Transfer of *Pseudomonas maltophilia* Hugh 1981 to the Genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov.

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Genotypic and phenotypic data (results of deoxyribonucleic acid-ribosomal ribonucleic acid and deoxyribonucleic acid-deoxyribonucleic acid hybridizations, guanine-plus-cytosine content, comparative enzymology, type of ubiquinones, cellular fatty acid composition, growth, niches, and several other characteristics) showed that *Pseudomonas maltophilia* (Hugh 1981) is generically misnamed and should be transferred to *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov.

An organism named "*Bacterium bookerii*" was isolated from pleural fluid by J. L. Edwards in 1943 in England. This strain and several similar ones were classified as *Pseudomonas maltophilia* by Hugh and Ryschenkow (19). The latter species name was not on the Approved Lists of Bacterial Names (29) but was revived by Hugh (16).

Strains of this species have often been associated with nosocomial infections; they have been isolated most frequently from clinical specimens, but have also been obtained from soil, water, frozen foods, and plant material.

The exact generic position of this species is not altogether clear. A number of arguments in the literature (see below) point to similarities between *P. maltophilia* and *Xanthomonas*; nevertheless, a definite transfer of this organism to the genus *Xanthomonas* was not made.

In this paper we summarize the available data in favor of the transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov.

DNA-rRNA hybridizations. Deoxyribonucleic acid (DNA)-ribosomal ribonucleic acid (rRNA) hybridization techniques are very reliable for assessing taxonomic relatedness at generic and suprageneric levels (6–8, 15; De Vos and De Ley, Int. J. Syst. Bacteriol., in press). Palleroni et al. (27) showed that the present genus *Pseudomonas* consists of five rRNA homology groups. For one of these, group V, *P. maltophilia* strain Stanier 67^T (type strain) (= ATCC 13637^T) and three strains of its closest neighbor, *Xanthomonas*, were examined. More and foreign strains were required to reach a conclusion on the exact taxonomic position of *P. maltophilia*.

In an extensive survey of the genus *Pseudomonas*, in which a different DNA-rRNA hybrid-

ization technique was used (5) and which included hundreds of strains belonging to many genera and species, De Vos and De Ley (submitted for publication) confirmed the existence of the five rRNA groups. The taxonomic distances between each pair of the five groups and their distances to other genera were determined. From Fig. 1 it appears that the five *Pseudomonas* groups are less related to each other than to other genera. Consequently, the generic splitting of the present genus *Pseudomonas* becomes unavoidable (De Vos and De Ley, in press). Figure 1 also shows that the rRNA cistrons of *P. maltophilia* are most similar to those of *Xanthomonas*.

DNAs from 27 strains of different *Xanthomonas* species displayed a thermal denaturation midpoint temperature ($T_{m(e)}$) range of 78.8 to 81.5°C with reference ¹⁴C-labeled 23S rRNA from *Xanthomonas campestris* pv. *campestris* NCPPB 528^T. [$T_{m(e)}$ is the temperature at which 50% of the hybrid is denatured.] The following strains were used: *X. campestris* NCPPB 528^T, ICPB A121, ICPB C110, ICPB C144, ICPB C5, ICPB G1, ICPB H110, ICPB M16, ICPB P121, ICPB P137, ICPB P10, ICPB T11, ICPB L1, and ICPB V136; *Xanthomonas fragariae* NCPPB 1822; *Xanthomonas albilineans* NCPPB 2503; *Xanthomonas axonopodis* NCPPB 457^T; and *Xanthomonas populi* Bt₃, Spm11, M1j, PC3, S8, 175, 45.51, Sma, BII, and NCPPB 2432. Four *P. maltophilia* strains (ATCC 13637^T, ATCC 17448, ATCC 17806, and CIP 5960) displayed $T_{m(e)}$ values of 76.5 to 78.0°C under the same conditions. From our extensive experience in this area (6–8, 15), we conclude from the $T_{m(e)}$ data that *P. maltophilia* Hugh 1981 is a member of *Xanthomonas*, albeit a slightly unusual one.

Guanine-plus-cytosine contents. The guanine-

FIG. 1. rRNA cistron similarities, expressed as $T_{m(e)}$ values of DNA-rRNA hybrids (5), between different *Pseudomonas* groups (boxes) and a variety of other gram-negative bacteria. Not all known finer divisions of the branches are represented. Data were obtained from the following sources: 1, De Ley et al. (6); 2, De Vos and De Ley, in press; 3, De Ley et al., unpublished data; 4, De Smedt et al. (7); 5, Swings et al. (32). The asterisk indicates that *Pseudomonas diminuta* and *Pseudomonas vesicularis* are at this level, together with the *Acetobacteraceae*, and *Rhodospirillaceae*, *Agrobacterium*, *Rhizobium*, *Zymomonas*, and a few other organisms (8, 15; De Vos and De Ley, in press). Gram pos, Gram positive bacteria.

plus-cytosine contents that have been determined for various *P. maltophilia* strains by different authors (20, 22, 24; De Vos and De Ley, in press) range from 63 to 67.5 mol% and thus fit well in the range that has been determined for *Xanthomonas* (63 to 70 mol%) (4, 10; De Ley and De Vos, unpublished data). These guanine-plus-cytosine values are not inconsistent with reclassification of *P. maltophilia* into the genus *Xanthomonas*.

DNA-DNA homology. The DNA-DNA homologies among the three *P. maltophilia* strains and four *Xanthomonas* strains (*X. campestris* pv. *translucens*, *X. campestris* pv. *oryzae*, *X. campestris* pv. *pruni*, and *X. campestris* pv. *hyacinthi*), as determined by the method of Murata and Starr (26), were very low, ranging from 4 to 15% (mean, 8.9%); there was one exception of 21%. However, the values for "*Xanthomonas ampelina*" (which is not a member of the genus

Xanthomonas at all) also fell in the same range. Such low values are at the border of or below the sensitivity of this method. Ikemoto et al. (20) obtained DNA homologies ranging from 37 to 100% among several *P. maltophilia* strains and two members of *Xanthomonas* (*X. campestris* pv. *campestris* and *X. campestris* pv. *citri*). The latter values were significantly higher than those between *P. maltophilia* and *Pseudomonas aeruginosa*.

Comparative enzymology. It has been suggested that alternative routes for tyrosine biosynthesis from prephenic acid via prephenate or arogenate dehydrogenases are highly conserved (2). The distribution of arogenate and prephenate dehydrogenases, the regulatory properties of these enzymes, and their cofactor specificity allowed differentiation of five groups within the genus *Pseudomonas*, corresponding to the five

groups recognized by DNA-rRNA hybridization (27; De Vos and De Ley, in press). Both *P. maltophilia* and *Xanthomonas* are unusual in that they lack nicotinamide adenine dinucleotide phosphate-linked dehydrogenases and possess nicotinamide adenine dinucleotide-specific prephenate and arogenate dehydrogenases for tyrosine biosynthesis; the prephenate dehydrogenase is highly sensitive and the arogenate dehydrogenase is highly insensitive toward feedback inhibition by tyrosine.

There are strongly conserved control patterns for the enzyme 3-deoxy-D-arabinoheptulosonate-7-phosphate synthetase among the species of a genus (21). *P. maltophilia* (three strains) and *Xanthomonas* (six strains) both possess a group V 3-deoxy-D-arabinoheptulosonate-7-phosphate synthetase which is sensitive to sequential feedback inhibition by chorismate and has no requirement for divalent cations (34). The occurrence of this control mechanism, which is apparently unique in nature, supports a close taxonomic relationship between these two taxa.

Upon immunological comparison, the glutamine synthetases of one strain of *P. maltophilia* and two strains of *Xanthomonas* behaved in an identical fashion, which was distinctly different from the behavior of the enzymes of more than 30 other gram-negative organisms (1).

Ubiquinones. The structural types of isoprenoid or respiratory quinones are valuable markers for elucidating taxonomic relationships among bacterial groups (3). Both *P. maltophilia* and *Xanthomonas* contain ubiquinones with eight isoprene units (Q-8), whereas all other *Pseudomonas* strains tested generally contain ubiquinones with nine isoprene units (Q-9) as major components (3, 20).

Cellular fatty acid composition. Ikemoto et al. (20) showed that the cellular fatty acid composition of *P. maltophilia* is similar to that of *X. campestris* pv. *campestris* KS 0198 and *X. campestris* pv. *citri* KS 0199 but different from that of *X. campestris* pv. *oryzae* KS 0200.

The patterns of the fatty acids tightly bound to other cellular compounds from *Xanthomonas* and *P. maltophilia* strains are very similar and clearly distinct from those found in *Pseudomonas* species, members of the *Enterobacteriaceae*, and many other gram-negative bacteria (25, 28). The following branched-chain hydroxy acids occur in both *Xanthomonas* and *P. maltophilia*: 2-OH-9-CH₃-decanoic acid, 3-OH-9-CH₃-decanoic acid, 3-OH-11-CH₃-dodecanoic acid, and 9-CH₃-decanoic acid. These four acids occur in the lipid A component and are bound through ester and amide linkages to glucosamine residues. 9-CH₃-decanoic acid and 13-CH₃-tetradecanoic acid are not found in several authentic *Pseudomonas* species. These data are

not inconsistent with inclusion of *P. maltophilia* in *Xanthomonas*.

Phage typing. According to Starr (31), phage typing studies have provided evidence for a relationship between *P. maltophilia* and *Xanthomonas*.

Growth and pigments. *P. maltophilia* cultures do not show the yellow mucoid growth typical of *Xanthomonas*. On medium containing 1% glucose, 0.5% yeast extract, 3% CaCO₃, and 2% agar (GYCA medium), the growth of *P. maltophilia* is nonpigmented to yellowish and shiny but not mucoid. A still-incomplete characterization of the pigments of *P. maltophilia* led to the tentative conclusion (31) that these pigments are "not xanthomonadins or at least that they are not closely related to the xanthomonadins already explored." On GYCA medium, most *Xanthomonas* colonies, except those of *X. albilineans*, are very mucoid due to the production of extracellular xanthan gum. When large numbers of *Xanthomonas* strains are inspected, it becomes clear that colony color varies broadly from white to deep yellow. Not all *Xanthomonas* strains show the typical bright yellow color; e.g., colonies of *X. campestris* pv. *manihotis* NCPPB 1834, 1161, and 2443, *X. campestris* pv. *azadirachtae* NCPPB 2388, *X. campestris* pv. *pedalii* NCPPB 2368, *X. campestris* pv. *phyllanthi* NCPPB 2066, and *X. campestris* pv. *ricini* NCPPB 1063 are white. The colonies of a number of other *Xanthomonas* strains are faintly yellowish.

Some *P. maltophilia* strains characteristically form brownish grey water-soluble pigments. Brown, water-soluble pigments on GYCA medium were also formed by 5 of the 244 true *Xanthomonas* strains observed by us (*X. campestris* pv. *bauhiniae* NCPPB 1335, *X. campestris* pv. *laureliae* NCPPB 1155, *X. campestris* pv. *pruni* NCPPB 416, *X. campestris* pv. *punicae* NCPPB 466, and *X. campestris* pv. *ricini* NCPPB 1063). In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (10), *Xanthomonas phaseoli* var. *fuscans* was differentiated from *X. phaseoli* f. sp. *vignicola* by the production of a diffusible brown pigment. Three strains received by us as *X. phaseoli* pv. *fuscans* produced a brown pigment (strain Xpf from A. W. Saettler, strain PD24 from H. J. Miller, and strain HM144 from H. Maraite). Dye (9) doubted the stability and taxonomic utility of this character within *Xanthomonas*.

Niches. *P. maltophilia* is an ubiquitous bacterium. It occurs free living in nature, in animals, and in various areas of the human body, where it is sometimes associated with opportunistic infections (13, 17). It has been recovered from plants (e.g., the internal tissue of banana pseudostems, cotton seeds, bean pods, and tobacco

seedlings [17]) and from rice paddies (22). At present nothing is known about the phytopathogenic capacity of *P. maltophilia* strains. However, this should not prevent the inclusion of *P. maltophilia* in the genus *Xanthomonas*. Whereas all known *Xanthomonas* strains are highly specialized plant pathogens, it is not clear whether they also occur in a free-living state. Furthermore, it would certainly not be easy to identify the specific host plant(s) for a *Xanthomonas* strain not isolated from diseased plants.

Hypersensitive reactions on tobacco leaves occur only with a limited number of *Xanthomonas* strains and appear in 1 to 4 days. The remaining *Xanthomonas* strains and *P. maltophilia* (five strains) cause only different degrees of chlorosis.

Other morphological, physiological, and biochemical characteristics. For phenotypic characterization of *P. maltophilia*, we refer to the original description by Hugh and Ryschenkow (19), and to the studies of Hugh and Leifson (18), Stanier et al. (30), Gilardi (11–14), Komagata et al. (22), Hugh and Gilardi (17), and Ikemoto et al. (20). The data of these authors, together with our own unpublished data, show that *P. maltophilia* and *Xanthomonas* strains share a number of characteristics, including: gram-negative rod-shaped cells with polar flagella; growth in peptone broth or nutrient agar at pH 5.6 but not at pH 4.5; no growth in the presence of 6.5% NaCl; no hemolysis; acid produced oxidatively from glucose, lactose, fructose, and maltose but not from rhamnose, mannitol, ribitol, galactitol, inositol, inulin, or salicin; positive *o*-nitrophenyl- β -D-galactopyranoside reaction; oxidase negative or weak; indole, acetoin, urease, and ornithine decarboxylase are not produced; resistant to lincomycin; and hydrolysis of esculin. All *P. maltophilia* strains hydrolyze Tween 80, but this characteristic occurs in only 82% of the 233 *Xanthomonas* strains examined. From the phenotypic data on *P. maltophilia* and *Xanthomonas* given by various authors, a simple matching similarity coefficient (S_{SM}) of approximately 75% was calculated.

Reclassification of *P. maltophilia* as a new *Xanthomonas* species, *Xanthomonas maltophilia* (Hugh 1981) comb. nov. A new description of the genus *Xanthomonas* is being prepared as the result of a very extensive phenotypic study (Van den Mooter, Swings, and De Ley, manuscript in preparation). Our improved definition comes closest to, but is different in a number of aspects from, the description given by Dye and Lelliott (10). Our laboratory definition is that all *Xanthomonas* strains tested (including *P. maltophilia* and "*X. populi*," but excluding "*X. ampelina*") fall in the $T_{m(e)}$ range from 76 to 81°C when their DNAs are hybridized with labeled rRNA from

type strain *X. campestris* pv. *campestris* NCPPB 528. The $T_{m(e)}$ values for all other organisms which have been tested are below 72°C.

The arguments summarized above show that *P. maltophilia* should be transferred to the genus *Xanthomonas*. The inclusion of *P. maltophilia* within one of the existing *Xanthomonas* species cannot be justified, as several characteristics differentiate the *P. maltophilia* strains from all other *Xanthomonas* species. These strains are multitrichously flagellated, whereas *Xanthomonas* species have only one polarly inserted flagellum; in DNA-rRNA hybridization *P. maltophilia* strains are at the lower edge of *Xanthomonas*; these strains reduce nitrates to nitrites and produce lysine decarboxylase, whereas other *Xanthomonas* species do not; these strains do not hydrolyze starch, a characteristic found in 60% of *Xanthomonas* strains. *P. maltophilia* appears to be more resistant than other *Xanthomonas* species to antibiotics (33), dyes, and metals. All *P. maltophilia* strains are resistant to 0.01% methyl green, 0.01% thionine, and 0.01% lead acetate, whereas at least 90% of the *Xanthomonas* strains which have been tested are susceptible. The DNA homologies between *P. maltophilia* strains and *Xanthomonas* are low. All of the facts described above justify inclusion of *P. maltophilia* Hugh 1981 in the genus *Xanthomonas* as a separate species, *Xanthomonas maltophilia* (Hugh 1981) comb. nov., according to Rule 41a of the *International Code of Nomenclature of Bacteria* (23).

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