NOTE

***Mycoplasma alligatoris* sp. nov., from American alligators**

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Mycoplasmas were isolated from multiple tissues of diseased American alligators (*Alligator mississippiensis*). This paper presents biochemical, serological and molecular genetic characterizations of a lethal pathogen of alligators for which the name *Mycoplasma alligatoris* sp. nov. is proposed. The type strain is A21JP2^T (ATCC 700619^T).

Keywords: mycoplasma, alligator, crocodilian, reptile

An epizootic of pneumonia with polyserositis and multifocal arthritis emerged in captive alligators in Florida, USA, in 1995 (Brown *et al.*, 1996). Mycoplasmas were cultured from multiple organs, peripheral blood, synovial fluid and cerebrospinal fluid of affected alligators. Nine isolates cloned from single colonies obtained from different tissues of six animals appeared to be identical as determined by growth characteristics and biochemical tests. In this paper strain A21JP2^T is fully characterized and compared with previously described mollicutes.

Cultivation of mycoplasmas

The mycoplasmas characterized in this paper were isolated from tracheal swabs, minced lung, limb joint synovial fluid or cerebrospinal fluid of alligators that died during the 1995 epizootic. Primary isolates were cultured at 30 °C in an atmosphere containing 5% CO₂ on ATCC medium 988 (Tully, 1995) agar with 10⁵ U penicillin G l⁻¹, 10⁵ U polymyxin B l⁻¹, 65 mg cefoperazone l⁻¹ and 20% (v/v) fetal bovine serum, or in ambient air in ATCC medium 988 broth with those supplements. Nine isolates (A21CSFP3, A21JP2^T, A21JP3, A22JP2–1A, N95–701LPA2N, N95–713LP3, N95–713TP3, N95–714LPA1N and N95–740LP1)

cloned from single colonies obtained from different tissues of six animals were characterized. To determine the temperature range for growth and reproduction, the isolates were incubated in 10-fold serial dilutions of broth at 4, 22, 25, 30, 34, 37 and 42 °C. Anaerobic growth on agar at 30 and 37 °C was assessed by using the GasPak system (Becton Dickinson). Primary isolates grew extremely rapidly for mycoplasmas, reaching mid-exponential growth phase as indicated by acidification of the medium within 8 h at 30 °C. Optimum growth occurred at temperatures of 30 and 34 °C. The organisms grew slowly at 22 and 25 °C, and did not grow at 4, 37 or 42 °C. The organisms grew well under anaerobic conditions at 30 °C but did not grow anaerobically at 37 °C.

Colonies of the organisms on agar were examined at up to ×100 magnification by using a compound microscope. The cellular morphology of the organisms was assessed by Gram stain (Barile, 1983b) at ×1000 magnification and by transmission electron microscopy of tissues from infected alligators. Colonies were easily visible on agar under a dissecting microscope within 24 h of incubation at 30 °C and had a typical fried-egg appearance at ×20 magnification (Fig. 1). Gram staining showed faintly stained minute pleomorphic bodies. Ultrathin sections of infected alligator tissues showed that the mycoplasmas lacked a cell wall and were non-helical (Fig. 2).

Filtration studies

Cultures were diluted 1:10 in broth medium and then passed sequentially through membrane filters (Gelman) with pore diameters of 800, 450 and 200 nm.

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The GenBank accession number for the 16S rRNA gene sequence of strain A21JP2^T is U56733.

Fig. 1. Typical colony morphology of A21JP2^T after 48 h growth on ATCC medium 988 at 30 °C in an atmosphere containing 5 % CO₂. Typical colony diameter, 200–300 µm.

Fig. 2. Transmission electron micrograph of alligator synovium infected with *Mycoplasma alligatoris*, showing absence of a cell wall and non-helical cell morphology of the mycoplasmas. Bar, 500 nm.

The number of colonies of the unfiltered cultures and the filtrates was determined by inoculating 10-fold serial dilutions onto agar and counting the colonies. Filtration reduced the number of colonies of a representative culture from 1.2×10^{11} c.f.u. ml⁻¹ in the unfiltered culture to 2.2×10^{10} c.f.u. ml⁻¹ in the 800 nm filtrate, 1.0×10^7 c.f.u. ml⁻¹ in the 450 nm filtrate and 2.1×10^5 c.f.u. ml⁻¹ in the 200 nm filtrate. The filtration characteristics were similar for all nine isolates.

Reversion experiments

Each isolate was diluted 1 : 10 in broth medium without antibiotics and incubated at 30 and 37 °C. When growth was detected by acidification of the medium, each isolate was passed again to fresh broth without antibiotics for a total of 10 passages. Each passage was subcultured on agar without antibiotics. Cultures were examined for differences in colony morphology and broth turbidity. No reversion was observed.

Sterol requirement

A requirement for sterol was established by using two different methods: by testing susceptibility of the isolates to digitonin and by attempted cultivation of the isolates in ATCC medium 988 lacking serum or any other cholesterol supplements (Tully, 1983). Cultures were serially diluted 10-fold in broth medium without serum and incubated at 30 and 37 °C. All isolates were sensitive to digitonin with zones of growth inhibition between 6 and 9 mm wide. All isolates grew in broth containing 20 and 2 % fetal bovine serum, but none grew in broth containing only 0.2 % serum.

Biochemical tests

The mycoplasma isolates were examined for metabolism of glucose, mannose, lactose and sucrose (Razin & Cirillo, 1983); for hydrolysis of arginine, aesculin and urea (Barile, 1983a; Razin, 1983); for phosphatase activity (Bradbury, 1983); and for production of film and spots (Freundt, 1983) by using standard methods. Appropriate control cultures of *Acholeplasma axanthum*, *Mycoplasma alkalescens*, *Mycoplasma bovis*, *Mycoplasma pulmonis* and *Ureaplasma urealyticum* were obtained from ATCC. All alligator isolates fermented glucose, mannose, lactose and sucrose. They did not hydrolyse arginine, aesculin or urea. All isolates possessed phosphatase activity. They did not produce film and spots.

SDS-PAGE and Western immunoblotting

Whole-cell lysates (Horowitz & Cassell, 1978) of A21JP2^T and six other isolates were separated on denaturing 10 % polyacrylamide gels and stained with Coomassie blue or electroblotted onto nitrocellulose membranes by using standard methods. Rabbit polyclonal antiserum, made against A21JP2^T whole-cell lysate by using standard methods of immunization

Fig. 3. Coomassie stain of whole-cell lysates of seven isolates of *Mycoplasma alligatoris* separated by SDS-PAGE. Lanes: 1, molecular mass markers; 2, N95-713TP3; 3, A21JP2^T; 4, N95-714LPA1N; 5, N95-713LP3; 6, N95-701LPA2N; 7, A21JP3; 8, N95-740LP1.

Fig. 4. Western blot of whole-cell lysates of seven isolates of *Mycoplasma alligatoris* probed with polyclonal antiserum against A21JP2^T. Lanes: 1, molecular mass markers; 2, N95-713TP3; 3, A21JP2^T; 4, N95-714LPA1N; 5, N95-713LP3; 6, N95-701LPA2N; 7, A21JP3; 8, N95-740LP1.

(Pel-Freez Biologicals), was used to probe the Western blots. Bound antibodies were detected by using alkaline phosphatase-conjugated polyclonal donkey anti-rabbit antibodies (Pierce) and nitro blue tetrazolium chloride plus 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt as substrate. The uniformity of the Coomassie staining patterns of whole-cell lysates of the isolates separated by PAGE is shown in Fig. 3.

Minor variation among the isolates in the patterns of the predominant antigens recognized by rabbit polyclonal antiserum against A21JP2^T is shown in Fig. 4.

Determination and analysis of partial 16S rRNA gene sequence

The 16S rRNA gene of A21JP2^T was amplified by using PCR with primers complementary to terminal sequences conserved among mollicutes (van Kuppeveld *et al.*, 1992; Kirchhoff *et al.*, 1997). The nucleotide sequence of both strands was determined by automated dideoxy DNA sequencing methods without molecular cloning. The 16S rRNA gene nucleotide sequence of A21JP2^T was released to GenBank with accession number U56733 under the provisional name '*Mycoplasma lacerti*' sp. nov. The sequence obtained was compared to all sequences held at GenBank by using BLAST (Bilofsky & Burks, 1988; Altschul *et al.*, 1997). Pairwise nucleotide sequence similarity scores were computed with default gap and gap length weights. A score of 0 indicated that two sequences had only random sequence similarity and a score of 1 indicated that sequences were identical (Brown *et al.*, 1995).

The partial 16S rRNA gene nucleotide sequence of A21JP2^T (1161 contiguous bp including all variable regions, approximately 77% of the gene) did not completely match any other sequence in GenBank. However, the sequence diverged from the 16S rRNA gene nucleotide sequence of *Mycoplasma crocodyli* (U63137) by only 2%. Pairwise nucleotide sequence comparisons with the 16S rRNA genes of other mycoplasmas indicated that A21JP2^T was a member of the *Mycoplasma fermentans* phylogenetic clade (Maniloff, 1992), the next closest relative being the avian mycoplasma *Mycoplasma sturni* (91% sequence similarity; U22013). For perspective, the sequence similarity was 87% to *Mycoplasma fermentans* (M24289) and 72% to *Lactobacillus casei* (M58815).

Genome size determination and restriction endonuclease analysis

The genome size of A21JP2^T was estimated by using PFGE analysis after cleavage with restriction endonucleases *Bss*HII and *Sma*I (Neimark & Carle, 1995). Size standards used were cleaved *Saccharomyces cerevisiae* chromosomes (Bio-Rad) and concatenated ladders of bacteriophage λ DNA (New England Biolabs). Genome fragments were separated on 0.8% Chromosomal Grade Agarose gels (Sigma) by using a clamped homogeneous electrical field (CHEF) PFGE system (Bio-Rad). Electrophoresis conditions were 40 s pulse time at 200 V for 24 h with 0.5 $\times$ TBE buffer cooled to 14 °C. The gels were stained with ethidium bromide and visualized by photography under shortwave UV illumination. The genome cleavage patterns of restriction endonucleases *Apa*I, *Bam*HI, *Kpn*I, *Sac*II, *Sal*I and *Xho*I were similarly analysed.

Fig. 5. PFGE of restriction endonuclease digests of the genome of A21JP2^T. Lanes: 1, *Saccharomyces cerevisiae* standard; 2, bacteriophage λ standard; 3, *Apa*I; 4, *Bam*HI; 5, *Sma*I; 6, *Xho*I; 7, *Bss*HII; 8, *Sac*II; 9, *Sal*I; 10, bacteriophage λ standard.

The results of CHEF PFGE of A21JP2^T cells after lysis and restriction endonuclease digestion are shown in Fig. 5. Restriction endonucleases *Bss*HII and *Sma*I yielded fragment patterns reliable for genome size estimations of 1060 and 1040 kb, respectively, which were in the middle of the range of chromosome sizes of *Mycoplasma* species. No recognition sequences for *Apa*I or *Bam*HI were detected in the chromosome, while *Kpn*I, *Sac*II, *Sal*I and *Xho*I produced many small fragments.

Serological studies

Rabbit polyclonal antisera against A21JP2^T and against the mollicutes listed in Table 1 were used to perform conventional growth inhibition tests of A21JP2^T (Clyde, 1983). In addition, reciprocal metabolism inhibition tests were performed with *Mycoplasma crocodyli* MP145^T (Taylor-Robinson, 1983). The growth inhibition tests revealed that A21JP2^T was serologically unique. No growth inhibition was observed. In particular, A21JP2^T was serologically distinct from *Mycoplasma crocodyli*, which was in agreement with the finding of Kirchhoff *et al.* (1997). That finding was confirmed by results of reciprocal metabolism inhibition tests, which yielded titres of 1: < 16 for heterologous antisera and 1: > 4096 for homologous antisera for the two species.

Pathogenicity

The nine isolates described were obtained from alligators that died during an epizootic characterized by pneumonia with fibrinous polyserositis and multifocal arthritis. Thirty-three 200–300 kg adult male alligators

died and 13 moribund alligators were euthanized within 1 month of the index case. No mycoplasma could be isolated from unaffected healthy alligators. In a preliminary experimental inoculation study, healthy seronegative (titres 1: < 10) 1 m long alligators were inoculated with A21JP2^T. Alligators received 10⁶ c.f.u. in a 1 ml volume by intracoelomic injection ($n = 2$) or by instillation through the glottis ($n = 2$). A control alligator received sterile broth. Three of four inoculated alligators died between 1 and 3 weeks post-inoculation with systemic mycoplasmosis. Regardless of inoculation route, the mycoplasmas were re-isolated post-mortem by culture from multiple organs, blood, synovial fluid and cerebrospinal fluid. The identity of the re-isolated mycoplasmas was confirmed by restriction endonuclease analysis of the amplified 16S rRNA gene. The 6 week post-inoculation titre of the surviving alligator rose to 1:640 and remained constant for the following 8 weeks. Mycoplasmas were not recovered from any site of the surviving alligator at necropsy after 14 weeks. The control remained seronegative and free of mycoplasmas at necropsy. The Henle–Koch–Evans postulates were therefore fulfilled for A21JP2^T as the aetiology of fatal mycoplasmosis of alligators (Evans, 1976).

Taxonomic assignment

The properties of strain A21JP2^T described in this paper fulfil revised criteria for new species descriptions in the class *Mollicutes* (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes, 1995). Properties mandating assignment to this class include absence of a cell wall, filterability and penicillin resistance. The non-helical

Table 1 *Mycoplasma* species tested by growth inhibition for cross-reactivity with *Mycoplasma alligatoris* A21JP2^T

<i>Mycoplasma agalactiae</i> PG2 ^T	<i>Mycoplasma crocodyli</i> MP145 ^T	<i>Mycoplasma lipofaciens</i> R171 ^T
<i>Mycoplasma agassizii</i> PS6 ^T	<i>Mycoplasma cynos</i> H831 ^T	<i>Mycoplasma moatsii</i> MK405 ^T
<i>Mycoplasma agassizii</i> Utah C	<i>Mycoplasma dispar</i> 462/2 ^T	<i>Mycoplasma mobile</i> 163K ^T
<i>Mycoplasma alvi</i> Ilsley ^T	<i>Mycoplasma edwardii</i> PG24 ^T	<i>Mycoplasma molare</i> H542 ^T
<i>Mycoplasma anatis</i> 1340 ^T	<i>Mycoplasma elephantis</i> E42 ^T	<i>Mycoplasma mustelae</i> MX9 ^T
<i>Mycoplasma bovigenitalium</i> PG11 ^T	<i>Mycoplasma equigenitalium</i> T37 ^T	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> PG1 ^T
<i>Mycoplasma bovirhinis</i> PG43 ^T	<i>Mycoplasma fastidiosum</i> 4822 ^T	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> UM30847
<i>Mycoplasma bovis</i> Donetta ^T	<i>Mycoplasma feliminutum</i> Ben ^T	<i>Mycoplasma mycoides</i> subsp. <i>capri</i> PG3 ^T
<i>Mycoplasma bovoculi</i> M165/69 ^T	<i>Mycoplasma felis</i> CO ^T	<i>Mycoplasma neurolyticum</i> Type A ^T
<i>Mycoplasma buteonis</i> Bb/T2g ^T	<i>Mycoplasma fermentans</i> PG18 ^T	<i>Mycoplasma ovipneumoniae</i> Y98 ^T
<i>Mycoplasma californicum</i> ST-6 ^T	<i>Mycoplasma flocculare</i> Ms42 ^T	<i>Mycoplasma oxoniensis</i> 128 ^T
<i>Mycoplasma canis</i> PG14 ^T	<i>Mycoplasma gallinaceum</i> DD ^T	<i>Mycoplasma penetrans</i> GTU54 ^T
<i>Mycoplasma capricolum</i> subsp. <i>capricolum</i> Calif.kid ^T	<i>Mycoplasma gallisepticum</i> PG31 ^T	<i>Mycoplasma pirum</i> 70–159 ^T
<i>Mycoplasma capricolum</i> subsp. <i>capripneumoniae</i> F38 ^T	<i>Mycoplasma gallopavonis</i> WR1 ^T	<i>Mycoplasma pneumoniae</i> FH ^T
<i>Mycoplasma caviae</i> G122 ^T	<i>Mycoplasma genitalium</i> G37 ^T	<i>Mycoplasma pullorum</i> CKK ^T
<i>Mycoplasma cavipharyngis</i> 117C ^T	<i>Mycoplasma glycyphilum</i> 486 ^T	<i>Mycoplasma pulmonis</i> PG34 ^T
<i>Mycoplasma citelli</i> RG-2C ^T	<i>Mycoplasma hyopneumoniae</i> J ^T	<i>Mycoplasma putrefaciens</i> KS-1 ^T
<i>Mycoplasma collis</i> 58B ^T	<i>Mycoplasma hyorhinis</i> BTS7 ^T	<i>Mycoplasma sturni</i> UCMF ^T
<i>Mycoplasma columborale</i> MMP-4 ^T	<i>Mycoplasma imitans</i> 4229 ^T	<i>Mycoplasma sualvi</i> Mayfield B ^T
<i>Mycoplasma conjunctivae</i> HRC581 ^T	<i>Mycoplasma iowae</i> 695 ^T	<i>Mycoplasma synoviae</i> WVU 1853 ^T
<i>Mycoplasma corogypsi</i> BV1 ^T	<i>Mycoplasma iowae</i> PPAV	<i>Mycoplasma testudinis</i> 01008 ^T
<i>Mycoplasma cottewii</i> VIS ^T	<i>Mycoplasma leonicaptivi</i> 3L2 ^T	<i>Mycoplasma verecundum</i> 107 ^T
<i>Mycoplasma cricetuli</i> CH ^T	<i>Mycoplasma leopharyngis</i> LL2 ^T	<i>Mycoplasma yeatsii</i> GIH ^T

morphology of strain A21JP2^T, optimum growth temperature of 30–34 °C and inability to hydrolyse urea place the organism within the order *Mycoplasmatales*, family *Mycoplasmataceae*. A sterol requirement and the conserved nucleotide sequences of the 16S rRNA gene indicate the organism belongs in the genus *Mycoplasma*. Finally, the lack of a serologic relationship of strain A21JP2^T with the type strains of previously established *Mycoplasma* species, in particular *Mycoplasma crocodyli*, demonstrates its stature as a distinct species. Accordingly, we propose the designation of *Mycoplasma alligatoris* for this organism, in consideration of the initial isolation from an alligator. The English word alligator is from the Spanish *el lagarto* (Latin *ille Lacertu* the lizard). However, the specific epithet *lacerti*, originally proposed for this taxon (Brown *et al.*, 1996, 1997), was ultimately rejected because of the modern phylogenetic distinction between lizards (Lacertilia) and crocodilians (Crocodylia). The taxonomic description below summarizes the properties of the organism.

Description of *Mycoplasma alligatoris* sp. nov.

Mycoplasma alligatoris (all.ig.a.tor'is. L. gen. n. *al-ligatoris* from an alligator).

Cells lack a true cell wall and are surrounded only by cytoplasmic membrane. Non-helical and non-motile. Cells filterable through 200 nm membranes. Colonies on solid medium with 0.8% agar exhibit typical fried-

egg morphology at low magnification. Chemo-organo-troph. Acid produced from glucose. Does not hydrolyse arginine nor urea. Serum or sterol required for sustained growth. Temperature range 22–34 °C, with optimum at 30–34 °C. Serologically distinct from all other established *Mycoplasma* species. Genome size 1040–1060 kb. Isolated from multiple organs, blood, synovial fluid and cerebrospinal fluid of diseased American alligators (*Alligator mississippiensis*) in Florida. The organism is a lethal pathogen of alligators. The 16S rRNA gene nucleotide sequence is unique. The type strain is A21JP2^T (= ATCC 700619^T).

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References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Barile, M. F. (1983a). Arginine hydrolysis. *Methods Mycoplasmol* **1**, 345–350.
- Barile, M. F. (1983b). Gram staining technique. *Methods Mycoplasmol* **1**, 39–41.
- Bilofsky, H. S. & Burks, C. (1988). The GenBank genetic sequence data bank. *Nucleic Acids Res* **16**, 1861–1864.
- Bradbury, J. M. (1983). Phosphatase activity. *Methods Mycoplasmol* **1**, 363–366.
- Brown, D. R., Crenshaw, B. C., McLaughlin, G. S., Schumacher, I. M., McKenna, C. E., Klein, P. A., Jacobson, E. R. & Brown, M. B. (1995). Taxonomic analysis of the tortoise mycoplasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16S rRNA gene sequence comparison. *Int J Syst Bacteriol* **45**, 348–350.
- Brown, D. R., Clippinger, T. L., Helmick, K. E., Schumacher, I. M., Bennett, R. A., Johnson, C. M., Vliet, K. A., Jacobson, E. R. & Brown, M. B. (1996). Mycoplasma isolation during a fatal epizootic of captive alligators (*Alligator mississippiensis*) in Florida. *Int Org Mycoplasmol Lett* **4**, 42–43.
- Brown, D. R., Brown, M. B. & Tully, J. G. (1997). Comparison of mycoplasma isolated from alligators and crocodiles. *Am Soc Microbiol Gen Mtg Abstr* **97**, 281.
- Clyde, W. A. (1983). Growth inhibition tests. *Methods Mycoplasmol* **1**, 405–410.
- Evans, A. S. (1976). Causation and disease: the Henle–Koch postulates revisited. *Yale J Biol Med* **49**, 175–195.
- Freundt, E. A. (1983). Film and spot production. *Methods Mycoplasmol* **1**, 373–374.
- Horowitz, S. A. & Cassell, G. H. (1978). Detection of antibodies to *Mycoplasma pulmonis* by an enzyme-linked immunosorbent assay. *Infect Immun* **22**, 161–170.
- International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes (1995). Revised minimum standards for the description of new species of the class Mollicutes (Division Tenericutes). *Int J Syst Bacteriol* **45**, 605–612.
- Kirchhoff, H., Mohan, K., Schmidt, R. & 7 other authors (1997). *Mycoplasma crocodyli* sp. nov., a new species from crocodiles. *Int J Syst Bacteriol* **47**, 742–746.
- van Kuppeveld, F. J., van der Logt, J. T., Angulo, A. F., van Zoest, M. J., Quint, W. G., Niesters, H. G., Galama, J. M. & Melchers, W. J. (1992). Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. *Appl Environ Microbiol* **58**, 2606–2615.
- Maniloff, J. (1992). Phylogeny of mycoplasmas. In *Mycoplasmas: Molecular Biology and Pathogenesis*, pp. 549–559. Edited by J. Maniloff, R. N. McElhaney, L. R. Finch & J. B. Baseman. Washington, DC: American Society for Microbiology.
- Neimark, H. & Carle, P. (1995). Mollicute chromosome size determination and characterization of chromosomes from uncultured mollicutes. *Mol Diagn Procedures Mycoplasmol* **1**, 119–131.
- Razin, S. (1983). Urea hydrolysis. *Methods Mycoplasmol* **1**, 351–354.
- Razin, S. & Cirillo, V. P. (1983). Sugar fermentation. *Methods Mycoplasmol* **1**, 337–344.
- Taylor-Robinson, D. (1983). Metabolism inhibition tests. *Methods Mycoplasmol* **1**, 411–417.
- Tully, J. G. (1983). Tests for digitonin sensitivity and sterol requirement. *Methods Mycoplasmol* **1**, 355–362.
- Tully, J. G. (1995). Culture medium formulation for primary isolation and maintenance of mollicutes. *Mol Diagn Procedures Mycoplasmol* **1**, 33–39.