Taxonomic Note: Implementation of the Provisional Status *Candidatus* for Incompletely Described Procaryotes

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The International Committee on Systematic Bacteriology recommended that the category *Candidatus*, recently proposed by Murray and Schleifer (Int. J. Syst. Bacteriol. 44:174–176, 1994) to record the properties of putative taxa of procaryotes, should be implemented. This category should be used for describing procaryotic entities for which more than a mere sequence is available but for which characteristics required for description according to the *International Code of Nomenclature of Bacteria* are lacking. In addition to genomic information, such as sequences apt to determine the phylogenetic position of the organism, all information, including structural, metabolic, and reproductive features, should be included in the description of a provisional taxon, together with the natural environment in which the organism can be identified by in situ hybridization or other similar techniques for cell identification.

The International Committee on Systematic Bacteriology discussed the proposal of Murray and Schleifer (9) for a new procaryotic category, *Candidatus*, during their meeting in Prague, Czech Republic, in July 1994 (7). The members of this committee agreed that such a category would be useful and timely because of the increasing involvement of sequencing technology in the characterization of procaryotes that are difficult to cultivate and can only be described in limited terms. The International Committee on Systematic Bacteriology has stated previously that “the integrated use of phylogenetic and phenotypic characteristics, or polyphasic taxonomy, is necessary for the delineation of taxa at all levels from kingdom to genus” (8). We should be reminded in this context that the *International Code of Nomenclature of Bacteria* (12) permits the naming of taxa even for organisms that cannot be maintained in laboratories as pure cultures, provided that a description, preserved specimen, or illustration is provided as a type (rule 16a). Examples of this are *Prochloron didemni*, *Planctomyces bekefi*, and *Pasteuria penitans*, as well as *Buchnera* and *Holospira* species.

Although the *International Code of Nomenclature of Bacteria* allows systematists to include in a description taxonomic features that can be recognized only in situ, at the present time microbiologists are not encouraged to base a taxonomic description solely on a phylogenetic placement inferred from a comparison of nucleotide sequences. We feel that decisions concerning the roles of DNA and RNA data in taxonomy are important with respect to the evolution of taxonomic rules in response to progress in methods and increases in knowledge. We expect that some of what we know about the interactions and activities of bacteria will come from biota that have not been cultivated yet and in situ observations, and this information needs to be recorded. But it is cultivation that has allowed assessment and comparison among species of actual and potential capacities and has allowed us to understand what it is that evolution has brought about. After cultivation, more precise descriptions and a nomenclature that allows effective

communication become effective. In the meantime, as molecular biology settles into the fabric of bacteriology, various attitudes concerning the changes will be expressed (2, 5, 6).

Current wisdom suggests that a description of a potential new procaryotic genus and species would be inadequate for formal naming under the following circumstances: (i) when information comes exclusively from an analysis of clones generated from DNA that has been isolated directly from a natural sample; (ii) when the authenticity of the genetic material is verified in the environment by reamplification of DNA with sequence-specific PCR primers, but the presence of cells containing that DNA is not confirmed by microscopy or isolation; (iii) when the origin of genetic material from living cells is detected in a natural sample, but the authenticity of the bacterial cells in the host is not proven by in situ hybridization, as in the case of the agent of bacillary angiomatosis (10) and Whipple's disease bacillus (11); and (iv) when the origin of genetic material from living cells is detected in a natural sample by in situ hybridization, but information concerning properties other than phylogenetic position and morphology is lacking. The essence of this strategy, with a cloning step omitted, has been used in naming obligate intracellular symbionts, which are often small in number and size. As an unambiguous sequence is most likely obtained only in the absence of large numbers of contaminating cells, a single readable sequence may be indicative of the presence of a dominating population (3, 4).

The status *Candidatus* has been discussed as a possible taxonomic status for uncultured procaryotic cells for which relatedness has been determined (e.g., relatedness to the nearest neighbor) and authenticity has been verified by in situ probing or a similar technique for cell identification (additional studies will be necessary to investigate whether reamplification of a sequence determined with sequence-specific primers is sufficient proof of authenticity). In addition, it is mandatory that information concerning phenotypic properties, such as structural peculiarities and certain metabolic and physiological features, is made available as a starting point for further investigation and eventual description and naming. It is obvious that at the present time it is mainly symbionts and parasites of eucaryotic cells, as well as cultures obtained from special enrichment preparations, that are candidates for this provisional status because such organisms can be recognized by their molecular structures but cannot be assigned to a known

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TABLE 1. Items for inclusion in the codified record of a provisional taxon^a

Order of mention	Example responses
Status.....	<i>Candidatus</i>
Vernacular epithet.....	"another"
Phylogenetic lineage or possible genus	e.g., <i>δ-Proteobacteria</i> , possible (probable) <i>Desulfovibrio</i>
Cultivation	Cultivated or Not Cultivated
Gram reaction.....	G+, G-, Variable, or Not Applicable
Morphology.....	Rod, Coccus, Filamentous, Mycolial, Other, Unknown
Basis of assignment	Nucleid Acid Sequence (data bank #), Morphology, etc.
Specific identification of morphotype	Probe identity
Habitat, association, or host	Symbiotic (name host and tissue), Free-Living (sea, etc.), etc.
Metabolism and unusual features	Aer., Anaer., Microaer., etc.
Growth temperature.....	Meso-, Psycho-, Thermophilic
Source	From ileal cells of pigs
Author(s)	Essential reference

^a Modified from reference 9.

genus because of the lack of enough distinguishing characteristics. Formal recognition will come when new observations allow; hence, the designation *Candidatus*, which is not a rank but a status that is not formally recognized in the *International Code of Nomenclature of Bacteria* (5).

The previous proposal (9) included a format for condensed descriptions that will be appropriate, when the time comes, for insertion into a computer-linked database. There is a defined order of presentation for recording a vernacular name, the basis of the characterization (i.e., the discovery), and the discoverers. The order and appropriate abbreviations were given previously and are shown in Table 1. It should be noted that sequences of various macromolecules could be used, but the 16S rRNA sequence should be mandatory and should contain at least 1,000 bases. Listed in the text by Murray and Schleifer (9) but not included in the table of codified items was identification of a morphotype with a specific probe (probe identity) inferred from a characteristic sequence of the putative taxon by in situ probing or other similar techniques for cell identification. Undoubtedly, contentious discussion will focus on whether amplification of DNA or RNA with oligonucleotide primers assumed to be specific for the organisms being targeted will allow unambiguous characterization of potential *Candidatus* status organisms.

The increasing application of molecular methods to the assessment of diversity of procaryotic populations in nature and to the study of complex symbioses (1) makes it likely that numerous *Candidatus* examples will be recorded. At this time the number of such organisms is small, as the list below attests, and it seems appropriate to record them in lists appearing in the *International Journal of Systematic Bacteriology* when they are presented to the editor in suitable form; each submission should include a reprint to back it up.

***Candidatus* list.** Below is a list of sequence-based potential taxa with properties suitable for recording under the designation *Candidatus*. The abbreviated format described in the original proposal (9) is used, and an appropriate trivial or vernacular name is provided, along with information concerning the original publication. The provisional status *Candidatus*

is intended to record discoveries and to provide a basis for recognizing putative taxa that are equivalent to genus or higher rank.

Workers who have described a bacterial *Candidatus* taxon should submit to the *International Journal of Systematic Bacteriology* a reprint along with an entry in the appropriate format and a cover letter. Lists will appear at appropriate intervals (annually at the outset).

"Candidatus intracellularis" [(*δ-Proteobacteria*) NC; G-; R; NAS (GenBank number L15739), oligonucleotide sequence complementary to unique region of 16S rRNA 5'-GTCTTGAGGCTCCCCGAAAGGCACCTC TTAATC-3'; S (*Sus*, ileum); MJ. Gebhart et al., *Int. J. Syst. Bacteriol.* **43**:533, 1993.

"Candidatus magnetobacterium" [(new subclass of *Proteobacteria* or new lineage) NC; G-; R; NAS (EMBL number X71838), oligonucleotide sequence complementary to unique region of 16S rRNA 5'-GCCATCCCTC GCTTACT-3'; FL (freshwater lake sediment); microaer., magnetosomes, sulfur inclusions; MJ. Spring et al., *Appl. Environ. Microbiol.* **59**:2397, 1993.

"Candidatus Liberobacter asiaticum" [(*α-Proteobacteria*) NC; G-; F; NAS (GenBank number L22532), oligonucleotide sequence complementary to unique region of 16S rRNA 5'-GCGCGTATGCAATACGAGCGGCA-3'; S (*Citrus*, phloem; *Diphorina citri* (Psyllidae), hemolymph, salivary glands); MJ. Jagoueix et al., *Int. J. Syst. Bacteriol.* **44**:386, 1994.

"Candidatus Liberobacter africanum" [(*α-Proteobacteria*) NC; G-; F; NAS (GenBank number L22533), oligonucleotide sequence complementary to unique region of 16S rRNA 5'-GCGCGTATTTTATACGAG CGGCA-3'; S (*Citrus*, phloem; *Trioza erytreae* (Psyllidae), hemolymph, salivary glands); MJ. Jagoueix et al., *Int. J. Syst. Bacteriol.* **44**:386, 1994.

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REFERENCES

1. Amann, R., W. Ludwig, and K. H. Schleifer. 1994. Identification of uncultured bacteria: a challenging task for molecular taxonomists. *ASM News* **60**:360-365.
2. Caldwell, D. E. 1994. End of pure culture era? *ASM News* **60**:360.
3. Distel, D. L., and A. P. Wood. 1992. Characterization of the gill symbiont of *Thyasira flexuosa* (Thyasiridae: Bivalvia) by use of polymerase chain reaction and 16S rRNA sequence analysis. *J. Bacteriol.* **174**:6317-6320.
4. Finlay, B. J., T. M. Embley, and T. Fenchel. 1993. A new polymorphic methanogen, closely related to *Methanococcus parvum*, living in stable symbiosis within the anaerobe ciliate *Trimyxa* sp. *J. Gen. Microbiol.* **139**:371-378.
5. Guest, H. 1993. Bacterial growth and reproduction in nature and in the laboratory. *ASM News* **59**:542.
6. Guest, H. 1993. More on uncultivated bacteria. *ASM News* **60**:403.
7. Judicial Commission of the IJSB. 1995. Minutes of the meetings, 2 and 6 July 1994, Prague, Czech Republic. *Int. J. Syst. Bacteriol.* **45**:195-196.
8. Murray, R. G. E., D. J. Brenner, R. R. Colwell, P. de Vos, M. Goodfellow, P. A. D. Grimont, N. Pfennig, E. Stackebrandt, and G. A. Zarvarzin. 1990. Report of the Ad Hoc Committee on Approaches to Taxonomy within the *Proteobacteria*. *Int. J. Syst. Bacteriol.* **40**:213-215.
9. Murray, R. G. E., and K. H. Schleifer. 1994. Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. *Int. J. Syst. Bacteriol.* **44**:174-176.
10. Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tomkins. 1990. The agent of bacillary angiomatosis. *N. Engl. J. Med.* **323**:1573-1580.
11. Relman, D. A., T. M. Schmidt, R. P. MacDermott, and S. Falkow. 1992. Identification of the uncultured bacillus of Whipple's disease. *N. Engl. J. Med.* **327**:293-301.
12. Sneath, P. H. A. (ed.) 1992. International code of nomenclature of bacteria. 1990 Revision. American Society for Microbiology, Washington, D.C.