Phylogenetic Evidence for the Transfer of *Pseudomonas cocovenenans* (van Damme et al. 1960) to the Genus *Burkholderia* as *Burkholderia cocovenenans* (van Damme et al. 1960) comb. nov.

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***Pseudomonas cocovenenans*, the producer of bongkreikic acid and toxoflavin, has been described previously by us (N.-X. Zhao, M.-S. Ma, Y.-P. Zhang, and D.-C. Xu, Int. J. Syst. Bacteriol. 40:452–455, 1990) in terms of more than 180 phenotypic traits, G+C content, and DNA relatedness. The bacterium conformed to section II of the genus *Pseudomonas*. Here, its partial 16S rRNA gene sequence was compared with those of *Burkholderia* spp., and the results verified that *P. cocovenenans* is a *Burkholderia* species. On the basis of the phenotypic and genetic characteristics, *P. cocovenenans* is transferred to the genus *Burkholderia* as *Burkholderia cocovenenans* comb. nov.**

The name *Pseudomonas cocovenenans* was first proposed for a food-poisoning bacterium in 1960 by van Damme et al. (12). The 1980 Approved Lists of Bacterial Names (8) included *P. cocovenenans*, and the article cited for a description of this organism was the article in which the name was proposed. However, in Bergey's Manual of Systematic Bacteriology (4) the following was said concerning this organism: "to our knowledge, no description of this species is available. . ."

In 1982 and 1988 (17) we described 17 strains of food-poisoning bacteria isolated from sour fermented corn flour, a homemade product in the countryside of northeastern China, and showed that these conformed to *Pseudomonas* characteristics. The bacteria were also the cause of food poisoning caused by eating deteriorated (mildewed) *Tremella fuciformis* (white fungus). In cultivated *T. fuciformis* the contamination rate of the organism was ca. 50%. The bacterium was not infectious to man and animals but its toxic metabolites induced the poisoning.

In 1990 we comparatively described *P. cocovenenans* NCIB 9450^T and our 17 strains of the poisoning bacteria (16). Morphological, physiological, and biochemical reactions, the substrate utilization pattern, patterns of resistance and sensitivity to antibiotics, G+C content of DNA, and ca. 200 traits showed that the strains were similar. Furthermore, the antigenic relatedness and DNA relatedness showed the strains were closely homologous. From these findings, we concluded that (i) these strains are members of the same species, (ii) they conform to the definition of *Pseudomonas* spp., and (iii) they belong to Palleroni's section II of the genus. These strains were very similar to *P. cepacia* except for a few biochemical reactions and the outstanding property of producing bongkreikic acid and toxoflavin.

In 1988 Gwynn et al. (3) also identified and described *P. cocovenenans*, as a producer of a new member of the monobactam family of antibiotics, and their experimental results were very similar to ours (16, 17).

In 1992 Yabuuchi et al. (13) proposed a new genus *Burkholderia* for the RNA homology group II of the genus *Pseudomonas* on the basis of the 16S rRNA sequences, DNA-DNA homology values, cellular lipids, fatty acid composition, and phenotypic characteristics. Seven species in the group were

transferred to the new genus with *Burkholderia cepacia* as the type species. In 1994 Urakami et al. transferred *P. plantarii* and *P. glumae* to the genus *Burkholderia* and described *B. vandii* as a new species (11).

We have reexamined the phenotypic characteristics and examined the main respiratory quinone of *P. cocovenenans*, determined partial sequences of its 16S rRNA gene, and propose that *P. cocovenenans* be transferred to the genus *Burkholderia* as *Burkholderia cocovenenans* (van Damme et al. 1960) comb. nov.

The strains used for phenotypic reexamination were NCIB 9450^T, NCIMB 12451, and 16 other strains of *P. cocovenenans* (16, 17). The phenotypic characteristics were determined by methods described in the Manual of Methods for General Bacteriology (2) and the methods of Stanier et al. (9). The phenotypic characteristics were the same as those described previously by us (16) and others (3) except for some additional ones and revised ones as follows. Oxidase reaction was weakly positive as described in our previous article, but it was scored as negative, although it was different from the true negative reaction as in *Enterobacter* spp. Esculin was hydrolyzed. The strains did not utilize 2,3-butyleneglycol as a sole carbon source. Growth was positive at pH 4.0.

Respiratory quinone was assayed by the method of Collins (1a), and it was shown that the main quinone was ubiquinone Q-8.

The strain used for partial 16S rRNA gene nucleotide sequencing was *P. cocovenenans* NCIMB 12451 (T7707-a). This strain showed 95% DNA complementarity with the type strain of *P. cocovenenans* (NCIB 9450). The 16S rRNA gene of *P. cocovenenans* NCIMB 12451 was partially sequenced by the procedure of Young et al. (15). The primer Y1 (5'-TGGCT CAGAACGAACGCTGGCGGC-3') corresponds to positions 20 to 43 in the *Escherichia coli* 16S rRNA sequence, and the reverse primer, Y2 (5'-CCCACTGCTGCCTCCCGTAGGA GT-3'), corresponds to *E. coli* positions 361 to 338. Genomic DNA from NCIMB 12451 provided the template from which a 291-bp segment of the 16S rRNA gene was amplified by PCR. This segment was then cloned in phage M13, and single-stranded DNA was extracted as described by Sambrook et al. (7). This fragment was sequenced by a model ABI-370A DNA sequencer.

The sequence (corresponding to positions 44 to 337 in the *E.*

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FIG. 1. Sequence alignment for 16S rRNA gene fragments from *B. cocovenenans* NCIMB 12451, *B. cepacia* (Bc), *B. mallei* (Bm), *B. gladioli* (Bg), *B. solanacearum* (Bs), *B. pickettii* (Bp), *P. aeruginosa* (Pa), *C. testosteroni* (Ct), and *E. coli* (Ec). Dashes, identity with the *B. cepacia* sequence; dots, alignment gaps.

coli 16S rRNA sequence) of *P. cocovenenans* NCIMB 12451 was compared with published data on *Burkholderia* species (13), *E. coli*, *P. aeruginosa* (10), and *Comamonas testosteroni* (14). The partial 16S rRNA gene sequence and the alignment with sequences of other related bacteria are shown in Fig. 1. Positions 5, 6, 11, 12, and 37 to 58 were omitted because this loop varied in length among the sequences that were compared and could not be aligned unambiguously. Table 1 shows the distance matrix calculated from the sequences in Fig. 1. A phylogenetic tree of these species drawn from the distance matrix by the neighbor-joining method (6) is shown in Fig. 2.

P. cocovenenans is not a pseudomonad in the strict sense but could be a member of the genus *Burkholderia* because in terms of DNA relatedness it is closer to *B. cepacia* than to *P. aeruginosa* (16); its main respiratory quinone is ubiquinone Q-8; it contains intracellular poly- β -hydroxybutyrate, does not pro-

duce phenazine or fluorescent pigment, has a weak oxidase reaction, grows at a low pH, is resistant to polymyxin; and its substrate utilization pattern is very similar to that of *B. cepacia*. The classificatory deduction from phenotypic characteristics and DNA relatedness was verified by a genomic phylogenetic study of the 16S rRNA gene fragment data. It was shown that *P. cocovenenans* is a close relative of *B. gladioli* and *B. cepacia*. The distance between them was less than those between *B. gladioli* and *B. pickettii*. We therefore propose that *P. cocovenenans* be transferred to the genus *Burkholderia* as *Burkholderia cocovenenans* (van Damme et al. 1960) comb. nov.

Description of *Burkholderia cocovenenans* (van Damme, Johannes, Cox, and Berends 1960) comb. nov. *Burkholderia cocovenenans* (co.co.ve'ne.nans. M.L. n. *Cocos*, genus of coconut; L. v. *veneno*, to poison; M.L. part. adj. *cocovenenans*, coconut poisoning). The description below is based on studies by us

TABLE 1. Genetic distances^a

Species or strain	No. of nucleotide differences with or evolutionary distance from: ^b								
	1	2	3	4	5	6	7	8	9
1. <i>B. cepacia</i> ATCC 25416		8	4	5	29	32	71	39	65
2. <i>B. cocovenenans</i> NCIMB 12451	2.9		9	6	32	30	71	40	63
3. <i>B. mallei</i>	1.4	3.2		6	25	30	68	41	63
4. <i>B. gladioli</i>	1.8	2.2	2.2		30	31	72	41	63
5. <i>B. solanacearum</i>	10.5	11.6	9.0	10.8		8	66	38	61
6. <i>B. pickettii</i>	11.2	10.8	10.8	11.2	2.9		68	37	59
7. <i>B. aeruginosa</i>	25.1	25.1	24.0	25.4	23.3	24.0		76	68
8. <i>C. testosteroni</i>	14.1	14.4	14.8	14.8	13.7	13.3	26.8		55
9. <i>E. coli</i>	23.5	22.7	22.7	22.7	22.0	21.3	24.0	19.8	

^a For demonstrated partial 16S rRNA gene sequences (Fig. 1) (omitting positions 5, 6, 11, 12, and 37 to 58).

^b Above and below the diagonal, respectively.

FIG. 2. Relationship of strain NCIMB 12451 to some related species.

TABLE 2. Diagnostic characteristics for distinguishing *B. cocovenenans* from other *Burkholderia* species^a

Test or characteristic	<i>B. cocovenenans</i> ^b	<i>B. gladioli</i> ^c	<i>B. cepacia</i> ^c	<i>B. caryophylli</i> ^c	<i>B. pseudomallei</i> ^c	<i>B. mallei</i> ^c	<i>B. solanacearum</i> ^c	<i>B. pickettii</i> ^c	<i>B. plantarii</i> ^d	<i>B. glumae</i> ^d	<i>B. vandii</i> ^d
Denitrification	—	—	—	+	+	v	+	+	+	+	+
Oxidase	—	v	v	—	+	+	+	+	+	v	+
Arginine dihydrolase	—	—	—	+	+	+	—	—	—	—	—
Hydrolysis of gelatin	+	+	v	—	+	+	—	—	+	+	+
Hydrolysis of starch	—	—	—	—	+	v	—	—	—	—	—
Utilization of:											
L-Rhamnose	—	—	v	+	—	—	—	—	w	—	—
Maltose and starch	—	—	(—)	—	+, —	—	—	—	—	—	—
Citrate	+	+	+	+	+	—	+	—	+	—	—
Mucate	+	+	+	+	—	—	+	+	+	—	—
Malonate	+	+	+	+	—	v	—	+	+	—	—
Glutarate	—	v	+	—	v	v	—	v	—	—	—
Adipate	+	+	+	—	+	+	—	+	+	+	+
Azelaate and sebacate	+	+	+	—	+	v, —	v	+	w	+	—, +
D-Tartrate	+	+	—	—	—	—	—	—	+	—	+
Glycolate	—	—	v	+	—	—	v	+	+	w	—
Aconitate	+	+	+	+	+	—	+	+	+	—	—
Levulinate	—	—	+	—	+	—	v	+	—	—	—
Citraconate	+	+	+	—	—	—	—	+	w, +	w	+
Mesaconate	v	+	—	—	—	—	—	—	w	—	+
Erythritol	—	—	—	—	+	—	—	—	—	—	—
2,3-Butylene glycol	—	—	+	+	—	—	—	v	—	—	—
Benzoate	w	+	+	—	+	+	—	—	—	—	—
D-Alanine	+	+	+	—	+	+	+	+	—	—	—
L-Valine	v	+	v	v	+	v	—	+	—	—	—
Leucine	—	+	+	—	+	—	—	—	+	+	+
DL- α -Aminobutyrate	v	v	—	—	—	v	—	—	—	—	—
Kynurenate and anthranilate	+	+, v	+	—	+	—, v	—	+, —	—	—	—
Ethanolamine	+	v	+	—	+	—	—	—	—	+	—
Benzylamine	—	(—)	v	—	—	—	—	—	—	—	—
Acetamide	—	—	v	—	—	—	—	—	w	—	—
Nicotinate	+	—	v	—	—	—	—	—	+	w	+
G+C (mol%)	68	67.9	66.6	64.6	68	68	66.6	64	64	69.6	68.5

^a (—), present in more than 90% of the strains; v, present in 10 to 90% of the strains; w, weakly positive.^b 17 strains.^c The data are from reference 5.^d Type strain. The data are from reference 14.

(16) of the type strain, NCIB 9450, strain NCIMB 12451, 16 other strains, and two strains (including the type strain) described by Gwynn et al. (3).

Rods 0.3 to 0.5 by 1.6 to 2.0 μ m. Cells occur singly or in pairs and are motile by means of a tuft of (mainly 1 to 5) polar flagella. Cells accumulate poly- β -hydroxybutyrate as a carbon reserve material. No lipid soluble or fluorescent pigment, but has a greenish-yellow diffusive pigment (toxoflavin) after 1 to 2 days of incubation on potato dextrose agar or other medium. The growth temperature ranges from 6 to 41°C. The pH range of growth is 4.0 to 8.0 with an optimum of 6 to 7. Obligately aerobic. Metabolism is respiratory and never fermentative with oxygen as the terminal electron acceptor. Denitrification is negative; nitrate is reduced to nitrite. Catalase is positive and oxidase is scored as negative. No growth factor is required. Gelatin is liquefied. Lipase against Tween 80 and lecithinase are produced. Chemoorganotrophic, utilizing a wide range of carbohydrates, organic acids, and amino acids as sources of carbon and energy.

The following compounds are used by most of the species as sole carbon sources: acetate, acetylglucine, acetylsalicylate, aconitate, adipate, β -alanine, D- α -alanine, L- α -alanine, anthranilate, L-arabinose, L-arginine, asparagine, L-aspartate, azelate, butanone, butyrate, caprylate, cellobiose, choline, citraconate, citrate, ethanol, ethanolamine, D-fructose, D-fucose, L-fucose,

fumarate, D-galactose, gluconate, D-glucose, glucuronate, glycerol, β -glycerophosphate, guanosine, heptylate, L-histidine, DL- β -hydroxybutyrate, *i*-inositol, 2-ketoglutarate, kynurenate, DL-lactate, L-lysine, DL-malate, malonate, mannitol, mannose, melibiose, nicotinate, pelargonate, L-proline, protocatechuate, pyruvate, ribose, salicin, sarcosine, sebacate, serine, sorbitol, succinate, D-tartrate, L-tartrate, *meso*-tartrate, DL-threonine, trehalose, L-tyrosine, urate, xylitol, and D-xylose.

Table 2 lists the diagnostic characteristics for the differentiation of *B. cocovenenans* from other *Burkholderia* species (1, 5, 11).

The type strain is NCIB 9450. The following results were obtained for this strain by tests that are not universally positive or negative for the 18 strains of *B. cocovenenans*: the strain cannot utilize adonitol, α -aminovalerate, DL- α -aminobutyrate, DL- α -aminobutyrate, benzoate, catechol, hippurate, L-phenylalanine, or *p*-phthalate as sole carbon sources but is able to utilize mesaconate as a sole carbon source. The G+C content of the DNA is 69 ± 0.5 mol% (from thermal denaturation).

DNA-DNA hybridization experiments and partial 16S rRNA gene sequence (positions 44 to 337 in *E. coli*) indicate that this species is closely related to *B. gladioli* and *B. cepacia*.

Strains have been isolated from local fermented coconut food (bongkreng) in Indonesia, fermented cornmeal in China, deteriorated white fungus (*T. fuciformis*), and soil.

Nucleotide sequence accession number. The partial sequence of the *B. cocovenenans* 16S rRNA gene has been deposited in EMBL under accession number X87334.

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