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**Abstract —** Improvement of the taxonomy of the genus *Xanthomonas* and especially of *Xanthomonas campestris*, which is subdivided into more than 125 pathovars, is discussed. Recent contributions to the taxonomy of *Xanthomonas* are reviewed, and on the basis of these data and unpublished data from several laboratories, the usefulness of different phenotypic, chemotaxonomic, and genotypic techniques is discussed. The heterogeneity of several X. *campestris* pathovars has been demonstrated by sodium dodecyl sulfate electrophoresis of whole-cell proteins and fatty acid fingerprinting. The host selectivity of the pathovars is not correlated with their relationships as revealed by DNA-DNA hybridization experiments. In order to reveal the phylogenetic relationships among X. *campestris* pathovars and their relationships to other *Xanthomonas* species, it will be necessary to perform extensive DNA-DNA homology studies as an essential part of a polyphasic approach. At present, six DNA homology groups within *X. campestris* have been delineated. A systematic approach to improve the taxonomy of the genus *Xanthomonas* is proposed.

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**Improvement of the taxonomy of the genus *Xanthomonas* and especially of *Xanthomonas campestris*, which is subdivided into more than 125 pathovars, is discussed. Recent contributions to the taxonomy of *Xanthomonas* are reviewed, and on the basis of these data and unpublished data from several laboratories, the usefulness of different phenotypic, chemotaxonomic, and genotypic techniques is discussed. The heterogeneity of several *X. campestris* pathovars has been demonstrated by sodium dodecyl sulfate electrophoresis of whole-cell proteins and fatty acid fingerprinting. The host selectivity of the pathovars is not correlated with their relationships as revealed by DNA-DNA hybridization experiments. In order to reveal the phylogenetic relationships among *X. campestris* pathovars and their relationships to other *Xanthomonas* species, it will be necessary to perform extensive DNA-DNA homology studies as an essential part of a polyphasic approach. At present, six DNA homology groups within *X. campestris* have been delineated. A systematic approach to improve the taxonomy of the genus *Xanthomonas* is proposed.**

The genus *Xanthomonas* consists mainly of plant-pathogenic bacteria that occur worldwide and cause diseases of diverse plants. Because of its economic importance, this genus has been subjected to many taxonomic and determinative studies. DNA-rRNA hybridizations (4, 17) have revealed that the genus constitutes a separate rRNA branch in the gamma taxon of the *Proteobacteria* (sensu Stackebrandt et al. [21]). In *Bergey's Manual of Systematic Bacteriology*, the genus is divided into the following five species (1): *Xanthomonas albilineans*, *Xanthomonas ampelina*, *Xanthomonas axonopodis*, *Xanthomonas campestris* (type species), and *Xanthomonas fragariae*. Recently, the following proposals have been formulated in order to improve the classification of the genus *Xanthomonas*: (i) the name "*Xanthomonas populi*" (18) has been proposed, but has not been validated yet; (ii) Swings et al. transferred *Pseudomonas maltophilia* to the genus *Xanthomonas* as *Xanthomonas maltophilia* (24); (iii) in an extensive phenotypic study of 268 *Xanthomonas* strains, *X. campestris* pv. *graminis* and *X. campestris* pv. *oryzae* were clearly separated from other *X. campestris* pathovars, and Van den Mooter et al. proposed that these pathovars should be reclassified as separate species ("*Xanthomonas graminis*" and "*Xanthomonas oryzae*") (29); and (iv) Willems et al. proposed that *X. ampelina* does not belong to the genus *Xanthomonas* and reclassified this organism as *Xylophilus ampelinus* (32).

Consequently, Van den Mooter et al. (29) proposed that the genus *Xanthomonas* contains the following eight species: *X. albilineans*, *X. axonopodis*, *X. campestris*, *X. fragariae*, "*X. graminis*," *X. maltophilia*, "*X. oryzae*," and "*X. populi*." *X. campestris* is by far the most complex of these species because it has been subdivided into 123 pathovars as described by Dye et al. (5) (this number has increased by an additional 20 pathovars since the study of Dye et al. [J. M. Young, personal communication]). The term pathovar was established in a special-purpose nomenclature (5) to satisfy the needs of plant pathologists for names of pathogens that are, or are believed to be, specific for certain hosts or diseases. This special-purpose classification does not meet the requirements for naming taxa according to the *International Code of Nomenclature of Bacteria* (13). It is a nomenclatural compromise which is open to criticism (6, 20, 23, 29). In a general taxonomic environment, there is no reason to assign crucial importance to a single phenotypic feature (in this case, phytopathogenicity). "Modern bacterial systematists should not be content to work with two types of classification: an artificial one for practical purposes only and a natural one without practical implication. Rather, the ultimate goal of bacterial taxonomists should be to define taxa on the basis of their genealogical relationships" (13). An important consequence of pathovar classification in the genus *Xanthomonas* is that nonpathogenic xanthomonads (e.g., epiphytic isolates) cannot be classified in the system.

It is generally accepted that the taxonomy of the genus *Xanthomonas* and especially of *X. campestris* needs revision. The results of some recent studies of *X. campestris* taxonomy are summarized in Table 1.

In our opinion, the development of improved *Xanthomonas* taxonomy will depend on satisfying the two following criteria: examination of large, representative sets of *Xanthomonas* strains and use of a number of discriminating techniques. In the most extensive taxonomic study performed so far, 268 strains belonging to 107 *X. campestris* pathovars (29) were examined. This means that on average fewer than three

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TABLE 1. Recent contributions to the taxonomy of the genus *Xanthomonas*[a]

| Pathovar(s) | No. of strains investigated | Reference | Main method(s) | Main conclusions |
|---|---|---|---|---|
| *oryzae*, *oryzicola* | 49 | 30 | Phenotypic features and soluble protein PAGE | Both rice pathogens are differentiated phenotypically and by protein electrophoresis |
| *campestris* | 35 | 25 | Serological techniques, SDS-PAGE of membrane proteins | Strain identification by Ouchterlony double diffusion is possible; *X. campestris* pv. *campestris* is distinguished from other *X. campestris* pathovars by SDS-PAGE of membrane proteins |
| *manihotis*, *cassavae* | 43 | 27 | Phenotypic features, soluble protein PAGE, and DNA-DNA hybridizations | The cassava pathogens are differentiated from each other by protein electrophoresis and phenotypic analysis; DNA-DNA hybridizations reveal low levels of homology (18 to 37%) among *X. campestris* pv. *manihotis*, *X. campestris* pv. *cassavae*, and *X. campestris* pv. *campestris* |
| *graminis*, *phleipratensis*, *poae*, *arrhenatheri* | 35 | 28 | Phenotypic features and soluble protein PAGE | In protein electrophoresis each of the four pathovars forms a separate cluster; in the phenotypic analysis *X. campestris* pv. *arrhenatheri* and *X. campestris* pv. *poae* cluster together; *X. campestris* pv. *graminis* and *X. campestris* pv. *phleipratensis* occur in two separate phena |
| 107 pathovars | 268 | 29 | Physiological and biochemical tests | One *X. campestris* cluster exists without internal differentiation, except for *X. campestris* pv. *graminis* and *X. campestris* pv. *oryzae*, which occur in separate phena |
| 26 pathovars | 117 | 14 | RFLP analysis of plasmid DNA | Not all *X. campestris* strains contain plasmids; plasmid-containing strains are distinguishable on the pathovar level |
| 26 pathovars | 93 | 15 | RFLP analysis of genomic DNA | Differentiation among pathovars is possible; RFLP analysis is suitable for identifying unknown *Xanthomonas* strains |
| *citri* | 30 | 10 | Total genomic fingerprinting by DNA restriction analysis | Two clonal *X. campestris* pv. *citri* groups correspond to pathological groups A and (B+D+C); pathological group E is heterogenous |
| *citri* | 24 | 7 | RFLP analysis of genomic DNA | Clonal variation occurs among *X. campestris* pv. *citri* strains, analogous to the results of Hartung and Civerolo[b] |
| *citri*, *phaseoli*, *alfalfae*, *glycines*, *cyamopsidis*, *dieffenbachiae*, *pisi* | | 8 | RFLP analysis of genomic DNA, phytopathological tests | Reinstatement of *X. campestris* pv. *citri* pathological group A to *X. citri* and reclassification of pathological groups (B+D+C) and E as *X. campestris* pv. *aurantifolii* and *X. campestris* pv. *citrumelo*, respectively; reinstatement of American *X. campestris* pv. *phaseoli* strains to *X. phaseoli* |
| 15 pathovars from grasses and cereals | 120 | 12 | SDS-PAGE of whole-cell proteins and DNA-DNA hybridizations | Various pathovars display a characteristic protein pattern; *X. campestris* pv. *translucens*, *X. campestris* pv. *hordei*, *X. campestris* pv. *cerealis*, *X. campestris* pv. *secalis*, *X. campestris* pv. *undulosa*, and *X. campestris* pv. *poae* cannot be differentiated by SDS-PAGE and are genomically highly related |
| 14 pathovars from grasses and cereals | 63 | 22 | Gas-chromatographic analysis of cellular fatty acids | Various pathovars display a distinct fatty acid pattern; *X. campestris* pv. *translucens*, *X. campestris* pv. *hordei*, *X. campestris* pv. *cerealis*, *X. campestris* pv. *secalis*, and *X. campestris* pv. *undulosa* cannot be differentiated |

[a] Contributions since the publication of *Bergey's Manual* in 1984 (1).
[b] See reference 10.

strains per pathovar were included. On the basis of studies in which pathovars have been examined extensively by high-resolution techniques (e.g., sodium dodecyl sulfate [SDS] protein electrophoresis, fatty acid analysis, DNA restriction analysis), it appears that some pathovars are heterogeneous (see below). Valid taxonomic conclusions cannot be made unless large numbers of strains, representing the geographical distribution area of each pathovar, are examined. A number of methods discriminate groups of strains at the specific and infraspecific levels and have been used to study

*X. campestris* pathovars (e.g., serological techniques, phage typing, protein electrophoretic patterns, isoenzyme electrophoresis, phenotypic analysis, fatty acid composition, DNA restriction analysis). For classification purposes however, it is now considered necessary to calibrate these techniques against DNA-DNA hybridization, the conventional method used for the delineation of species (11, 19, 26, 31). It should be realized that in fact the complete nucleotide base sequence of genomic DNA is the absolute reference standard (31).

SDS-polyacrylamide gel electrophoresis (PAGE) of cellular proteins has demonstrated the homogeneity or heterogeneity of *X. campestris* pathovars (Table 1 and see below). The following pathovars have been shown to be internally homogeneous as determined by SDS-PAGE (Vauterin, unpublished data, except where indicated otherwise): *X. campestris* pv. *arrhenatheri* (7 strains) (12), *X. campestris* pv. *begoniae* (35 strains), *X. campestris* pv. *campestris* (112 strains), *X. campestris* pv. *cassavae* (18 strains), *X. campestris* pv. *cucurbitae* (8 strains), *X. campestris* pv. *glycines* (15 strains), *X. campestris* pv. *graminis* (14 strains) (12), *X. campestris* pv. *holcicola* (9 strains) (12), *X. campestris* pv. *hyacinthi* (11 strains), *X. campestris* pv. *manihotis* (19 strains), *X. campestris* pv. *melonis* (5 strains), *X. campestris* pv. *oryzae* (18 strains) (12), *X. campestris* pv. *oryzicola* (12 strains) (12), *X. campestris* pv. *pelargonii* (131 strains), *X. campestris* pv. *phlei* (7 strains) (12), *X. campestris* pv. *pruni* (14 strains), and *X. campestris* pv. *vitians* (10 strains). Although not all pathovars have been studied by SDS-PAGE, it is clear that several pathovars are heterogeneous and possess two or more protein electrophoretic types. The following pathovars have been shown to be heterogeneous by SDS-PAGE (Vauterin, unpublished data, except where indicated otherwise): *X. campestris* pv. *alfalfae* (10 strains), *X. campestris* pv. *citri* (43 strains), *X. campestris* pv. *corylina* (7 strains), *X. campestris* pv. *juglandis* (11 strains), *X. campestris* pv. *malvacearum* (11 strains), *X. campestris* pv. *phaseoli* (17 strains), *X. campestris* pv. *poinsettiicola* (9 strains), *X. campestris* pv. *ricini* (10 strains), *X. campestris* pv. *vasculorum* (24 strains) (12), *X. campestris* pv. *vesicatoria* (22 strains), and *X. campestris* pv. *vignicola* (12 strains). *X. campestris* pv. *cerealis* (8 strains), *X. campestris* pv. *hordei* (11 strains), *X. campestris* pv. *poae* (2 strains), *X. campestris* pv. *secalis* (4 strains), *X. campestris* pv. *translucens* (10 strains), and *X. campestris* pv. *undulosa* (6 strains) (12) cannot be differentiated from each other by SDS-PAGE of proteins.

Fatty acid analyses of lipopolysaccharides and membrane lipids of members of 60 species and pathovars within the genus *Xanthomonas* have demonstrated the great heterogeneity within the genus. Most species, except *X. campestris*, are relatively homogeneous, but even within some individual *X. campestris* pathovars there is great heterogeneity (22; Stead, unpublished data). Some pathovars (for example, *X. campestris* pv. *vasculorum*) comprise several discrete subgroups. Other heterogeneous pathovars are *X. campestris* pv. *citri*, *X. campestris* pv. *dieffenbachiae*, and *X. campestris* pv. *vesicatoria*.

Many taxa group together according to fatty acid profile type. Table 2 shows some *X. campestris* groups based on principal-component analysis. These groups are virtually identical to those based on DNA-DNA homology (Table 3) and SDS-PAGE (data not shown). Some pathovars are so closely related that there are no significant differences between profiles. These include (i) *X. campestris* pv. *holcicola* and *X. campestris* pv. *vasculorum* subgroup C and (ii) *X. campestris* pv. *translucens*, *X. campestris* pv. *cerealis*, *X. campestris* pv. *hordei*, *X. campestris* pv. *undulosa*, and *X. campestris* pv. *secalis*.

TABLE 2. Some fatty acid profile groups within *X. campestris*[a]

| Group | *X. campestris* pathovars | Host family |
|---|---|---|
| 1 | *phaseoli, alfalfae, glycines, vignicola, sesami, passiflorae* | Mainly Fabaceae |
| 2 | *campestris, raphani, armoraciae, barbareae, incanae* | Brassicaceae |
| 3 | *graminis, arrhenatheri, phlei, poae, cerealis, undulosa, secalis, hordei, translucens, phleipratensis* | Poaceae |
| 4 | *holcicola, vasculorum* subgroup C, *oryzae, oryzicola, cyamopsidis* | Mainly Poaceae |

[a] Stead, unpublished data.

The application of restriction fragment length polymorphism (RFLP) analysis also seems to offer new perspectives for *Xanthomonas* taxonomy (Table 1). Gabriel et al. (8) proposed reinstatement of *X. campestris* pv. *citri* strains to the species *Xanthomonas citri* and reinstatement of American *X. campestris* pv. *phaseoli* strains to the species *Xanthomonas phaseoli*. In both cases, the only differentiating criterion advanced by the authors was a "distinctive pattern of hybridizing DNA bands by RFLP analysis." Although we are aware of the possibilities that RFLP analysis offers for bacterial characterization, we do not agree with the reclassifications proposed by these authors, because the reinstatements of former pathovars or parts of them to species level are based essentially on RFLP differences. The similarities of RFLP patterns refer only to congruence of a restricted fragment of the entire genome. This could lead to the development of an RFLP system, which probably would be as unjustified as a system based on pathogenicity. If RFLP data are to be used in taxonomy, they should be complemented with total DNA-DNA homology studies.

DNA-DNA hybridization studies have demonstrated great variation in the relatedness of pathovars (12, 16, 27; Hildebrand, Palleroni, and Schroth, unpublished data; unpublished data obtained in the Laboratory of Microbiology, State University, Ghent, Belgium). A DNA homology value of 60 to 70% is the generally accepted minimum value for strains within a species (11, 19, 31). Many *X. campestris* pathovars have DNA-DNA homology values between 0 and 40% and thus are unlikely to belong to a single species, whereas other pathovars have levels of DNA-DNA homology (Table 3) higher than 60% and most likely belong to the same species. At present, six DNA homology groups have been delineated within *X. campestris*. The pathovar reference strains given by Dye et al. (5) for the pathovars listed in Table 3 are all representative and can be used as reference strains of the DNA homology groups, except for *X. campestris* pv. *vesicatoria* subgroup A. A representative strain of this subgroup is strain NCPPB 2573. At first, it was striking that some of the six groups shown in Table 3 comprise pathovars which attack plants of the same family. Reality is more complex however; there are pathovars that attack members of the same plant family but occur in different DNA homology groups, as well as pathovars that attack members of different plant families and occur in the same DNA homology group. A number of pathovars, including *X. campestris* pv. *eucalypti*, *X. campestris* pv. *papavericola*, *X. campestris* pv. *pisi*, *X. campestris* pv. *vesicatoria* subgroup B (including the pathovar reference strain), *X. campestris* pv. *hyacinthi*, and *X. campestris* pv. *cassavae*,

TABLE 3. DNA-DNA homology groups within *X. campestris*

| Group | *X. campestris* pathovars | Host family(ies) | DNA-DNA homology values (%) |
|---|---|---|---|
| 1 | *phaseoli*, *phaseoli* "var. *fuscans*," *alfalfae*, *glycines*, *cajani*, *vignicola*, *manihotis*, *vesicatoria* subgroup A, *malvacearum*, *begoniae*, *cassiae*, *melhusi*, *biophyti*, *khayae*, *physalidicola*, *fascicularis*, *nakataecorchori*, *lespedezae*, *maculifoliigardeniae*[c] | Fabaceae and others | 50–90[a,b] |
| 2 | *campestris*, *raphani*, *aberrans*, *armoraciae*, *barbareae*, *incanae*, *plantaginis* | Mainly Brassicaceae | 68–95[a,b] |
| 3 | *graminis*, *arrhenatheri*, *phlei*, *poae*, *cerealis*, *undulosa*, *secalis*, *hordei*, *translucens*, *phleipratensis* | Poaceae | 58–94[d] |
| 4 | *oryzae*, *oryzicola* | Poaceae | 80–100[d] |
| 5 | *taraxaci*, *carotae*, *pelargonii*, "*X. gardneri*" | Different families | 88–96[b] |
| 6 | *juglandis*, *celebensis* | Different families | 84[b] |

[a] L. Vauterin and B. Hoste, unpublished data.
[b] Hildebrand, Palleroni, and Schroth, unpublished data. DNA homology values were recalculated by using the methods of Grimont (9) and De Ley et al. (3).
[c] *X. campestris* pv. *vesicatoria* subgroup A does not include the pathovar reference strain according to Dye et al. (5).
[d] Data from reference 12.

do not fall into any of the groups listed and may represent additional DNA homology groups. It is also clear that in many cases the DNA homology groups are not consistent with the pathovar subdivision. Although several attempts have been made to characterize and isolate genes for pathogenicity (2), it is still not known whether phytopathological specialization is related to only a single gene or to many genes. The present data suggest that genes for pathogenicity constitute a rather small part of the genome or at least that phytopathogenic specialization is affected by few genes, as a number of pathovars with different host ranges share high levels of DNA homology. This also implies that host specificity is not a representative marker for phylogenetic classification.

It is evident from a survey of the recent literature, together with unpublished data from some of our laboratories, that the taxonomic structure of the genus *Xanthomonas* is very complex. The sometimes unexpected phylogenetic relationships between pathovars can be detected only by extensive DNA-DNA hybridizations.

Progress in *Xanthomonas* taxonomy will largely depend on a systematic, polyphasic, and comprehensive approach, involving (i) the constitution of a large, representative collection of strains, comprising fresh isolates and type, neotype, and collection strains from all *Xanthomonas* species and pathovars; (ii) screening of the entire collection for identical and closely related strains by appropriate methods (e.g., phenotypic tests, SDS-PAGE, fatty acid analysis, etc.); (iii) selection of one or two representative members from each group for genotypic analysis; (iv) construction of a DNA-DNA homology matrix, essentially containing representative strains of all pathovars of *X. campestris* and all species of *Xanthomonas*; (v) the search for phenotypic features allowing differentiation between the delineated DNA homology groups; and (vi) pathogenicity testing of the delineated DNA homology groups by inoculation and cross-inoculation tests.

As the investigation of all described *X. campestris* pathovars will be a very extensive task, a collaborative polyphasic approach will be needed to obtain a reliable classification of the genus *Xanthomonas*. Workers should refrain from advancing premature nomenclatural changes in the genus *Xanthomonas* on the basis of fragmentary data. Both the stability and the uniformity of the classification and the respectability of taxonomists will profit from this.

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