Analysis of the Phylogenetic Relationships of Strains of *Burkholderia solanacearum*, *Pseudomonas syzygii*, and the Blood Disease Bacterium of Banana Based on 16S rRNA Gene Sequences

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We determined nearly complete 16S rRNA gene sequences for 19 isolates of *Burkholderia solanacearum*, three isolates of the blood disease bacterium of bananas, and two isolates of *Pseudomonas syzygii*, the cause of Sumatra disease of cloves. The dendrogram produced by comparing all of these sequences revealed that there were two divisions, which corresponded to the results obtained previously in a restriction fragment length polymorphism analysis (D. Cook, E. Barlow, and L. Sequeira, *Mol. Plant Microbe Interact.* 2:113–121, 1989) and a total 16S ribosomal DNA (rDNA) sequence analysis of four isolates representing four biovars of *B. solanacearum* (X. Li, M. Dorsch, T. Del Dot, L. I. Sly, E. Stackebrandt, and A. C. Hayward, *J. Appl. Bacteriol.* 74:324–329, 1993). Division 1 comprised biovars 3, 4, and 5 and an aberrant biovar 2 isolate (strain ACH0732), and division 2 included biovars 1, 2, and N2, the blood disease bacterium, and *P. syzygii*. Specific nucleotides at positions 458 to 460 (UUC) and 474 (A) characterized division 2, whereas in division 1 the nucleotides at these positions were ACU and U, respectively. However, strain ACH0732 had a U at position 458, as did division 2 isolates, and G instead of U at position 474. Division 2 consisted of two subdivisions; one subdivision contained two *B. solanacearum* isolates that originated from Indonesia, *P. syzygii* strains, and blood disease bacterium strains, and the other subdivision contained all of the other division 2 isolates. Within division 1, the level of 16S rDNA sequence similarity ranged from 99.8 to 100%, and within division 2, the levels of 16S rDNA sequence similarity ranged from 99.1 to 100%. The division 1 isolates exhibited an average level of 16S rDNA sequence similarity to division 2 isolates of 99.3% (range, 99.1 to 99.5%). The occurrence of consistent polymorphisms in the 16S rDNA sequences of *B. solanacearum* strains, in particular unique 16S rDNA sequence differences in aberrant biovar 2 isolate ACH0732, and the occurrence of the Indonesian subdivision of division 2 suggest that this group is a rapidly evolving (tachytelic) group.

The use of rRNA sequences for classification and identification of microorganisms is now routine, and analysis of 16S rRNA gene sequences provides a powerful tool for determining the phylogenetic and evolutionary relationships of microorganisms (34). The rRNA genes are useful molecular chronometers not only because of their conserved nature, high information content, and universal distribution, but also because of the rapidly expanding 16S rRNA gene sequence database that can be used for phylogenetic comparisons.

Burkholderia solanacearum causes bacterial wilt of a wide range of crops and is one of the most important disease bacteria in tropical and subtropical regions and some of the warm temperate regions of the world (12). *B. solanacearum* belongs to pseudomonad rRNA homology group II as determined by rRNA-DNA homology (24) and to the beta subclass of the *Proteobacteria* (8, 18).

B. solanacearum is a heterogeneous species which exhibits significant phenotypic diversity (3, 12). Traditionally, *B. solanacearum* strains have been divided into five races on the basis of host range and into five biovars on the basis of biochemical properties (10, 12). Two clusters of *B. solanacearum* strains have been identified on the basis of restriction fragment length polymorphisms (3, 4) and on the basis of the 16S ribosomal DNA (rDNA) sequences of four isolates representing biovars 1, 2, 3, and 4 (18).

Pseudomonas syzygii, the causal agent of Sumatra disease of cloves (*Syzygium aromaticum*), is a close relative of *B. solanacearum*, as determined by phenotypic data and DNA-DNA hybridization data (25). The blood disease bacterium (BDB), the causal agent of blood disease of bananas, affects certain members of the Musaceae in Indonesia (1, 6). The BDB strains have a distinct host range and several physiological and nutritional characteristics that are quite distinct from characteristics of *B. solanacearum* (5, 6, 28). However, partial 16S rDNA sequence data for the BDB and *P. syzygii* have confirmed that these organisms are close relatives of *B. solanacearum* (28). Therefore, complete sequence data for the 16S rRNA genes of *P. syzygii* and the BDB could help resolve the relationships of these bacteria and *B. solanacearum*.

MATERIALS AND METHODS

Bacterial strains. The bacterial isolates used in this study are listed in Table 1. *B. solanacearum* and the BDB isolates were grown on sucrose-peptone agar (10) or tetracycline chlorid plates (16) and were incubated at 28°C for 48 to 72 h. *P. syzygii* isolates were cultured on Casamino Acids medium (25).

DNA purification. Genomic DNA was extracted and purified by using two methods, the method of Marmur (21) and the method of Boucher et al. (2).

PCR. The 16S rRNA gene was amplified in a 100- μ l (total volume) reaction mixture containing PCR buffer [67 mM Tris-HCl (pH 8.8), 16.6 mM (NH₄)₂SO₄, 0.45% (vol/vol) Triton X-100, 200 μ g of gelatin per ml], 1.5 mM MgCl₂, each deoxynucleoside triphosphate at a concentration of 200 μ M, 0.25 μ M primer 27f, 0.25 μ M primer 1525r (17), 100 ng of DNA, and 1 U of *Tth* Plus DNA polymerase (Biotech International, Ltd., Perth, Australia). Negative controls that contained all of the ingredients described above except for the template DNA were included in each experiment. Each reaction mixture was overlaid with 80 μ l of mineral oil to prevent evaporation.

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TABLE 1. *B. solanacearum* strains and related bacteria studied

Organism	Strain ^a	Location	Host	Biovar	Division or subdivision	Year isolated	Other designation(s) ^a	GenBank accession no.
<i>B. solanacearum</i>	R288	People's Republic of China	Mulberry	5	1		UW373	U27984
	ACH092	Australia	Ginger	4	1	1966		U27985
	ACH0171	Australia	Eggplant	3	1	1967		U27986
	R791	Indonesia	Tomato	3	1	1992		U27987
	CIP365	Philippines	Potato	5	1			U28220
	ACH0732 ^b	Australia	Tomato	2	1	1979		U27983
	PDDCC1727 ^T	United States	Tomato	1	2a		ATCC 1696 ^T , K60 ^T , ACM3851 ^T	U28221
	CIP 210	Brazil	Potato	1	2a	1976	Brazil 62, 1076	U28222
	R207	Belize	<i>Musa</i> ABB (Moko)	1	2a			U28223
	ACH0158	Australia	Potato	2	2a	1966		U28224
	Br 150	United Kingdom	<i>Solanum dulcamara</i>	2	2a			U28225
	CIP238	Chile	Potato	2	2a	1983		U28226
	CIP10	Peru	Potato	N2	2a	1979		U28227
	R483	Philippines	Banana (Bugtok)	1	2a			U28228
	R634	Philippines	Banana (Moko)	1	2a	1991	JEMOK6	U28229
	R633	Philippines	Banana (Moko)	1	2a	1991	JEMOK2	U28230
	R639	Philippines	Banana (Bugtok)	1	2a	1991	JEBUG1	U28231
	R780	Indonesia	Potato	N2	2b	1992		U28232
	R142	Indonesia	Clove	2	2b	1985		U28233
BDB	R506	Indonesia	Banana		2b		T340	U28234
	R233	Indonesia	Banana		2b	1986	T379	U28235
	R223	Indonesia	Banana		2b	1986	T439	U28236
<i>P. syzygii</i>	R001 ^T	Indonesia	Clove		2b	1980		U28237
	R058	Indonesia	Clove		2b	1985		U28238

^a Abbreviations: R, Rothamsted Experimental Station, Harpenden, Hertfordshire, United Kingdom; ACH, A. C. Hayward, Department of Microbiology, Centre for Bacterial Diversity and Identification, The University of Queensland, St. Lucia, Australia; ACM, Australian Collection of Microorganisms, Department of Microbiology, Centre for Bacterial Diversity and Identification, The University of Queensland, St. Lucia, Australia; CIP, International Potato Center, Lima, Peru; ATCC, American Type Culture Collection, Rockville, Md.; PDDCC, Culture Collection of Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand.

^b Atypical biovar 2 strain.

PCR amplifications were performed with a Perkin-Elmer Cetus model 480 thermal cycler programmed as follows: an initial denaturation step at 96°C for 3 min, followed by 28 cycles consisting of 48°C for 1 min, 72°C for 2 min, and 93°C for 1 min, with a final extension step at 48°C for 1 min and at 72°C for 5 min to allow all extension products to be completed.

The PCR products were examined by electrophoresing 5 µl of each PCR product in 1% agarose gels at 4 V/cm for 10 min and then at 10 V/cm for 30 min. The PCR amplification products were visualized by staining with ethidium bromide.

The remaining 95 µl of each PCR product was purified by using a Promega Magic PCR Prep DNA purification kit according to the manufacturer's instructions.

DNA sequencing. A Taq DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems, Foster City, Calif.) was used as recommended by the manufacturer with primers 27f, 357r, 519r, 907r, 926f, 1100r, 1114f, 1392r, 1406f, and 1525r (17), as well as primers 787r and 803f (29), to directly determine the sequences of purified PCR products. The extension products were purified by phenol-chloroform extraction according to the manufacturer's instructions. The sequences of the products were determined with an Applied Biosystems model 373A DNA sequencer.

Analysis of data. The 16S rDNA sequences of different strains of *B. solanacearum*, *P. syzygii*, and the BDB were manually aligned with the sequences of the type strain of *B. solanacearum* (strain PDDCC1727) and other species belonging to the beta subclass of the *Proteobacteria* by using the AE2 sequence editor (20). The evolutionary distances between sequences were computed by using the algorithm of Jukes and Cantor (15) and the DNADIST program of the PHYLIP phylogenetic analysis software package (version 3.5) (7). A dendrogram was constructed from evolutionary distance values by using the neighbor-joining method of Saitou and Nei (26) contained in the NEIGHBOR program of PHYLIP, version 3.5. The most-parsimonious tree was also constructed by using the PAUP software package (33). The topologies of the distance and parsimony trees were tested by 100 bootstrap resamplings of the data.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study have been deposited in the GenBank data library under the accession numbers shown in Table 1.

RESULTS

We determined nearly complete 16S rDNA sequences for 19 isolates of *B. solanacearum*, three isolates of the BDB, and two isolates of *P. syzygii*. The dendrogram (Fig. 1) that was produced by comparing 1,422 nucleotide positions (all ambiguous nucleotides were omitted from the comparisons) contains two divisions, which is consistent with the findings of Cook et al. (3) and Li et al. (18). *B. solanacearum* biovar 3, 4, and 5 strains and an aberrant biovar 2 isolate (strain ACH0732) made up division 1, and *B. solanacearum* biovar 1, 2, and N2 strains, the BDB strains, and *P. syzygii* strains made up division 2. Two subdivisions were recognized within division 2, and these subdivisions were designated subdivisions 2a and 2b. Subdivision 2b comprised *P. syzygii*, the BDB, and two Indonesian *B. solanacearum* isolates, strains R780 and R142. Subdivision 2a comprised all other biovar 1, 2, and N2 isolates. As determined by bootstrap values, the branch point separating divisions 1 and 2 was well supported (100% of evolutionary distance-based trees and 92% of parsimony-based trees). However, the branch point separating the two subdivisions of division 2 was not well supported (59% of evolutionary distance-based trees and 53% of parsimony-based trees) (Fig. 1).

The sequence differences and their positions (in the *Escherichia coli* numbering system [35]) are shown in Table 2. Positions 458 to 460 (UUC) and 474 (A) characterized the division 2 isolates. The nucleotides at the same positions in the division 1 isolates were ACU and U, respectively, except in aberrant biovar 2 isolate ACH0732. Strain ACH0732 had a U at posi-

FIG. 1. Distance matrix tree showing the phylogenetic relationships of *B. solanacearum* strains and strains of the closely related organisms *P. syzygii* and the BDB based on 16S rDNA sequence comparisons. *Pseudomonas pickettii* and *Alcaligenes eutrophus* were used as outgroups in this analysis. The numbers at the branch points are the percentages of bootstrap replicates in which the clusters were found; in each case the upper value is the percentage of distance trees in which the branch point occurred, and the lower value is the percentage of parsimony trees in which the branch point occurred.

tion 458, as did the division 2 isolates, and a G at position 474 (Table 2). The division 1 and subdivision 2b isolates had the same nucleotides at positions 1424 (C), 1428 (A), 1456 (A), and 1472 (U) and differed at these positions from the subdivision 2a isolates (Table 2). The sequences of *P. syzygii* strains were characterized by a G at position 328 and a deletion at position 205. The sequences of the BDB strains had unique nucleotides at positions 848 and 1441. A G at position 264 was found only in the sequence of the single biovar 4 isolate tested; the sequences of all of the other isolates examined had an A at this position. However, partial sequencing of other biovar 4 isolates failed to confirm that this nucleotide is biovar 4 specific (data not shown). The type strain of *B. solanacearum*, strain PDDCC1727 (= ATCC 11696 = K60 = ACM3851), clustered in subdivision 2a but differed from all other members of subdivision 2a by having the same nucleotides at positions 167 and 649 as subdivision 2b strains. The sequences of isolates R483, R634, R633, and R639 differed from the sequences of all other isolates at position 1208. The sequences of isolates ACH0732, CIP210, R207, and PDDCC1727^T (T = type strain) had a U at position 269, in contrast to all other isolates, which had a C at this position. Strains PDDCC1727^T and R207 also had a U at position 1451, while all other isolates had a C at this position. The sequence of BDB isolate R223 had a unique nucleotide at position 1402.

The 16S rDNA sequences of the division 1 isolates exhibited levels of similarity that ranged from 99.8 to 100%. Within division 2 (including subdivisions 2a and 2b) the levels of sequence similarity ranged from 99.1 to 100%. Within subdivision 2b, the levels of sequence similarity ranged from 99.7 to 100%, and within subdivision 2a the levels of sequence simi-

larity ranged from 99.6 to 100%. The division 1 isolates exhibited an average level of sequence similarity of 99.3% (range, 99.1 to 99.5%) with the division 2 isolates. The average level of sequence similarity for the members of the two subdivisions of division 2 was of 99.3% (range, 99.1 to 99.4%). Division 1 isolates exhibited average levels of similarity of 99.3% (range, 99.1 to 99.4%) and 99.4% (range, 99.2 to 99.5%) with members of subdivisions 2a and 2b, respectively. The levels of 16S rDNA sequence similarity between the BDB and *B. solanacearum* isolates ranged from 99.1 to 99.9%. The sequences of the *B. solanacearum* R780 and R142 isolates were most similar to the sequences of the BDB strains; all of these organisms were isolated from plants in Indonesia (Table 1). The levels of sequence similarity between the *P. syzygii* isolates and the *B. solanacearum* isolates sequenced ranged from 99.2 to 99.9%. Again, the *B. solanacearum* isolates which exhibited the highest levels of similarity to *P. syzygii* isolates were strains R780 and R142.

The sequences of strains ACH0158, ACH092, ACH0171, and PDDCC1727^T determined previously by Li et al. (18) were confirmed, except that one extra G occurred at position 1456 in strains PDDCC1727^T and ACH0158.

DISCUSSION

We determined the 16S rRNA gene sequences of 19 strains of *B. solanacearum* that were isolated from various hosts and geographical regions (Table 1) and represented all known biovars (10, 14). The results of our sequence analysis and comparisons expressed in the dendrogram in Fig. 1 confirm and extend the results of Li et al. (18). The two main clusters, designated divisions 1 and 2 (Fig. 1), which were identified in this study and by Li et al. (18) on the basis of 16S rDNA sequences correspond to the two divisions defined by Cook et al. (3) on the basis of restriction fragment length polymorphism analysis data. Cook et al. (3) showed that the similarity coefficient based on pairwise comparisons of all restriction fragment length polymorphism groups for groups division 1 (biovars 3, 4, and 5) and division 2 (biovars 1, 2, and N2) was only 13.5%.

The divergent phylogeny observed with *B. solanacearum* strains corresponds to a level of DNA reassociation that is less than the level of DNA reassociation expected within a species. Strains of the same species are expected to exhibit DNA reassociation values of more than 70% (30), whereas the levels of DNA-DNA hybridization between the members of different biovars of *B. solanacearum* have commonly been substantially less than 70% (23, 25). However, Roberts et al. (25) and Palleroni and Doudoroff (23) found that the techniques used to measure levels of DNA-DNA hybridization of *B. solanacearum* strains resulted in inherent variability (25) or produced inconsistent results (23) and may therefore only be useful as a guide to the relatedness of strains. It is evident from restriction fragment length polymorphism analysis data, 16S rRNA gene sequence data, and phenotypic differences that the two divisions of *B. solanacearum* may represent subspecies, as suggested by Li et al. (18), or separate species. Below we refer to the species *B. solanacearum*, as currently described phenotypically, as a species complex to reflect this intraspecific variation.

B. solanacearum ACH0732, which clusters with biovars 3, 4, and 5 in division 1, was originally isolated from a tomato plant in Darwin, Northern Territory, Australia, in October 1978. The characteristics of this isolate are for the most part typical of biovar 2; strain ACH0732 produces acid from lactose, maltose, and cellobiose, but not from the hexose alcohols mannitol, sorbitol, and dulcitol, and does not produce gas from nitrate.

TABLE 2. 16S rDNA sequence differences in 24 isolates of *B. solanacearum* and related bacteria

Isolate	Nucleotide(s) at position(s) ^a :																				Division or subdivision
	167	204	205	264	269	328	458–460	474	649	669	737	848	1208	1402	1424	1428	1441	1451	1456	1472	
R288	A	G	A	A	C	C	ACU	U	G	A	U	C	C	C	C	A	A	C	A	U	1
ACH092	A	G	A	G	C	C	ACU	U	G	A	U	C	C	C	C	A	A	C	A	U	1
ACH0171	A	G	A	A	C	C	ACU	U	G	A	U	C	C	C	C	A	A	C	A	U	1
R791	A	G	A	A	C	C	ACU	U	G	A	U	C	C	C	C	A	A	C	A	U	1
CIP365	A	G	A	A	C	C	ACU	U	G	A	U	C	C	C	C	A	A	C	A	U	1
ACH0732	A	G	A	A	U	C	UCU	G	G	A	U	C	C	C	C	A	A	C	A	U	1
PDDCC1727 ^T	G	G	A	A	U	C	UUC	A	G	A	U	C	C	C	U	G	A	U	G	C	2a
CIP210	A	G	A	A	U	C	UUC	A	A	A	U	C	C	C	U	G	A	C	G	C	2a
R207	A	G	A	A	U	C	UUC	A	A	A	U	C	C	C	U	G	A	U	G	C	2a
ACH0158	A	G	A	A	C	C	UUC	A	A	A	U	C	C	C	U	G	A	C	G	C	2a
Br 150	A	G	A	A	C	C	UUC	A	A	A	U	C	C	C	U	G	A	C	G	C	2a
CIP238	A	G	A	A	C	C	UUC	A	A	A	U	C	C	C	U	G	A	C	G	C	2a
CIP10	A	G	A	A	C	C	UUC	A	A	A	U	C	C	C	U	G	A	C	G	C	2a
R483	A	G	A	A	C	C	UUC	A	A	A	U	C	U	C	U	G	A	C	G	C	2a
R634	A	G	A	A	C	C	UUC	A	A	A	U	C	U	C	U	G	A	C	G	C	2a
R633	A	G	A	A	C	C	UUC	A	A	A	U	C	U	C	U	G	A	C	G	C	2a
R639	A	G	A	A	C	C	UUC	A	A	A	U	C	U	C	U	G	A	C	G	C	2a
R780	G	G	A	A	C	C	UUC	A	G	G	C	C	C	C	C	A	A	C	A	U	2b
R142	G	G	A	A	C	C	UUC	A	G	G	C	C	C	C	C	A	A	C	A	U	2b
R223	G	G	A	A	C	C	UUC	A	G	G	C	U	C	A	C	A	G	C	A	U	2b
R506	G	G	A	A	C	C	UUC	A	G	G	C	U	C	C	C	A	G	C	A	U	2b
R233	G	G	A	A	C	C	UUC	A	G	G	C	U	C	C	C	A	G	C	A	U	2b
R001 ^T	G	C		A	C	G	UUC	A	G	G	C	C	C	C	C	A	A	C	A	U	2b
R058	G	N		A	C	G	UUC	A	G	G	C	C	C	C	C	A	A	C	A	U	2b

^a *E. coli* numbering (35).

Strain ACH0732 produces acid from trehalose, unlike all other biovar 2 isolates found in Australia (14), and for this reason has been considered aberrant. No other biovar 2 isolates have been isolated from the Northern Territory. In Australia, biovar 2 isolates are isolated almost exclusively from potatoes or a few solanaceous weeds in southern and eastern states (11), whereas strain ACH0732 came from a lowland tropical region with no previous history of potato cultivation. In a comparison of whole-cell protein profiles, ACH0732 occupied a position intermediate between the clusters representing division 1 (biovars 3 and 4) and division 2 (biovars 1 and 2), but it was more similar to biovars 3 and 4 than to biovars 1 and 2 (19). In this study we found that ACH0732 has a unique nucleotide sequence at key positions 458 to 460, as well as different nucleotides at positions 269 and 474 than those present at these positions in division 1 strains. The occurrence of an organism that has a phenotype similar to that of a biovar 2 organism but a protein profile (19) and 16S rDNA sequence similar to (but distinct from) the protein profiles and 16S rDNA sequences of biovar 3 and 4 strains underscores the level of heterogeneity present in the *B. solanacearum* species complex.

Two close relatives of *B. solanacearum*, the BDB, which causes blood disease of bananas in Indonesia (6, 32), and *P. syzygii*, which causes Sumatra disease of cloves in Indonesia, were included in this study to clarify their taxonomic status. *P. syzygii* and the BDB are found only in Indonesia (5), have similar fatty acid profiles (25, 31), and are serologically related (1, 25) to *B. solanacearum*. DNA homology studies have shown that *P. syzygii* is closely related to *B. solanacearum* (25), and partial sequencing of the 16S rRNA gene has confirmed the close relationship of *B. solanacearum*, the BDB, and *P. syzygii* (28). Partial sequencing of the 16S rRNA gene of the BDB by Seal et al. (28) showed that the first 292 bases are identical to the bases found in some *B. solanacearum* isolates, including isolate R142 (which was also included in this study), and that *P. syzygii* has the same sequence as the BDB and R142 but its

sequence is one base shorter. The occurrence of a deletion in *P. syzygii* (at position 205) was confirmed in this study (Table 2). The sequence of *P. syzygii* which we determined differs from the sequence of Seal et al. (28) by a single base at position 204 in strain R001; this strain has a C rather than the G which is found in all other *B. solanacearum* and BDB isolates sequenced by us and Seal et al. (28).

We found that *P. syzygii* and the BDB cluster within the *B. solanacearum* species complex (Fig. 1). *P. syzygii*, the BDB, and two isolates of *B. solanacearum*, strains R142 and R780, form a separate cluster within division 2, designated subdivision 2b (Fig. 1), which contains only isolates from Indonesia. Isolate R780 is a biovar N2 isolate and was obtained from a potato. Isolate R142 was isolated from cloves, which is not a common host of *B. solanacearum* biovar 2 isolates. This strain is an atypical biovar 2 isolate which does not belong to race 3 and exhibits the same tRNA fingerprint as the BDB, but has the host range and biochemical characteristics of *B. solanacearum* (27, 28).

The subgroup containing Indonesian isolates (subdivision 2b) (Fig. 1) exhibits sequence homology with division 1 isolates at positions 649, 1424, 1428, 1456, and 1472 (V7 and V5 region of Gray et al. [9]) and with subdivision 2a isolates at positions 458 to 460 and 474. Division 1 isolate ACH0732 is an exception; this isolate has the same base as division 2 isolates at position 458 and has a unique base at position 474. Furthermore, subdivision 2b isolates have two unique nucleotides, at positions 669 and 737. Although the branch point between subdivisions 2a and 2b is not stable (Fig. 1) (the bootstrap values are less than 60%), the nucleotide positions listed above unify the group. Phenotypically, the subdivision 2b organisms are divergent, and they belong to two valid species (*B. solanacearum* and *P. syzygii*) and one species (BDB) which has not been validly described, although they have very similar 16S rDNA sequences.

Despite the high levels of 16S rDNA sequence similarity

between *P. syzygii* strains and members of the *B. solanacearum* species complex, recognition of *P. syzygii* as a separate species is justified because it has a clearly distinct phenotype (5, 25). In contrast, the phenotype of the BDB is similar to the phenotype of *B. solanacearum* (5). In a numerical analysis of more than 70 phenotypic properties, Eden-Green (5) showed that the BDB forms a distinct phenon that is intermediate between the clusters corresponding to divisions 1 and 2 of *B. solanacearum*. Also, the symptoms of blood disease of banana in Indonesia are similar to the symptoms of the insect-transmitted Moko disease caused by strains of *B. solanacearum* (e.g., *B. solanacearum* race 2 strain SFR) (32). However, the nine nucleotide differences (Table 2, positions 649, 669, 737, 848, 1424, 1428, 1441, 1456, and 1472) between the sequences of three BDB strains and three Moko disease strains of *B. solanacearum* and the phenotypic differences between these organisms provide evidence that the BDB is distinct from the *B. solanacearum* species complex strains that cause Moko disease in Central and South America and The Philippines. Stover and Espinoza (32) concluded that Moko disease and blood disease of banana are closely related. Moko disease-causing *B. solanacearum* strains and the BDB have independently evolved similar pathologies on jungle *Heliconia* species in Central America and probably on wild *Musa* species in southern Sulawesi (Indonesia), respectively (32). In addition, our results also suggest that both the BDB and *P. syzygii* evolved from the same progenitor organism as *B. solanacearum*, which includes the Moko disease-causing organism. It is likely that in Indonesia host specialization resulted in the phenotypically distinct species *P. syzygii* on cloves and BDB on bananas.

Eden-Green (5) hypothesized that Bugtok disease of cooking bananas in The Philippines and Moko disease of dessert bananas are caused by the same organism. Eden-Green (5) also suggested that, as the first reports of Bugtok disease pre-date the reported introduction of Moko disease into The Philippines, either Moko disease was introduced into The Philippines at an earlier date or Central American strains that cause Moko disease may have originated in Southeast Asia. Our results which show that Bugtok and Moko disease isolates from The Philippines have the same 16S rDNA sequence and have a single unique nucleotide at position 1208 (Table 2) support Eden-Green's hypothesis that Moko and Bugtok disease organisms from The Philippines may be the same organism. However, we can make no conclusion concerning the similarity of the rest of the genomes of these organisms.

The polymorphisms found in the 16S rDNAs of members of the *B. solanacearum* species complex and the levels of 16S rDNA sequence similarity between members of the *B. solanacearum* species complex and related organisms (BDB and *P. syzygii*), together with the phenotypic variation among these organisms, suggest that they belong to a rapidly evolving (tachytelic) group. Alternatively, it is also possible that *B. solanacearum*, as defined phenotypically, represents more than one species. If this is the case, *B. solanacearum* would appear to be a rapidly evolving species as the strains which we compared may actually belong to different species. Further work on the DNA-DNA relatedness of strains belonging to the *B. solanacearum* species complex and related bacteria will be required to resolve this issue. The high level of variability within the *B. solanacearum* species complex and related organisms is most evident near the equator in the Americas and Southeast Asia. Martin et al. (22) found that the genetic diversity of *B. solanacearum* was greatest in the tropical lowlands of Brazil, Costa Rica, and Peru and apparently lower farther from the equator. Similarly, Southeast Asia, including Indonesia, where blood disease of bananas and Sumatra disease of cloves have

evolved (13), appears to be a region where rapid evolution occurs. *B. solanacearum* is unique among bacterial plant pathogens because of the amount of biomass generated in the xylem vessels of susceptible plant hosts. In many parts of the lowland tropics access to crop plant and weed hosts is uninterrupted by seasonal variation, and the bacterial biomass is proportionately greater than the bacterial biomass observed in areas where hosts are not as abundant.

Cook et al. (3) concluded that members of their division 2, equivalent to our subdivision 2a, may have originated in the Americas and that members of division 1 may have originated in Asia. The occurrence of biovar 2 isolates of the *B. solanacearum* species complex in Indonesian subdivision 2b casts doubt on this proposal. The Indonesian isolates of *B. solanacearum* belonging to division 2 have 16S rDNA sequences that are very similar to BDB and *P. syzygii* sequences. The latter two organisms are found only in Indonesia and must therefore have originated in Indonesia. It is also likely that the *B. solanacearum* strains belonging to subdivision 2b also originated in Indonesia. If all of the strains that belong to subdivision 2b originated in Indonesia, then it is likely that both divisions of *B. solanacearum* originated in Asia. To further clarify the complex phylogenetic relationships of this closely related group of organisms, other genes are being sequenced.

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