Characterization and Taxonomic Status of Tick Spiroplasmas: A Review

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Three serologically distinct groups of spiroplasmas have been recovered from ticks. *Spiroplasma mirum* strains (from rabbit ticks, *Haemaphysalis leporispalustris*) and Y32 group (VI) spiroplasmas (from *Ixodes pacificus*) are the only spiroplasmas to have a clear association with these arthropods. Group (VI) spiroplasmas are distinguished by an unusual nonhelical morphology and their capacity to hemadsorb guinea pig erythrocytes. *S. mirum* strains are unique in their ability to induce cataracts or lethal brain infections in a number of young vertebrates and in their virulence for the chick embryo. The 277F spiroplasma, while initially recovered from a pool of rabbit ticks (*H. leporispalustris*), is related by certain serological and genetic properties to spiroplasmas in the *S. citri* complex (serogroup I). These relationships suggest that the 277F spiroplasma may not be a natural inhabitant of the rabbit tick.

INTRODUCTION

Spiroplasmas are a cluster of helical, wall-free prokaryotes (mycoplasmas) recovered from a variety of plants and insects. The discovery of spiroplasmas in ticks added an important habitat to the known range of these organisms. The pathogenicity of at least one tick spiroplasma for vertebrates has stimulated exploration of possible tick-spiroplasma-man relationships. However, only limited information is currently available on the natural diversity, prevalence, host range, distribution, and potential pathogenicity for vertebrates of tick-associated spiroplasmas. The recent development of several laboratory techniques for the direct cultivation and recovery of these organisms from ticks has provided an effective means for studying some of the relevant issues in tick-spiroplasma interactions.

Host Range of Established Tick Spiroplasmas

The occurrence of spiroplasmas in ticks was first noted in 1976, when an organism previously described as a virus was shown to possess a helical structure and to have

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other attributes of spiroplasmas [1]. This organism, the suckling mouse cataract agent (SMCA), was initially recovered by the inoculation of embryoated chicken eggs with pooled extracts of rabbit ticks (Haemaphysalis leporispalustris) collected in Georgia (U.S.A.) in 1964 [2,3]. Subsequent studies [4] showed that two strains of the organism (SMCA and GT-48) could be grown on a newly developed culture medium (SP-4), and the cultivated organisms produced the same lethal infection and/or cataracts in rats as that produced by isolates grown only in chick embryos. Serological characterization of the SMCA and GT-48 strains indicated that they constituted a new serological group (V) distinct from all other plant and insect spiroplasmas [4–6]. A third strain (TP-2) of this group of spiroplasmas was recently isolated directly in the SP-4 culture medium from H. leporispalustris ticks collected in Maryland [7]. The three strains in the serogroup V cluster were characterized in more detail and eventually named Spiroplasma mirum [8].

A second, serologically distinct, spiroplasma (277F) also was apparently isolated from rabbit ticks. As with the SMCA isolate, this organism was also confused with another microbe. The 277F agent was first recovered in culture medium inoculated with extracts of rabbit ticks collected in Montana in 1968, and was described as a spirochete [9]. Following reports of the occurrence of spiroplasmas in a variety of insects and in other rabbit ticks, Brinton and Burgdorfer reexamined the morphology and ultrastructure of the 277F agent and confirmed that the organism possessed features characteristic of spiroplasmas [10]. Serological analysis of the 277F spiroplasma showed that while it was distinct from the SMCA group, it shared some serological properties with several plant and insect spiroplasmas assigned to the S. citri complex [6,11,12]. It was proposed that this spiroplasma be designated subgroup 4 in serogroup I [12,13]. The relationship of 277F to the plant-insect spiroplasmas in serogroup I suggests that this spiroplasma may not be of arthropod origin. However, until other spiroplasmas with the serological and genetic properties of 277F are recovered, its true host origin cannot be ascertained.

More recently, at least eight spiroplasma strains have been recovered from extracts of Ixodes pacificus ticks collected in Oregon [14]. These strains, which have some unique morphological features, were recovered directly in SP-4 culture medium after incubation of tick extracts for periods of 20 to 45 days. Isolations were made from both male and female ticks, but only ticks examined between March and May were found to carry spiroplasmas. Serological characterization of the eight strains indicated that they were all closely related, but serologically distinct from other groups of spiroplasmas. They were proposed as representing a new serogroup (VI) [13,14].

Limited cultivation attempts, using the SP-4 medium, have been performed on other ticks [Yunker CE, Tully JG: unpublished; Anderson JF, Tully JG: unpublished], including the following Ixodidae (hard ticks) (numbers in parentheses indicate number of ticks sampled): Ixodes dammini (663), I. spinipalpis (65), H. leporispalustris (188 +), Dermacentor andersoni (277), D. occidentalis (630), and D. variabilis (247). Several Argasidae (soft ticks) were also tested: Argas cooleyi (179), Ornithodoros concanensis (440), and O. rostratum (30). Although spiroplasmas were not isolated in these latter trials, the pools mostly contained large numbers of ticks which were collected at one time of the year. A more systematic approach, in which culture attempts could be made on small tick pools collected from individual species over weekly or monthly intervals, might yield important data on the timing of acquisition of spiroplasmas and host identity.
**Morphology of Tick Spiroplasmas**

The morphology of *S. mirum* strains and the 277F spiroplasma is similar to that of most other spiroplasmas. Short helical forms predominate in young broth cultures and the helices increase in length as the culture reaches the logarithmic phase of growth. There is much less tendency for organisms in these two groups to revert to spherical cells in the stationary growth phase, as is observed, for example, with *S. floricola*. As the cultures age, the cells may lose some of their helicity, and these partly helical filamentous forms may tend to clump in large masses [8]. Group (VI) *Ixodes* spiroplasmas, on the other hand, tend to occur predominantly as nonhelical filaments [14]. A logarithmic phase broth culture of representative strains (Y32, for example) examined by dark field microscopy shows characteristically large numbers of nonhelical filaments, many of which are in clumps. Individual filaments, whether free in broth or attached to the periphery of the clumps, display extensive flexional movement. The occasional group (VI) organism that is helical behaves as do other spiroplasmas, exhibiting rapid rotary motility and flexional movements. Examination of Y32 spiroplasmas by negative staining techniques and electron microscopy showed that some of the short, apparently nonhelical forms observed in dark field microscopy were actually very tightly coiled helical organisms [14]. However, this ultrastructural study confirmed that all eight strains recovered from *Ixodes pacificus* ticks were predominately nonhelical, wall-less organisms.

**Cultivation and Biological Characteristics of Tick Spiroplasmas**

The SP-4 formulation [4,14,15], which is essentially composed of a protein base, yeast extract, fetal bovine serum, and the 1066 tissue culture supplement, is the most useful maintenance medium for organisms in the three tick spiroplasma groups. This medium has been shown to be successful in primary isolation of both *S. mirum* strains and the Y32 spiroplasmas. Primary isolation of the 277F spiroplasma occurred in a medium prepared from chick embryo fluids, amino acids from casein, albumin, and so on [9]. Since only a single isolate of this group has been recovered, the value of SP-4 (or other spiroplasma media) to support primary isolation is not known. Most of the strains in the three groups also grow well in M1A medium [15], but this medium has not been used for primary isolation attempts. Much work remains to be done to define the essential growth factors in SP-4 medium for tick spiroplasmas. Studies on *S. mirum*, however, did establish the critical nature of the 1066 tissue culture supplement in primary recovery of the SMCA strain from chick embryo fluids [8]. Similarly, the Y32 spiroplasma appears to vary in its growth response to different commercial lots of fetal bovine serum and to alterations in the combination of fresh yeast extract and yeastolate in complete SP-4 medium [16]. More accurate definition of the essential growth factors in the 1066 medium for both SMCA and Y32 spiroplasmas might enable the cultivation of other tick spiroplasmas and, perhaps, some new fastidious mycoplasmas.

Aside from serological differences between the three tick spiroplasma groups, few biological or biochemical distinctions have been noted. The ability of Y32 colonies to hemadsorb guinea pig erythrocytes [16] is a most unusual characteristic, which not only distinguishes Y32 from other tick spiroplasmas, but also from other plant and arthropod spiroplasmas. Different guanine + cytosine (G + C) values of spiroplasma DNA have been reported for *S. mirum* (G + C of 29–31 moles %) [12] and the 277F spiroplasma (G + C of 26–27 moles %) [12]. The G + C value for the Y32 spiroplasma has not been determined.
Finally, some comment on the sterol requirements of tick spiroplasmas is relevant to biological characterization of these organisms and may be useful for cholesterol tests with other fastidious spiroplasmas. The SMCA and Y32 spiroplasmas grew so poorly on media formulations with limited sterol, such as M1B medium [16] (contains 5 percent bovine serum fraction in place of fetal bovine serum), that we could not perform the standard mycoplasma cholesterol test [17]. The test was therefore modified to examine the growth response (in color-changing units/ml) when spiroplasmas were added to SP-4 base medium (without fetal bovine serum), or to SP-4 base medium supplemented with various concentrations of either cholesterol or fetal bovine serum. The results with *S. mirum* [8] indicated that quantities of 1 µg/ml cholesterol or 10 percent fetal bovine serum greatly promoted the growth of SMCA in comparison to that observed in the serum-free SP-4 base medium. The Y32 strain exhibited somewhat similar results, with quantities of 10–20 µg/ml cholesterol or 10–20 percent serum stimulating increased growth of the organisms.

**Serology of Tick Spiroplasmas**

As noted above, serological distinctions among the tick spiroplasmas are among the most useful characteristics for clearly separating these agents. Several serological techniques have been applied to these and other spiroplasmas, including, in order of increasing sensitivity, growth inhibition [8,18], deformation [5], and metabolism inhibition [6,19]. A summary of recent metabolism inhibition tests with representative strains is presented in Table 1.

**Pathogenicity Tests with Tick Spiroplasmas**

Finally, pathogenicity tests performed in embryonated chicken eggs and in suckling rats are useful in making distinctions among tick spiroplasma groups. Only *S. mirum* strains have been shown to be virulent for chick embryos [1–4,7] and to in-

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**TABLE 1**

Metabolism Inhibition Tests with Tick Spiroplasmas

| Antiserum (strain designations) | Serogroup | Spiroplasma Antigens |
|-------------------------------|-----------|----------------------|
|                              |           | 277F | SMCA | Y32* |
| *S. citri* (Maroc)            | I-1       | N*   | N    | N    |
| Honeybee (BC3)                | I-2       | N    | N    | N    |
| Corn stunt (E275)             | I-3       | 4,374<sup>c</sup> | N    | N    |
| Tick (277F)                   | I-4       | 39,000 | N    | N    |
| Green leaf bug (LB-12)        | I-5       | 54   | N    | N    |
| Coconut palm (N525)           | I-6       | 162  | N    | N    |
| Flower (M55)                  | I-7       | 162  | N    | N    |
| Sex ratio organism (WSRO)     | II        | N    | N    | N    |
| *S. floricola* (OBMG)         | III       | N    | N    | N    |
| Bee-flower (B31)              | IV        | N    | N    | N    |
| Tick *S. mirum* (SMCA)        | V         | N    | 13,000 | N    |
| Tick (Y32)                    | VI        | N    | N    | 4,374<sup>c</sup> |
| Wasp (*Monobia*) (MQ-1)       | VII       | N    | N    | N    |
| Syrphid fly (*Eristalis*) (EA-1) | VIII   | N    | N    | N    |
| Beetle (*Cotinus*) (CN-5)     | IX        | N    | N    | N    |

*Tests performed without guinea pig complement.
*<sup>a</sup>MI titer less than 1:18.
*<sup>c</sup>Reciprocal of antiserum dilution inhibiting metabolism.
duce cataracts or lethal brain infections when introduced intracerebrally into suckling rats [1–4,7,20,21], suckling rabbits [22,23], or suckling hamsters [24].

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