specific strains, or whether the determining factor is the environment. The principle objective of this project was to compare some strains of *P. solanacearum* originating from different host and different localities at the DNA level. The DNA fragment fingerprints may help us to understand the variability in *P. solanacearum* in relation to host-pathogen interaction.

Methods

1. DNA Extraction

The cultures were grown in Casamino acid Peptone Glucose broth (Kelman, 1954) at 28 °C for 24 h with shaking. The cells were harvested by pelleting 1.5 ml of culture in 1.5 ml Eppendorf microcentrifuge tubes. The cells were washed in 1 ml of 50 mM Tris-HCl pH 8, and centrifuge for 5 min. The cells were resuspended in 0.7 ml 50 mM Tris-HCl and 50 mM EDTA containing 2 mg/ml lysozyme and incubated at room temperature for 30 min. A 10 μl volume of 20% SDS and 50 μl volume of 1% pronase E were added to the tubes followed by incubation at 37 °C for 30 min. A 0.7 ml volume of phenol saturated in 50 mM Tris-HCl was added and the tubes hand shaken vigorously followed by 30 min incubation at 37 °C. The tubes were centrifuged for 5 min to separate the phases. The aqueous layer was transferred to a fresh tube and the tube filled with chloroform, vortex for 1 min, and centrifuge for 5 min. The upper layer was transferred to a fresh tube, 3 drops of 5 M ammonium acetate were added and the tubes were filled up with cold absolute ethanol. Precipitated DNA was spooled using a glass rod. The DNA on the glass rod was rinsed 3 times with 70% ethanol and dissolved in 400 μl of mM Tris-HCl and 1 mM EDTA pH 8.

2. DNA Digestion and DNA Electrophoresis

The DNA samples were digested using several restriction enzymes like *Eco* RI, *Not* I, *Bam* HI, *Hae* III or *Sal* I. A complete digestion was done for the DNA samples.

The DNA fragments were electrophoresed in 0.7% agarose gel using a Max Submarine Agarose Gel Unit at 30 volt for 14 – 16 h. The DNA fragments on the gel were stained using 0.5 μg/ml ethidium bromide for 20 min. The DNA fragments on the gel was visualized using a UV transilluminator and photographs taken using a polaroid camera.

The molecular weight of the DNA fragments of each strain was estimated by comparison to the λ-*Hind* III molecular weight markers run on the same gel. The $\log_{10}$ of the distance traveled in mm by the λ-*Hind* III molecular weight markers was plotted against the $\log_{10}$ value of the molecular weight of the λ-*Hind* III molecular weight markers. A third level polynomial equation to fit the resultant line was derived and the molecular weight of each of the first 12 – 15 bands from the *Sal* I digest of the DNA from each of the strains was estimated by measuring the distance traveled by these bands from the well and using the equation derived above. TAXAN version 4.0 was

used to calculate the similarity between the isolates based on the DNA banding patterns resulting from *Sal* I digestion.

Table 1. **The List of Isolates Used in the Experiments**

Isolate	Host plant	Locality	Biovar
001	Tomato	Redland Bay, Qld	3
0234	*Pultenaea villosa*	Nambour, Qld	3
0171	*Solanum melongena*	Nambour, Qld	3
0190	*Xanthium pungens*	Nambour, Qld	3
0369A	Tomato	Banora Point, NSW	3
01017S	*Solanum nigrum*	Maroota, NSW	3
01023S	*Streilitzia reginae*	Somersby, NWS	3
0732	Tomato	Darwin, NT	Ab. 2
0505	Potato	Gatton, Qld	2
EW0290	Peanut	Tangerang, West Java	3
1105 B	Peanut	Bogor, West Java	3
1405 WS	Peanut	Pasaman, West Java	3
1905 Mr	Peanut	Maros, South Sulawesi	3
T435	Chili	Wates, Central Java	3

Results

The restriction enzymes of *Bam* HI or *Sal* I gave better results for the digestion of our DNA preparation compare to those resulting from *Eco* RI or *Hae* III digestion. The results suggested that there was correlation between the DNA banding patterns with the geographical origin of the isolates rather than host plant of origin.

The DNA banding patterns resulting from *Bam* HI digestion suggested that the isolates of *P. solanacearum* biovar 3 have very similar DNA banding patterns, but completely different from those of biovar 2 isolate and aberrant biovar 2 isolate. *Sal* I digestion showed similar results that there were slightly differences in DNA banding patterns between the isolates of biovar 3, and different from those of biovar 2 and aberrant biovar 2 isolates (Figures 1 and 2). All of the isolates of biovar 3 originating from Indonesia and Australia has very similar DNA banding patterns resulting from *Sal* I but not identical. On the other hand, all of the isolates of biovar 3 originating from different localities in Australia have identical DNA banding patterns. The similarity between isolates of biovar 3 ranged from 81.3% to 96.9%. The similarity between the isolates of biovar 3 and biovar 2 isolate ranged from 40.6% to 50.0%, and between the isolates of biovar 3 and aberrant biovar 2 isolate ranged from 46.9 to 56.3%.

The similarities between the isolates of biovar 3 vary. Non peanut isolates of biovar 3 originating from Australia have a similarity coefficient of 78.1% with the Indonesian peanut isolate EW0290 and

87.5% with peanut isolate 1905 Mr. The similarity coefficient between the Australian non peanut isolates and the Indonesian non peanut isolate (T435) was 90.6%. Two peanut isolates originating from different localities in Indonesia (isolates EW0290 and 1905 Mr) have different fragment patterns with the similarity coefficient of 84.8%.

The dendogram of the similarity between the isolates is shown in Figure 3. All of biovar 3 isolates formed a cluster, whereas, isolate of biovar 2 and aberrant biovar 2 were not related according to restriction endonuclease analysis.

Discussion

The restriction endonuclease analysis suggested that all biovar 3 isolates formed a cluster, however there were differences between the isolates in the DNA banding patterns. The differences indicated that there was correlation between the fragment patterns and the geographical origin rather than the pathogenicity of the isolates. Two peanut isolates originating from different island in Indonesia (EW0290 and 1905) have different DNA banding patterns. Non peanut isolates originating from different localities in Australia have identical DNA banding patterns. Non peanut isolate from Indonesia (T435) has different DNA banding patterns from those of peanut isolates from Indonesia and non peanut isolates from Australia.

Similar results have been reported by Cook *et al.* 1989 and Cook *et al.*, 1990 They used nine probes to analyze the restriction fragment length polymorpism within *P. solanacearum* isolates. They found 33 distinct RFLP patterns of *P. solanacearum* isolates that could be grouped into two different divisions with similarity coefficients of only 13.5% between them. Within the division the similarity between the isolates were high. Many of the RFLP groups were highly correlated with geographical location and less frequently with host of origin. Division I contains all members of race 1, biovar 3, 4 and 5 and 90% of the isolates were from Asia and Australia. Division II contains all members of race 1, biovar 1 and races 2 and 3, and 98% of the isolates were from the Americas. Peanut isolates originating from different localities in China fell into different RFLP groups. However, Ma (1990) found that 12.8kb DNA sequence pattern appeared to be present only in *P. solanacearum* strains which could cause wilt on peanut and not in strains which were not pathogenic on peanut.

Conclusion

Sal I digestion can be used for fingerprint analysis of DNA fragments of *P. solanacearum* isolates using agarose gel electrophoresis. There was a correlation between the fragment patterns and the geographical origin rather than the pathogenicity of the isolates.

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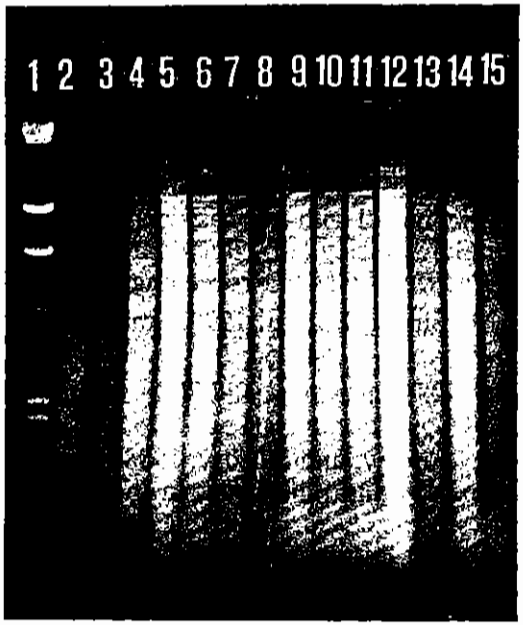
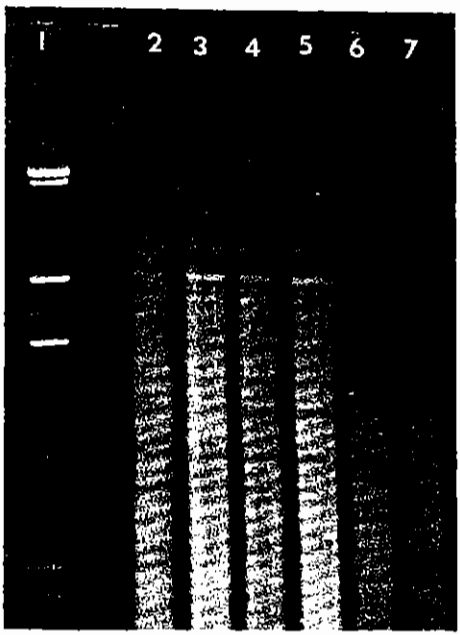


1. λDNA-Hind III
2. 1105 Mr
3. 1405 WS
4. EW0290
5. 1905 Mr
6. 0171
7. 0190
8. 0234
9. 001
10. 0369A
11. 01017S
12. 010123S
13. T 435
14. 0732
15. 0505

Figure 1. **Chromosomal DNA fragments of 14 isolates of *P. solanacearum* resulting from *Sa1* I digestion**



1. λDNA-Hind III
2. EW0290
3. 0171
4. 001
5. 01023S
6. 0732
7. 0505

Figure 2. **Chromosomal DNA Fragments of Six Isolates of *P. solanacearum* Resulting from *Sal* I Digestion.**

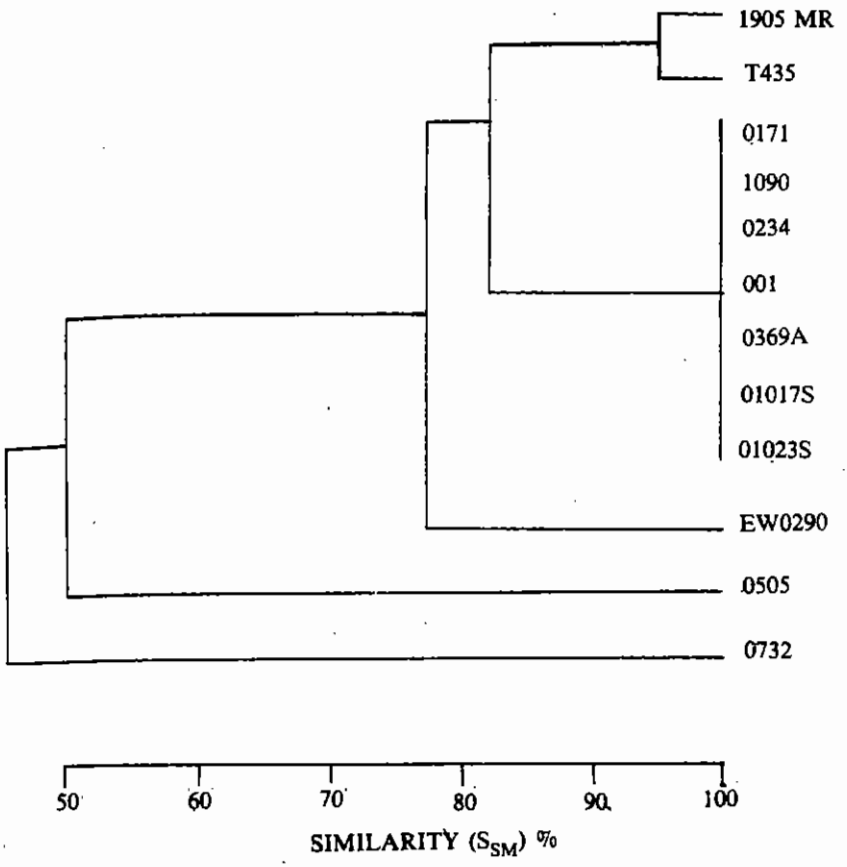


Figure 3. **The Dendrogram of Similarity Between the Isolates of *P. solanacearum* Based on the Chromosomal DNA Fragments Resulting From *Sal* I Digestion.**