Proposed Criteria for an Interim Serogroup Classification for Members of the Genus Spiroplasma (Class Mollicutes)†

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Criteria for description of new groups of the serogroup classification scheme for the Spiroplasma genus are proposed. New groups, if shown to be referable to the Mollicutes class and Spiroplasmataceae family, should be shown to differ from established groups by (i) reciprocal deformation tests and (ii) one of three other serologic tests (growth inhibition, metabolism inhibition, or enzyme-linked immunosorbent assay). Also, the ability or inability to utilize glucose, arginine, and urea should be determined. Determination of guanine-plus-cytosine content is strongly recommended but optional.

The genus Spiroplasma (class Mollicutes) was established in 1973 when the causal agent of citrus stubborn disease (Spiroplasma citri) was characterized (18) as the first genus and species of a new family (21) of helical, wall-less procaryotes. In the following decade, many new Spiroplasma species were recovered from flower surfaces and the hemolymph or gut fluids of a wide variety of insects and other arthropods (5). As with other members of the class Mollicutes, serologic techniques were first used to classify the Spiroplasma genus (29, 30). However, other molecular and genetic techniques were soon applied to this group of microorganisms, including analysis of guanine-plus-cytosine content, genome size of the deoxyribonucleic acid (DNA), DNA-DNA hybridization, DNA restriction endonuclease patterns, and polyacrylamide gel electrophoretic patterns of cell proteins (3). Results from molecular, genetic, and serologic analyses were complementary and consistent. Each technique indicated the existence of groups that differed in guanine-plus-cytosine content, had negligible genome size, and exhibited unique DNA-DNA homology, and exhibited unique polyacrylamide gel electrophoretic patterns (14, 15). It thus appeared that the genus contained species other than the type, Spiroplasma citri. On the other hand, a certain number of strains isolated from plant or arthropod sources showed partial serologic or molecular interrelationships with S. citri (2).

These observations led to a concept of Spiroplasma classification based upon both serologic and molecular genetic data. In the first scheme, proposed in 1980 (14), five groups (I to V) and four subgroups (I-1 to I-4) were defined. This proposal gained general acceptance and has been subsequently revised (26, 27) to accommodate discovery of new strains. The current classification recognizes 11 groups; group I now contains eight subgroups, including the recently added subgroup I-8 (20). Three of the groups (III, IV, and V) have been given species designations (Spiroplasma floridica, Spiroplasma apis, and Spiroplasma mirum, respectively).

Initially, the subcommittee recommended that elevation of the group I subgroups (other than the type species, S. citri) to species status should be postponed until more information on subgroup interrelationships became available (10). In 1982, however, the subcommittee proposed (11) that subgroups could be elevated to species under certain conditions. The most important requirement was that the level of DNA-DNA homology between the candidate subgroup and all other subgroups was less than 70%. Strains of subgroups I-2 and I-3 met the recommended conditions and have been characterized recently as Spiroplasma melliferum (6) and Spiroplasma kunkelii (25), respectively.

Within the past few years, Spiroplasma strains that represent 13 additional groups have been isolated, primarily from insects (C. Chastel, D. Devaux, F. Le Goff, A. M. Simitzis-Le Flohic, R. Ruffaz, G. Kerdraon, and B. Gilot, Isr. J. Med. Sci., in press; T. B. Clark, R. B. Henegar, L. Rosen, K. J. Hackett, R. F. Whitcomb, J. E. Lowry, C. Saillard, J. M. Bové, J. G. Tully, and D. L. Williamson, Isr. J. Med. Sci., in press). At its interim meeting in Jerusalem, Israel, in June 1984, the subcommittee acknowledged (12) that the currently informal serogroup system of classification had a number of obvious advantages for the genus Spiroplasma. Although workers were encouraged to assign Latin binary combinations to properly characterized Spiroplasma species, it was recognized that the large number of emerging (and putative) species might delay or indefinitely postpone development of a useful classification system for these organisms. An ad hoc committee was therefore appointed by the subcommittee to develop a series of recommendations for standard criteria for a serogroup designation for these organisms. The subcommittee emphasized that this scheme would serve only as an interim system in lieu of established specific epithets.

The ad hoc committee convened on 11 June 1985 and developed the following recommendations.

1. Cloning of the organism, by standard techniques (9, 23), should constitute the first step in designation of a new Spiroplasma serogroup.
2. It must be demonstrated that the organism is a member of the class Mollicutes (8, 17). This requires (i) demonstration (9) that the organism has an absolute susceptibility

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to penicillin, and (ii) demonstration, by thin-section electron microscopic techniques (7), that the helical cells are bounded by a single limiting cytoplasmic membrane and are devoid of a cell wall and periplasmic fibrils. These latter morphologic features would be observed with spirochetes (13) that might be cultivated on artificial media and confused with a spiroplasma.

3. It must be demonstrated that the organism is referable to the genus *Spiroplasma* by (i) examination of a broth culture of the organism by dark-field microscopy (22) to confirm that helicity and motility are present in at least some stage of the growth cycle or cultural history and (ii) demonstration of the ability of the organism to utilize glucose (16) and its ability (or inability) to utilize (a) arginine (1) and (b) urea (9). In conjunction with experiments demonstrating the status as a member of the *Mollicutes*, this morphologic and biochemical evidence establishes that the organism is a member of the genus *Spiroplasma* and the family *Spiroplasmataceae*.

4. Establishment of a new *Spiroplasma* serogroup requires fulfillment of the following additional criteria. (i) The organism must be shown to be serologically distinct from all other previously established serogroups or species in the genus *Spiroplasma* by a reciprocal deformation (DF) test (30-32) and by at least one of three other serologic tests: growth inhibition (GI) (28), metabolism inhibition (MI) (30), or enzyme-linked immunosorbent assay (ELISA) (19) tests. If the candidate organism shows, in any of the serologic tests selected, evidence of moderate sharing of serologic properties with a member of any other established serogroup or subgroup (for example, a DF titer of 1:40, an MI titer of 1:160, or a GI zone of 2 mm), the organism should not be described as a new serogroup or subgroup. To properly classify such an organism, antiserum must be prepared to the candidate strain, and reciprocal DF, MI, and GI tests must be performed between the candidate organism and the strain with which it shows partial crossing. Such tests could show (i) that the candidate organism is either a previously unrecognized subgroup (such as those in group I [2, 14, 27]) or a closely related serovar of the existing serogroup that does not merit recognition at the subgroup level (such as group IV strains [24]) or (ii) that the appearance of partial relationship seen in a given serologic test is exhibited only as one-way crosses or is not supported by results from other serologic tests. In the latter case, designation of a new serogroup may be justified.

5. The determination of guanine-plus-cytosine content of the DNA of the organism (4) is optional and may be postponed if the authors eventually wish to publish a taxonomic description and give a species designation to the organism. If there is no intention to provide a more extensive taxonomic description, the committee strongly recommends that this optional procedure be completed.

6. The candidate spiroplasma should be deposited in a recognized national culture collection and made available for circulation to other workers.

7. Characterization of *Spiroplasma* species will continue to be determined by the minimal standards for description of new *Mollicutes* species previously recommended by the subcommittee (9).

LITERATURE CITED


