Stenotrophomonas, a New Bacterial Genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983

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In consideration of the criticisms of the transfer of *Pseudomonas maltophilia* to the genus *Xanthomonas* proposed by J. Swings, P. De Vos, M. Van den Mooter, and J. De Ley (Int. J. Syst. Bacteriol. 33:409–413, 1983), a new generic name is created for this taxon. The name *Stenotrophomonas* is here proposed for the new genus, which includes a single species, *Stenotrophomonas maltophilia*. This proposal restores the genus *Xanthomonas* to its former definition (J. Bradbury, p. 199–210, in N. R. Krieg and J. G. Holt, ed., *Bergey's Manual of Systematic Bacteriology*, 1984) The arguments on which this proposal is based are presented.

As a result of the work of Swings et al. (28), in confirmation of previous findings (24), the two members (*Pseudomonas maltophilia* and *Xanthomonas* spp.) of group V of *Pseudomonas* described by Palleroni (20) were placed together under the generic name *Xanthomonas*. This decision did not receive universal approval (20, 21, 23), mainly because the two taxa differ in many basic properties that would justify different generic allocations. The problem was recently analyzed in depth by Van Zyl and Steyn (32), who shared this criticism. In what follows, we review the properties of the genus *Xanthomonas* and of the species *Xanthomonas maltophilia* in order to justify a proposal of a new genus for the latter.

Characteristics of the genus *Xanthomonas*. Following a suggestion by Burkholder (2), Dowson (4) created the name *Xanthomonas* for a well-defined subgroup of yellow-pigmented phytopathogenic pseudomonads which had been placed together with many other unrelated bacteria in the genus *Phytomonas*. Dowson (4) defined *Xanthomonas* as follows: "non-sporing, rod-shaped bacteria. Gram-negative, motile by one polar flagellum (rarely 2 present), or non-motile, yellow in the mass on nutrient agar and on sterile potato, on both of which abundant slimy growths are formed. Most species digest starch and produce acid in lactose; none produces acid in salicin." Two corrections were made later by Dowson: deletion of nonmotility and a change from nutrient agar to nutrient glucose agar (5).

In subsequent definitions of the genus, the following characteristics were added: absence of poly- β -hydroxybutyrate inclusions, production of highly characteristic brominated aryl-polyene yellow pigments (xanthomonadins), infrequent production of a diffusible, brown color in beef extract agar, digestion of proteins, production of hydrogen sulfide, inability to use asparagine as the sole source of carbon and nitrogen, absence of denitrification and of nitrate reduction, negative or weak oxidase reaction, inhibition of growth by 0.1% (and frequently by 0.02%) triphenyltetrazolium chloride, requirement for growth factors (usually methionine, glutamic acid, nicotinic acid, or a combination of these), optimal growth temperature of 25 to 27°C, DNA base composition (63 to 71 mol% G+C), and plant pathogenicity. Thus far, presence of pili has not been reported for any of the species (1). The slimes produced by various

species and pathovars were identified as anionic exopolysaccharides, to which the common name "xanthan gums" has been applied. These compounds have very interesting properties and may play a role in the interactions of the pathogens with their respective plant hosts (17). As pointed out by Dye (6), these organisms "form a well-defined remarkably uniform group from which other yellow-pigmented, Gram-negative, monotrichous, aerobic, non-sporing organisms can be distinguished in the laboratory without difficulty."

In *Bergey's Manual of Systematic Bacteriology* (1), five well-characterized species (*X. campestris*, *X. fragariae*, *X. albilineans*, *X. axonopodis*, and *X. ampelina*) are described; one of these, *X. campestris*, is subdivided into many pathovars that are named according to the host plant of origin. The five species accepted by Bradbury (1) were those already included in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (8), but a sixth species of doubtful affiliation (*X. populi*) was added. For the list of pathovars, Bradbury accepted the one given by Dye et al. (7) and added only two.

A review of the genus *Xanthomonas* was included in the first edition of the treatise *The Prokaryotes: a Handbook on Habitats, Isolation and Identification of Bacteria* (26). Unfortunately, aside from a summary of the general properties of *Xanthomonas* spp. by Palleroni (22), the special chapter on this important genus of gram-negative phytopathogens has been omitted from the second edition of the treatise.

Characteristics of *P. maltophilia*. *P. maltophilia* was named by Hugh and Ryschenkow (12) and later described in detail by the same authors (13). The name, accidentally omitted from the *Approved Lists of Bacterial Names* (24a), was revived by Hugh (10). Many additional phenotypic properties were added by Stanier et al. (25) in their extensive characterization of *Pseudomonas* species; these authors found *P. maltophilia* to constitute a very uniform taxon. The colonies of these organisms differ markedly in color and general appearance from the slimy, brightly yellow ones that are characteristic of many *Xanthomonas* strains. They may be faintly yellow, probably because of flavins (25). *P. maltophilia* strains have been isolated from very diverse materials, including plants, but they do not seem to have phytopathogenic propensities. However, this property has not been critically investigated. The properties of the strains are described in detail below, under the new genus name *Stenotrophomonas*.

Transfer of *P. maltophilia* to the genus *Xanthomonas*. De-

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spite the global resemblance of *Xanthomonas* and *Pseudomonas* spp. in their basic phenotypic properties, there was hardly any reason to suspect a priori any relationship between *Xanthomonas* spp. and any of the *Pseudomonas* species in particular. It must be admitted, however, that studies of the two taxa have not followed the same methodology, and therefore many details of the phenotypic descriptions cannot be subjected to comparison.

Murata and Starr (18) showed that there was very little overlap between the *Pseudomonas* species described by Stanier et al. (25) and *Xanthomonas* species, when the organisms were analyzed by the so-called "segmental DNA homology" procedure. However, a possible exception was *P. maltophilia*, which was found to be marginally related to *Xanthomonas* species. These results were the main reason for the inclusion of *Xanthomonas* strains by Palleroni et al. (24) in their work on similarities of rRNA cistrons by hybridization methods among pseudomonads. These studies clearly demonstrated a relationship between *P. maltophilia* and *Xanthomonas* spp. at the level of their rRNA gene sequences. Ten years later, Swings et al. (28) confirmed the relationship by using DNA-rRNA hybridizations and made their proposal to transfer *P. maltophilia* to the genus *Xanthomonas*.

From an examination of the properties of the two taxa, it is clear that *P. maltophilia* cannot be moved effortlessly into the genus *Xanthomonas* without a substantial redefinition of this genus. Consequently, the proposal by Swings et al. (28) has not been universally accepted. Basic objections are the inconvenience of defining bacterial genera mainly on the basis of molecular criteria, which implied in this particular case the tacit dismissal of a group of excellent characteristics for generic differentiation (20, 21).

Critical analysis of the transfer of *P. maltophilia* to the genus *Xanthomonas*. Recently, a most thorough analysis of this unsettled situation was published (32). Some of the arguments clearly point to the inconvenience of transferring *P. maltophilia* to the genus *Xanthomonas*, and they are summarized below. The original article should be consulted for more detail and as a source of references.

(i) There are many phenotypic differences between *P. maltophilia* and members of *Xanthomonas*. These include the type of flagellation, pigment production, nature of extracellular polysaccharides, optimal growth temperature, nitrate reduction, acid production from carbohydrates, starch hydrolysis, pathogenicity, resistance to antibiotics and other deleterious agents, and a substantial number of miscellaneous properties, all of which suggest more profound genomic differences than those normally distinguishing species of the same genus.

(ii) Both taxa vary in fatty acid composition, while ubiquinones with eight isoprene units are not limited to them but are found elsewhere in the family *Pseudomonadaceae*. These two criteria do not seem to give much support to the proposal.

(iii) The G+C content of the DNA of *P. maltophilia* falls within the range for *Xanthomonas* species, but similarities in DNA base composition can only be added to other criteria in support of presumed synonymy and are not valuable in themselves.

(iv) Perhaps more significantly, there are discrepancies among the results reported by workers of the same laboratory (3, 28) for identical strains. The melting temperature range of the hybrid nucleic acid molecules (one of the main criteria used by these workers for taxon circumscription) appears to have been expanded mainly with the intention of

accommodating *P. maltophilia* within the redefined genus *Xanthomonas*. It should be added that the similarity maps drawn by De Vos and De Ley (3) reinforce the idea of similarity, but not identity, of *P. maltophilia* and species of *Xanthomonas*.

Swings et al. (28) have calculated the similarity coefficient, S_{SM} , between *X. maltophilia* and other *Xanthomonas* species as 75%. In a comparative study carried out by Van Zyl and Steyn (31), an even higher value (79%) was obtained, but the relationship was lower than with the majority of *Pseudomonas* strains included in the study.

Additional comments on another article from the same laboratory deserve consideration here. Van den Mooter and Swings (29) reported a numerical analysis of 295 phenotypic traits of 266 *Xanthomonas* strains, of which 7 were labeled as *X. maltophilia*. Of these, five were authentic representatives of the species and two (the type strains of *P. betle* and of *P. hibiscicola*) were added because, in the authors' opinion, they seem to be members of the same phenon. The S_{SM} between this phenon and other *Xanthomonas* phenon was calculated as 65%.

It is our opinion that this extensive study gives only limited support to the transfer of *P. maltophilia* to the genus *Xanthomonas* for the reasons summarized below.

(i) The strains of *P. maltophilia* are described as negative for the capacity of growth at the expense of glucose, mannose, fructose, sucrose, trehalose, maltose (for which the species was originally named), cellobiose, D-alanine, L-alanine, and L-glutamic acid. The explanation that is given for these anomalous results with these substrates of high diagnostic value is that L-methionine had not been added as a growth factor to the basal medium used in the experiments. In fact, the addition of L-methionine would have made it possible to obtain nutritional data not only for the authentic *P. maltophilia* strains but also in support of the proposed synonymy of the three species lumped in this phenon. For instance, we do not know whether the two additional strains actually use the disaccharides characteristic of the nutritional spectra of authentic *P. maltophilia*. Moreover, it is very probable that the small amount of L-methionine required as a growth factor would have had little effect on the behavior of the nonexacting species.

(ii) In spite of the lack of a growth factor in the medium, the strains of *X. maltophilia* are reported in the article to be capable of growth on succinate, fumarate, malate, citrate, acetate, lactate, and propionate, all of which are qualified by the authors as having diagnostic value. Aside from the fact that the utilization of these organic acids is hardly diagnostic, in view of the fact that many other pseudomonads readily utilize them (25), one obvious question is whether the strains grow on these organic acids in a medium unsupplemented with L-methionine. There are no comments in the article about these anomalies.

(iii) It is also reported in the article that *X. maltophilia* was "shown to have xanthomonadin, justifying [its] inclusion in the genus." Reference is made to Starr et al. (27), who clearly state that "the pigments of the pale yellow *P. maltophilia* . . . are not xanthomonadins."

(iv) Data are presented in the article in percentages of positive strains, and therefore it is impossible for the reader to reconstruct the phenotype of individual strains. This is perhaps justifiable when one deals with many strains, but in this case there were only seven strains, of which five were authentic. We are informed that 71% of the strains (that is, five) were found to have oxidase activity and to grow at 37°C and that one strain (14% of 7) produced slimy colonies, but

TABLE 1. Differences between *S. maltophilia* and *Xanthomonas* spp.

Characteristic	Result for ^a :		Reference(s)
	<i>S. maltophilia</i>	<i>Xanthomonas</i> spp.	
No. of flagella	>1	1	1, 13
Fimbriae	+	—	1
Xanthomonadins	—	+	26
Xanthan gums	—	+	1
Asparagine as a C and N source	+	—	31
Nitrate reduction	(+) ^b	—	13
Inhibition of growth by 0.1% TPTC ^c	—	+ ^d	1
Starch hydrolysis	—	V ^e	1, 20
Chitin hydrolysis	+	—	9a
Growth at 37°C	+	—	13, 20
Oxidase reaction	(+)	(—)	1, 20
Plant pathogenicity	—	+	1
Association with human infections and clinical specimens	+	—	11
Growth in the presence of 0.01% methyl green, thionin, or lead acetate	+	—	28
Resistance to tetracycline, kanamycin, and erythromycin	+	— ^f	30
Resistance to novobiocin	+	— ^g	30

^a Parentheses indicate that the result applies to the majority of the strains.

^b Nitrate not used as a nitrogen source.

^c TPTC, triphenyltetrazolium chloride.

^d Frequently inhibited by 0.02% TPTC.

^e V, variable.

^f 97% of the strains tested are sensitive.

^g Only *X. albilineans* is resistant.

we are not told anything about the identities of the positive strains. Again, these points are worth clarification, in view of the allegation that the type strains of two species (*P. belle* and *P. hibiscicola*) are members of the species *P. maltophilia*.

In view of the above, the article by Van den Mooter and Swings (29) does not contribute solid evidence in support of the inclusion of *P. maltophilia* in the genus *Xanthomonas*.

Similarities between the two taxa. Aside from similarities in the sequences of rRNA cistrons, *P. maltophilia* and *Xanthomonas* spp. show resemblances in the utilization of certain substrates (although this point has not been exhaustively explored for *Xanthomonas* species), in the hydrolysis of Tween 80, in the sensitivity to some phages, and, more importantly, in details of the composition of the cell envelopes (19). However, in our opinion the differences outweigh the resemblances. At present, there is little difficulty in identifying *P. maltophilia* and *Xanthomonas* strains isolated from nature, although, to some extent, there may be some overlap in their natural habitats. As mentioned by Van Zyl and Steyn (32), after an improved characterization of *Xanthomonas* spp. has been achieved (29, 33), inclusion of *P. maltophilia* in the genus *Xanthomonas*, far from promoting taxonomic stability in this genus, will lead to confusion and will complicate the task of classifying and identifying isolates of the species.

Proposal of a new genus, *Stenotrophomonas*, for the species *X. maltophilia*. In view of the reasons expressed above, we propose to transfer *X. maltophilia* to the new genus

Stenotrophomonas (Ste.no.tro.pho.mo'nas. Gr. adj. *stenus*, narrow; Gr. n. *trophus*, one who feeds; Gr. n. *monas*, a unit, monad; M.L. fem. n. *Stenotrophomonas*, a unit feeding on few substrates). In the analysis by Stanier et al. (25) of many strains of *P. maltophilia*, it was found that they had a very limited nutritional spectrum, and this is reflected in the proposed new name. The proposal of the new genus name restores the genus *Xanthomonas* to its original definition (1).

The following is a description of the genus *Stenotrophomonas*. Gram-negative, nonsporulating cells, about 0.5 by 1.5 µm, motile with several polar flagella (13). They may produce fimbriae (9). Poly-β-hydroxybutyrate granules are not accumulated, and exogenous polymer is not hydrolyzed. The colonies are smooth, glistening, with entire margin, white, grayish, or pale yellow.

Strains require methionine for growth, but this is not a universal property (16). Nitrate is reduced, but it is not used as an N source. Denitrification and egg yolk reaction are negative. Liquefaction of gelatin and lipolytic reaction (Tween 80 hydrolysis) are positive. Optimal growth temperature, 35°C.

The nutritional spectrum is restricted. Of 145 organic compounds tested as the sole sources of carbon and energy, only 23 were used for growth (25). These include the disaccharides maltose, lactose, trehalose, and cellobiose and the glucoside salicin. Growth on lactose is poor, probably because of the inability to use galactose. Few fatty acids and amino acids were used for growth. None of the polyalcohols, aromatic compounds, or amines were used (25). Freshly isolated strains were found to use hydrocarbons, but the property was lost after subcultivation in common laboratory media (14, 15).

Isolated from assorted natural materials, human infections, and clinical materials. They are the most frequently isolated pseudomonads, after *P. aeruginosa*, in the clinical laboratory (11).

Characteristics useful for differentiating them from species of *Xanthomonas* are presented in Table 1. Since only a single species of *Stenotrophomonas* is known, its properties are the same as for the genus. The type strain is ATCC 13637 (Hugh 810-2, NCIB 9203, NCTC 10257, NRC 729).

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