Phylogenetic Evidence for Two New Insect-Associated Chlamydia of the Family Simkaniaceae

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Abstract. On the basis of 16S–23S ribosomal DNA analyses, the whitefly Bemisia tabaci (Sternorrhyncha, Aleyrodidae) and the eriococcid Eriococcus spurius (Sternorrhyncha, Eriococcidae) were each found to harbor novel related chlamydial species within the family Simkaniaceae. The generic designation Fritschea gen. nov. is proposed to accommodate the two species, F. bemisiae sp. nov. and F. eriococci sp. nov. The finding of chlamydial 16S–23S ribosomal DNA in B. tabaci is consistent with a previous electron microscopy study which found that bacteriocytes of this species contain structures that we consider to resemble the elementary and reticulate bodies of chlamydia (Costa HS, Westcot DM, Ullman DE, Rosell R, Brown JK, Johnson MW. Protoplasma 189:194–202, 1995). The cloning and sequencing of a 16.6 kilobase DNA fragment from F. bemisiae indicated that it contains six genes encoding for proteins similar to those found in other species of chlamydia. These results extend the range of organisms that harbor chlamydia.

The chlamydia are obligate intracellular, prokaryotic parasites which exist in two morphologically distinct stages designated as the reticulate body (RB) and the elementary body (EB) [14, 19]. The RB is the reproductive intracellular stage that resembles in its morphology Gram-negative bacteria. The accumulation of RBs within the cell is followed by their differentiation into EBs, metabolically inert, readily recognizable, electron-dense structures that are extruded from cells and cause the spread of the infection [14, 19]. Classically the chlamydia were thought to be agents of diseases of humans and animals [2]. Recently they have been found in amoebae, as a tissue culture contaminant, and in environmental samples [1, 8–12, 15]. Analyses of the 16S and 23S ribosomal DNA (rDNA) sequences of these organisms have shown that they are quite diverse [1, 6, 8, 10–12, 16]. On the basis of these molecular studies and other criteria, the taxonomy of chlamydia has recently been formalized [6]. Currently the order Chlamydiales contains four families (Fig. 3); the previously recognized human and animal pathogens are within the family Chlamydiales [6]. It has been suggested that chlamydia from other families may also be involved in human disease [1, 8, 12].

We have recently initiated a study of whitefly endosymbions. These organisms are housed within specialized cells, called bacteriocytes, that contain primary endosymbionts and secondary endosymbionts [3, 5]. Costa et al. [5] have found that Bemisia tabaci (biotype A) contains, besides these two endosymbionts, structures that they called “globular bodies” and another endosymbiont that they designated as the “C2” type. Both the “globular bodies” and the C2 endosymbiont were absent in the closely related Bemisia argentifolii (biotype B) [5]. Our reexamination of their work suggested that the “globular bodies” are chlamydial EBs, whereas the C2 endosymbionts are chlamydial RBs. In this study we have characterized chlamydial DNA fragments from Bemisia tabaci (Sternorrhyncha, Aleyrodidae) and Eriococcus spurius (Sternorrhyncha, Eriococcidae) and show that the chlamydia from these two insect species constitute two new species within a new genus of the family Simkaniaceae [6].
Materials and Methods

General methods. The methods used in these studies have been described in our past publications [4, 17, 18]. These include methods for total insect DNA purification, polymerase chain reaction (PCR) amplification of 16S–23S rDNA fragments and their cloning into pBluescript (Stratagene, La Jolla, CA), restriction enzyme and Southern blot analysis, electroelution of DNA fragments from agarose gels and their cloning into λZAP (Stratagene). The methods of phylogenetic analyses are the same as previously used [17, 18].

Source of insects. The Bemisia tabaci (biotype A) were reared in the laboratory of B. W. Falk. Eriococcus spurius was collected by P. J. Gullan from an elm tree in Davis, CA, in June 2001. The source of the remaining insects is described in our previous publications [17, 18].

Presence of chlamydial tree sequences. Primers U23F and 23SIGR, specific for the detection of chlamydial 23S rDNA [7], were used in PCR reaction mixtures (10 µl containing 150 nM Tricine buffer (pH9.1), 3 mM MgCl₂, 0.1 mM dNTPs, 5 pmol primer, 2 µg RNAse, 1 µg bovine serum albumin, 0.5 U Mastertaq (Eppendorf, Westbury, NY) and 20 ng of total insect DNA. The following conditions were used: initial denaturation at 95°C, 5 min; subsequent denaturations at 94°C, 30 s; annealing at 64°C, 30 s; elongation at 72°C, 45 s; total of 30 cycles followed by incubation at 72°C for 5 min. Besides the species listed in the legend to Fig. 1, the following additional insects were screened for the presence of chlamydial 23S rDNA sequences: whiteflies (Acantaltheryodes styraci, Aleurochiton aceris, Aleyrodes elevatus, A. proletella, Aleuropanaxus arctostaphylii, Aleyroplatus gelatinosus, Aleyroplatus sp., Dialeurodes hongkongensis, Siphomonas phillyreae, Trialeurodes acaciae, Trialeurodes hutchings, Vassavius concursus); psylids (Bactericera cockerelli, Calophya schini, Heteropsylla texana); and scale insects (Ceroplastes circipidiformis, Coccus hesperidum, Diaspis echinocaci, Eriococcus adenostomae Ferrisia sp., Icerya purchasi, Kuwania quercus, Melanococcus albizziae, Orthezia artemisiae, Pianococcus citri, Philephedra aphidae).

16S–23S rDNA fragments. Oligonucleotide primers 5'-GAG TTT GAT CAT GGC TCA GAT TG-3' and 5'-GCT CGC GTA CCA CTT TAA ATG GGC-3' were used in PCR reactions to obtain the 5.2-kb and 4.5-kb 16S–23S chlamydial rDNA fragments from the DNA of B. tabaci (Fig. 1A) and E. spurius (Fig. 1B), respectively.

GenBank accession numbers. The accession numbers for the 16.6-kilobase (kb) DNA fragment of Fig. 2A is AY140910, and the 4.5-kb DNA fragment from Fig. 2B is AY140911.

Results and Discussion

Survey for chlamydial 23S rDNA sequences. We screened DNAs from 16 whiteflies, 5 mealybugs, 5 psylids, 7 coccids, and 2 eriococci for chlamydial 23S rDNA sequences. Only Bemisia tabaci (biotype A) and Eriococcus spurius gave the expected 0.6-kb amplified DNA fragment (Fig. 1). Bemisia argentifolii (biotype B), an organism closely related to B. tabaci, did not have chlamydial sequences. This result is consistent with the electron microscope study of Costa et al. [5]. These investigators found that B. tabaci had in its bacteriocyte structures that we consider to resemble chlamydial EBs and RBs, while B. argentifolii lacked these structures [5].

Fig. 1. Results of a PCR screening for the presence of chlamydial sequences in DNA samples of various plant sap-sucking insects. Lanes 1 and 11, molecular size standards (1.33, 0.81, 0.46, and 0.24 kb): Whiteflies; lane 2, Bemisia tabaci; lane 3, B. argentifolii; lane 4, Neomaskelia andropogonis; lane 5, Tetraeurodes movi; Psylids: lane 6, Glycaspis brimblecombei; lane 7, Pachysyssa venusta. Mealybugs: lane 8, Dysmicoccus brevipes; lane 9, Macconellicoccus hirsutus. Eriococcids: lane 10, Eriococcus spurius. For additional insect species tested, see text.

Properties of the cloned DNA fragments. Figure 2A is a genetic map of the 16.6-kb DNA fragment from the chlamydia of B. tabaci. The guanine plus cytosine (G + C) content of the fragment is 40.0 moles %. If the rRNA genes are removed, the G + C content of the remaining DNA is 38.0 moles %. The coding regions presented in Fig. 2 and Table 1 constitute 76% of the DNA fragment. The G + C contents of the fully sequenced genomes of Chlamydia pneumoniae, C. trachomatis, and C. muridarum range from 40.3 to 41.3 moles %, similar to that of the DNA fragment from the chlamydia of B. tabaci.

In total, nine open reading frames (ORFs) were identified (Fig. 2A); six of these had homologs in the genomes of the three sequenced chlamydial species (Table 1) (GenBank AE001273, AE001363, AE002160). ORF-C and yjeE were adjacent in all of these chlamydial species. ORFs A, B, and D did not have homologs in the chlamydia data bases but had homologs in other organisms (amino acid sequence identity of 37–53%) (Table 1). This is not surprising since previous studies have indicated that the chlamydia are hybrid organisms containing genes from many different sources [13].

The G + C contents of the rDNA genes of chlamydia from B. tabaci and E. spurius were 50.1, 50.1% (16S rDNA) and 47.0, 47.6% (23S rDNA, without the intron), respectively. Simkania negevensis, the chlamydial tissue culture contaminant that may be involved in human disease [12], had the unusual property of having a 658-base pair (bp) Group I intron in the 23S rDNA [6]. The chlamydia from B. tabaci resembles this organism