Comment on the Reinstatement of *Xanthomonas citri* (ex Hasse 1915) Gabriel et al. 1989 and *X. phaseoli* (ex Smith 1897) Gabriel et al. 1989: Indication of the Need for Minimal Standards for the Genus *Xanthomonas*


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The proposals to reinstate *Xanthomonas citri* (ex Hasse 1915) Gabriel et al. 1989 and *X. phaseoli* (Smith 1897) Gabriel et al. 1989 are examined in terms of conventional criteria for describing new species. We suggest that the descriptions presented are insufficient in terms of modern practice for the purposes of formal classification in the genus *Xanthomonas*. To create guidelines for future reinstatements, the Judicial Commission of the International Committee on Systematic Bacteriology is requested to arrange for the preparation and promulgation of minimal standards for *Xanthomonas*. The pathovars proposed, *X. campestris* pv. aurantifolii Gabriel et al. 1989 and *X. campestris* pv. citrulmoelo Gabriel et al. 1989, are considered to be defective in terms of the International Society for Plant Pathology’s standards for naming pathovars.

*Xanthomonas citri* (Hasse 1915) Dowson 1939 and *X. phaseoli* (Smith 1897) Dowson 1939 were originally described as the pathogens that cause bacterial canker of citrus (*Citrus* spp.) and common blight of bean (*Phaseolus* spp.), respectively. These organisms were listed by Dye and Lelliott (5) as species whose descriptions did not allow them to be distinguished from *X. campestris* (Pammel 1895) Dowson 1939 or from each other except by host reactions. These species and most of the other *Xanthomonas* spp. accepted at the time did not fulfill the criteria established for validation in the Approved Lists of Bacterial Names (23). However, the names were retained (4) at the infrasubspecific level as pathovars of *X. campestris*. Recently, Gabriel et al. (8) proposed reinstatement of *X. phaseoli* from *X. campestris* pv. phaseoli (Smith 1897) Dye 1978 as the name for the pathogen of common blight of beans and reinstatement of *X. citri* as the name for the group A strains of *X. campestris* pv. citri (Hasse 1915) Dye 1978 which cause Oriental or Asian citrus canker. Gabriel et al. (8) also proposed the pathovar name *X. campestris* pv. aurantifolii for group B, C, and D strains and the pathovar name *X. campestris* pv. citrulmoelo for group E strains of *X. campestris* pv. citri.

**CLASSIFICATION**

The naming of a taxon according to the *International Code of Nomenclature of Bacteria* (19) presupposes that the taxon has been characterized by a range of methods which are sufficient to classify it as a distinct entity in terms of its overall characteristics and to distinguish it from all other named taxa (18, 21, 25, 26, 28). Published descriptions of taxa should be as complete as possible and are expected to include such information as morphological descriptions, physiological behavior, biochemical reactions, chemical composition, and nucleic acid comparisons. The general proposals to Trieper and Kramer (28) and Murray et al. (21) and the methodologies described by Johnson (14), Jones (15), Jones and Krieg (16), and Sneath (24) give clear indications of what is intended in a modern description. The modern description is an independent reference for a taxon. The *International Code* calls for the preparation of minimal standards (Recommendation 30b) to which species descriptions should conform in the future, which should "include tests for generic identity, and tests which would distinguish the species from others." The description of a given taxon can expand and become more detailed as closely related taxa are distinguished and as new methods are applied.

As an assessment of modern practices for naming new species, we examined a representative sample of 35 reports published in the *International Journal of Systematic Bacteriology* in the years from 1980 to 1988. This examination showed that, on average, for each species proposed approximately 16 strains were examined by using about 60 biochemical tests. An examination of all plant-pathogenic species named in the same period gave a similar result. Our examinations did not include polyphasic or multicharacter tests, such as electrophoresis of proteins, guanine-plus-cytosine ratios, or DNA-DNA hybridization studies. Commonly found in these descriptions was a list of procedures by which strains of the species could be distinguished from related species by using biochemical determinative tests.

One intention of the authors of the *International Code* in requiring adequate differentiation of species by modern descriptions was to provide independent references which workers could use to identify species and, in the wider context of bacterial taxonomy, which workers could use to...
identify strains as members of a known species or as members of previously unidentified taxa (18, 28). The collected descriptions of taxa in compendia, such as Bergey’s Manual of Systematic Bacteriology, should make it possible to identify any previously classified bacterium. This capability is essential for the future rational development of taxonomy.

The pathovar concept. During the period leading up to the compilation of the Approved Lists, there was much work done on the taxonomy of plant-pathogenic bacteria and much discussion of the impact of the changes in classification that would occur. Dye and Lelliott (5) had shown for the genus Xanthomonas and Sands et al. (22) and Doudoroff and Palleroni (2) had shown for the genus Pseudomonas that it would not be possible to retain species names for many plant-pathogenic bacteria recognized at that time. This caused concern among plant pathologists who believed that they had a need for names of bacteria as distinct pathogens (3, 12). Accordingly, a special-purpose nomenclature in which the term pathovar was used was proposed for those plant-pathogenic bacteria which did not meet the criteria for species designation (33, 34). This nomenclature provided names at the infrasubspecific level for pathovars that were distinguished on the basis of proved differences in pathogenicity, either in terms of host range or in terms of distinct symptoms.

Thus, the use of the term pathovar was established with formal standards (4) to satisfy the needs of plant pathologists for names of pathogens which do not meet the standards for higher taxa under the International Code. It was recognized that this classification at the infrasubspecific level is a nomenclatural compromise imposed by our present state of knowledge.

MOLECULAR BIOLOGICAL METHODS IN THE TAXONOMY OF PLANT-PATHOGENIC BACTERIA

Since the promulgation of the Approved Lists, the importance of the role of molecular biological methods in bacterial taxonomy has received increased recognition. Comparative studies of the levels of DNA-DNA homology of total bacterial genomes are used to differentiate taxa at the species level. The term genospecies has been defined by the Ad Hoc Committee on Reconciliation of Bacterial Systematics of the International Committee of Systematic Bacteriology (31) as a group of strains which exhibit 70% or greater DNA-DNA relatedness and have a thermal denaturation deviation value of <5°C (9). Implicit in this definition is the requirement for extensive genetic comparisons that have not yet been seen in practice. The fragmentary nature of DNA-DNA homology studies and the diversity of procedures and possible interpretations currently limit our ability to use such methods for species classification. Sneath (26) has noted the importance of standardizing procedures and the need for detailed analyses of results. Reviewing the complexity of genomic organization, Krawiec (17) suggested that DNA-DNA homology analyses may not reveal the elements of a genome which are taxonomically significant. If genospecies based on the results of DNA-DNA homology studies are to be established in nomenclature, then an orderly procedure which avoids introducing subjective synonymies is essential. It should involve DNA-DNA homology comparisons with all (or a wide range of) previously named species within a genus. The recommendation (31) that genospecies based on DNA-DNA homology data not be named unless there are phenotypic properties available for identification is consistent with earlier practice, and it preserves the ability to identify strains to the species level in laboratories by using published descriptions. This recommendation was endorsed by Murray et al. (21).

The results of the early DNA-DNA homology study of Murata and Starr (20) suggested that there are in the genus Xanthomonas distinguishable genospecies sensu Wayne et al. (31). For some pathogens in the genus Xanthomonas, Murata and Starr (20) showed that levels of DNA-DNA homology were sufficiently high (e.g., >50% for X. pruni compared with X. campestris, X. citri, X. oryzae, X. phaseoli, and X. pisi) that replicated studies with careful attention to method and details of stringency would be necessary to determine whether the organisms are separable as species (26). The otherwise inadequate descriptions of these taxa meant that there was no justification for proposing them as species until more general data were gathered (4).

Recent developments, such as genomic fingerprinting (10) and analysis of restriction fragment length polymorphisms (RFLP) (8), offer potentially useful diagnostic tools for identifying bacterial strains. However, these methods are not equivalent to total DNA-DNA homology studies, which were endorsed as the best applicable procedure for defining species in phylogenetic terms by Wayne et al. (31) and are considered to be of questionable value in deriving general conclusions in classification (21). Vauterin et al. (30) observed that the creation of species solely on the basis of RFLP analysis raises the same objections and poses the same problems for classification and nomenclature as does creating species solely on the basis of pathogenicity for particular plant hosts. It is generally recognized that an orderly nomenclature of plant-pathogenic bacteria will be best served if an accurate overview of the relationships of organisms at the molecular level is obtained and more phenetic data are gathered before individual plant-pathogenic species, presently named as pathovars, are reinstated.

PROPOSED REINSTATEMENTS OF X. CITRI AND X. PHASEOLI

The proposal of Gabriel et al. (8) to reinstate formally the species X. phaseoli and X. citri was based largely on observations that when RFLP analyses were performed, some strains identified as X. campestris pv. citri (group A strains) and strains identified as X. campestris pv. phaseoli formed distinct clonal groups which did not include the other strains of X. campestris examined. Gabriel et al. (8) claimed that these distinct groups identified by RFLP analysis could be regarded as separate species. We make the observations below in relation to the descriptions of these proposed species.

Numbers of strains. The report of Gabriel et al. (8) contains no systematic list of strains with sources, nor do the authors explicitly indicate which strains were included in tests. Although 7 strains of X. citri and 10 strains of X. phaseoli are described as having been tested, the only place where these strains are shown to have been used is in a computer-generated dendrogram of an analysis in which RFLP probes were used. For laboratory tests, results are given only for two strains of X. citri and one strain of X. phaseoli. This is substantially fewer than the number of strains usually included in systematic studies (see above).

Tests used. The preamble to the descriptions of X. citri and X. phaseoli as reinstated indicates that the data for these descriptions were derived collectively from the authors'
work and from the work of Bradbury (1). It must be deduced that the methods described in the text gave rise to the results in the description and that the remaining characteristics in the description are from the report of Bradbury (1). Reference to the general description of the genus, made on the basis of strains not included in this study, is not relevant to the description of the proposed new species, which should have been derived from tests on the strains used in the study.

**Genetic determination of Xanthomonas.** The characteristic determinative tests for the genus *Xanthomonas* are Gram reaction, cell morphology, flagellar insertion, oxygen requirement, presence of nitrate reductase, denitrification, triphenyltetrazolium chloride sensitivity, use of asparagine as a sole source of carbon, requirement for specific growth factors, and production of a distinctive pigment by a majority of the strains (1). It is only by routine confirmation of these tests for each new species that they can continue to be regarded as generic determinants. Unless these criteria are always met, further confusion can be expected, particularly between the genera *Xanthomonas* and *Pseudomonas*, as the numbers of species described increase.

**Pigment.** The absorption spectra of xanthomonadin pigments are important diagnostic criteria for the identification of xanthomonads. The only evidence presented by Gabriel et al. (8) that the strains of the proposed species and the supporting strains used in their study are xanthomonads is a reference to a single peak in methanol extracts of pigments, which corresponded to the presence of the xanthomonadin pigment. Bradbury (1) describes three peaks in petroleum ether extracts for *Xanthomonas* spp. The reference to a single peak appears to rely on the report of Irey and Stall (13) for its validity. Gabriel et al. (8) provide an unusual instance in which the generic characterization of a new species was based on one test. However, a confusing point in the report of Gabriel et al. (8) arises because it is unclear whether this test result is intended as proof of the identity of strains as members of the genus *Xanthomonas* or as members of the species *X. campestris*.

**Other characteristics.** Eight additional characteristics are reported for strains by Gabriel et al. (8). One of these characteristics reported in the species descriptions is devoid of data ("able to use a variety of carbohydrates and salts of organic acids as sole sources of carbon"). For another characteristic ("growth on minimal media greatly stimulated by addition of glutamic acid"), the source of the observation is obscure; the appropriate test is not listed in Materials and Methods, nor is this characteristic specifically referred to by Bradbury (1).

The tests listed in Materials and Methods for which specific results are actually reported do not distinguish *X. citri*, *X. phaseoli*, or the small number of strains of *X. campestris*. Amylace, casein, and lipase activities are identical for all of the strains tested. Pectinase activity is noted for single strains of *X. citri* and *X. phaseoli*, but as it is also variable between strains allocated to the species *X. campestris*, it is unclear whether this is a useful determinative characteristic.

**Species determinations.** Of the 17 determinative tests described by Bradbury (1) which distinguish existing *Xanthomonas* species, 3 (xanthomonadin production, starch hydrolysis, and milk proteolysis) were used by Gabriel et al. (8), and none of these usefully distinguished the species being studied. None of the 37 other species characteristics reported by Bradbury (1) were included.


The phenotypic descriptions of the reinstated species are indistinguishable from the description of the type species, *X. campestris*, and apart from a reference to differences in computer-analyzed RFLP patterns, contained no other information by which *X. citri* or *X. phaseoli* could be distinguished from other species of *Xanthomonas*. The two DNA probes reported were from strains of *X. campestris* whose provenance is unclear.

**Problems with species interpretations.** A problem in the taxonomic interpretation of the data presented by Gabriel et al. (8) arises because there is no clear presentation of comparative data that describe the type species of the genus *Xanthomonas* (*X. campestris*), or any other *Xanthomonas* sp. other than the two species proposed for reinstatement. *X. campestris* was represented in this study by 32 strains, 20 of which were not identified except on the basis of pigment production. Therefore, none of these strains was established as a member of *X. campestris* in the study. Twelve strains represented eight known pathovars, and although the type strain of *X. campestris* was included, there were no correlative data. Therefore, the actual representation of *X. campestris* strains in the study of Gabriel et al. (8) was small compared with the number of strains in other revisions of the species (29). If the concept that Gabriel et al. (8) proposed is accepted, that species are represented by clonal clusters in RFLP data, then presumably the species *X. campestris* is represented only by the 30 strains that lie between *X. citri* and *X. phaseoli* on the dendrogram. These strains are identified as members of six accepted pathovars (*X. campestris* pv. *alfalfae*, *X. campestris* pv. *campestris*, *X. campestris* pv. *cyamopsidis*, *X. campestris* pv. *dieffenbachiae*, *X. campestris* pv. *glycines*, and *X. campestris* pv. *macleaverum*) and two proposed new pathovars (*X. campestris* pv. *aurantifolii* and *X. campestris* pv. *citrumelo*).

**Genetic analysis of the genus Xanthomonas.** The RFLP data presented by Gabriel et al. (8) are combined data from two studies (7, 8). The two data sets reported include only 367 (24%) of the 1,128 similarity coefficients required to construct a complete matrix for all strains. It is not clear how Gabriel et al. (8) derived the dendrogram by using the Clustan program as reported, because this program does not function without complete data. If the dendrogram was generated by an amalgamation of separate analyses, then the procedure which proves the robustness of the method should be reported in detail. Without such explanation, there is doubt about the validity of the suggestion that "the criterion ... for inclusion of a *Xanthomonas* strain in a species is at least 80% similarity with the type strain as determined with test probes proven capable of revealing 20% or less similarity between species of a genus."

**Type strains.** (i) *X. campestris*. No comparative phenetic data are given for the type strain of *X. campestris*, and no evidence of an RFLP analysis of the type strain is found in an earlier reference that Gabriel et al. (8) cited. (ii) *X. phaseoli*. If designated type strain G27 of *X. phaseoli* is "probably synonymous" (8) with strain LB-2, the strain on which the RFLP analysis was conducted, then strain G27 is "probably" a strain which acts as type strain for the population described as *X. phaseoli*. There should be no doubt about the authenticity of type strains.

**Strain identification.** The descriptions of *X. citri* and *X. phaseoli* given by Gabriel et al. (8) do not allow the alloca-
tion of strains to the species, nor would the inclusion of such
descriptions in the taxonomic literature allow strains to be
identified as members of known species or as members of
previously unidentified taxa.

As a precedent, the proposal of Gabriel et al. (8) opens the
way for reinstatement of species in the genus *Xanthomonas*
and for proposals of new pathogenic species on what is for
all intents and purposes a “common name” basis (32).

Descriptions of species which give as the sole determination
“distinguished from . . . . (a list comprising other similarly
categorized *Xanthomonas* species) . . . by distinct pat-
terns of hybridizing bands by RFLP analysis” has little
descriptive content and no useful function for species iden-
tification.

**PATHOGENICITY**

It is not a requirement that Koch’s postulates (description
of the naturally occurring disease syndrome, isolation in
pure culture of microorganisms from the diseased host,
 inoculation of healthy hosts, re-establishment of the syn-
drome in a healthy host, and re-isolation of inoculated
organism) be conducted in the formal naming of a species.

However, if a species name is to be reinstated as the causal
pathogenic agent of a known disease, then Koch’s postulates
must be discussed, or there must be clear reference to a
publication which connects the disease syndrome and the
strains being studied.

Gabriel et al. (8) note pathogenic reactions for unspecified
strains of *X. citri*, but no collected description of the disease
syndromes, host ranges, and details of symptoms for the
strains being studied is presented.

A general description of citrus canker as the disease from
which some strains included in the study were originally
isolated is referred to but not reported in detail by Gabriel et
al. (8), and only modest descriptions of pathogenic reactions
following inoculation into citrus are given for the canker
pathogen. No specific statement is made that these reactions
correspond to citrus canker as it occurs in the field.

No description is given for symptoms of common blight of
bean (*X. phaseoli*), nor is there any substantive description
of pathogenic reactions of test plants that indicate similar-
ities with the disease as it occurs in the field.

**CONCLUSIONS REGARDING THE PROPOSALS TO
REINSTATE *X. CITRI* AND *X. PHASEOLI***

The following observations are pertinent to a discussion of the
validity of the new species described by Gabriel et al. (8).

(i) Valid publication of a new taxon requires (Rule 27) (a)
that the name be published in the International Journal
of Systematic Bacteriology and (b) that the publication of
the name in the International Journal of Systematic Bacteriology
be accompanied by a description of the taxon or by a
reference to a previously effectively published description of
the taxon.

(ii) The *International Code* states explicitly (Rule 28a,
Note 2) that reinstatement of a species is equivalent to
naming a new species.

(iii) Therefore, the report of Gabriel et al. (8) represents
the validation and effective publication of *X. citri* and *X.
phaseoli*, species names for the pathogens that cause bacte-
rial (Asian) canker of citrus and common blight of beans,
respectively.

Notwithstanding publication in the International Journal
of Systematic Bacteriology, it appears that the species are
not circumscribed in any recognizable sense (Principle 8).

The names are not accompanied by descriptions which
distinguish the species or allow identification of strains, in
that there is an almost total absence of confirmation of
generic identity, a very small number of supplementary
biochemical test results, none of which distinguishes the
species, and a complete reliance on RFLP analysis data. In
addition, the RFLP analysis data appear to be incomplete.
Therefore, the descriptions might be considered not to be in
comformity with Rule 27(2). The procedure which Gabriel et
al. used does not make clear (and obscures) the circumscrip-
tion and description of the type species, *X. campestris*.

A strict nomenclatural interpretation of the *International
Code* might find the names *X. citri* and *X. phaseoli* valid. The
*International Code* does not specify the requirements for
descriptions, and minimal standards have not been prepared
yet for the genus *Xanthomonas*. We suggest that if minimal
standards had been promulgated for the genus *Xantho-
monas*, they would give formal expression to a need for tests by
which strains of proposed species are allocated to the genus
and for tests that confirm that strains are distinguishable
from other species. It is unlikely that the proposals of
Gabriel et al. (8) to reinstate *X. citri* and *X. phaseoli* would
be consistent with such standards. To avoid confusion in the
nomenclature of the genus *Xanthomonas* in the future, the
Judicial Commission of the International Committee on
Systematic Bacteriology is therefore requested to institute
procedures to establish and promulgate minimal standards
for the genus *Xanthomonas* (Rule 30, Recommendation
30b). Without this step, further reinstatements of species in
the genus *Xanthomonas* on the basis of RFLP data or other
incomplete criteria could lead to a disorganized and confusing
nomenclature.

**NOTE ON THE PATHOVAR NAMES PROPOSED BY
GABRIEL ET AL.**

Gabriel et al. (8) also proposed the name *X. campestris* pv.
aurantifolii for the group B, C, and D strains and the name *X.
campestris* pv. *citri* for the group E strains of *X.
campestris* pv. *citri*. Although pathogenic distinctions be-
tween group B, C, and D strains and group E strains were
noted, no collected descriptions of the disease syndromes,
host ranges, and details of pathogenicity methods were
presented.

*X. campestris* pv. *aurantifolii*. The proposal of Gabriel et al.
(8) to name *X. campestris* pv. *aurantifolii* is defective in
terms of the Standards for Naming Pathovars (4) for the
reasons given below.

(i) The proposed pathovar is not fully described in a way
that allows its allocation to a particular species [Standard
17(2)]. The only evidence that the strains are members of the
genus *Xanthomonas* is the demonstration that they produce
a pigment which may correspond to that produced by
*Xanthomonas* spp.

(ii) No report is made and no literature citation is given
which proves the distinctive pathogenicity of the identified
strains of this pathovar (Standard 5). Several accounts
describing the cancrosis group B pathogen have been pub-
lished. Gabriel et al. (8) do not give reasons for amalgamat-
ing these strains with group C and D strains in a single
pathogenic variant, nor do they present evidence that the
strains which they used are the same as those for which
pathogenicity data are reported elsewhere.

(iii) No pathotype strain was designated [Standards 17(3)
and 17(4)].
In the absence of a formal pathovar designation, the International Society for Plant Pathology Subcommittee on the Taxonomy of Plant Pathogenic Bacteria proposes that workers continue to refer to the group B, C, and D strains of X. campestris pv. citri until their taxonomic status is clarified.

X. campestris pv. citrumelo. The proposal to name X. campestris pv. citrumelo is defective in terms of the Standards for Naming Pathovars (4) for the reasons given below.

(i) The proposed pathovar is not fully described in a way that allows its allocation to a particular species (Standard 17(2)). The only evidence that the strains are members of the genus Xanthomonas is the demonstration that they produce a pigment which may correspond to that produced by Xanthomonas spp.

(ii) No report is made and no literature citation is given which proves the distinctive pathogenicity of this pathovar (Standard 5). A detailed description of methods of inoculation of a range of hosts, together with an account of the resulting reactions, should have been a part of the report. Gabriel et al. (8) repeatedly refer to the affinities in pathogenic reactions which strains of X. campestris pv. citrumelo have with strains of X. campestris pv. alfafae. On the basis of this report, X. campestris pv. citrumelo appears to encompass a collection of strains, some of which (group E2 strains) were not distinguished in pathogenic terms from X. campestris pv. alfafae. Gabriel et al. (8) based their pathovar names solely on the hosts from which the strains were isolated, citing Starr (27). However, Starr (27) provided no pathovar concept, making only incidental reference to the term. If strains from one host are indistinguishable in their pathogenic reactions from strains of an earlier named pathovar, then they take the name of that pathovar.

The work of Gabriel et al. (6, 8) indicates that there is a hitherto unreported complexity in the host range of strains of X. campestris that are pathogenic for Citrus spp. and bean, to which it is difficult to give expression by using the existing nomenclature. It could be concluded from their work that the group E strains of X. campestris pv. citri are members of X. campestris pv. alfafae and that the host range of this pathovar should be extended to include the citrus nursery strains. Such a step could wait for the confirmation of alfalfa (Medicago sativa L.) as an alternative host for this citrus pathogen in Florida and more detailed comparative studies of these pathogen.

An alternative proposal has been made by Hartung and Civerolo (11), who believe that the group E strains do not constitute an element of X. campestris pv. citri. These authors do not report the pathogenicity of their strains, but they propose that strains of this pathogen should be referred to as "X. campestris-citrus bacterial spot."

Further work is needed to establish the details of relationships among these xanthomonads in terms of DNA-DNA homology and phenetic data to enable formal reclassification to be considered. The International Society of Plant Pathology Subcommittee on the Taxonomy of Plant Pathogenic Bacteria believes that the existing pathovar designations, X. campestris pv. phaseoli and X. campestris pv. citri, along with the designations for groups of strains (groups A, B, C, D, and E), offer an interim nomenclatural compromise.

REFERENCES


