***Acholeplasma vituli* sp. nov., from bovine serum and cell cultures**

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Organisms isolated from commercial foetal bovine serum and from cell culture lines containing such serum supplements were found to consist of non-helical, non-motile, pleomorphic coccoid forms. One strain (FC 097-2^T) cultivated directly from foetal bovine serum was selected for characterization. In ultrastructural examination, individual round cells lacked cell wall structures and cells varied in size, with a mean diameter of about 700 nm. However, variable numbers of cells were filterable through membranes of 300 nm. Optimum growth occurred between 30 and 37 °C. The organism fermented glucose, fructose and mannose, but did not hydrolyse arginine. The strain was insensitive to 500 U penicillin ml⁻¹ and was capable of growing in the absence of serum or cholesterol. The organism was serologically distinct from all 13 currently described species in the genus *Acholeplasma* and from other sterol-requiring species in the genus *Mycoplasma*, using growth inhibition, immunoperoxidase and immunofluorescence tests. Strain FC 097-2^T was found to have a DNA G+C composition between 37.6±1 mol% and 38.3±1 mol%. The genome size was determined to be 2095 kbp. The 16S rDNA sequence of strain FC 097-2^T was compared to 16S rDNA sequences of other mollicutes in nucleotide databases. No deposited sequence was found to be identical; the closest relatives were several members of the genus *Acholeplasma*. On the basis of these findings and other similarities to acholeplasmas in morphology and growth, the absence of a sterol requirement for growth, and similar genomic characteristics, the organism was assigned to the genus *Acholeplasma*. Strain FC 097-2^T is designated the type strain (ATCC 700667^T) of a new species, *Acholeplasma vituli*.

Keywords: *Acholeplasma vituli* sp. nov., taxonomy, description

INTRODUCTION

One of the more important sources of mollicute contamination in eukaryotic cell culture systems comes from *Mycoplasma* or *Acholeplasma* species present in bovine or other animal serum supplements used in such cell cultivation. Although significant improvement in eradicating mollicutes from serum supplements has come about with ultrafiltration (passage

through pore diameters of 35–40 nm) of some commercial foetal bovine serum products (Tully, 1996), the cost and other availability factors have frequently limited the use of such products. It is therefore important to continue to identify and characterize new mollicutes occurring in animal serum products in order to provide a rapid and comprehensive detection scheme to identify the origin of such cell culture contaminants. A mollicute was isolated more than five times from several lots of foetal calf serum and later from at least six eukaryotic cell cultures from various laboratories in the period 1979–1992. All of these isolates presented similar characteristics and appeared to be identical in several serological tests. In this paper, the first isolate in this series is described and charac-

Abbreviations: Bd, buoyant density method; Tm, melting temperature method.

The GenBank accession number for the 16S rRNA gene sequence of strain FC 097-2^T is AF031479.

terized and a species designation within the genus *Acholeplasma* is proposed.

METHODS

Isolation procedure. Strain FC 097-2^T was isolated from a foetal calf serum lot, using a technique whereby 15–20% of the test animal serum was incorporated into a standard mycoplasma liquid medium formulation in place of the usual serum supplement (Barile & Kern, 1971). The liquid medium contained 0.2% arginine but was without further serum supplements (Polak-Vogelzang *et al.*, 1980). Following incubation at 37 °C, subcultivations were made to agar plates for colony growth and for selection of individual colonies, with plates being incubated aerobically at 37 °C. Small pieces of agar containing single mollicute colonies were selected and streaked onto fresh agar plates. After incubation of these plates for 4 d, this process was repeated and the colonies obtained from the second agar passage were inoculated into the broth medium. After 3 d incubation at the temperature noted, each broth culture was filtered through membrane filters (Sartorius) with a pore diameter of 300 nm. The filtrate was diluted tenfold and each dilution was inoculated onto agar plates to obtain single colonies. This cloning was repeated once. One of the clones was designated FC 097-2^T.

Culture medium and cultivation techniques. The media used in this study for the culture of strain FC 097-2^T and for the mycoplasmas and acholeplasmas employed in the serological testing was Herderschée medium containing 20% horse serum (Polak-Vogelzang *et al.*, 1980) or two other formulations described previously (Hill, 1971; Taylor-Robinson *et al.*, 1968). The purified agar in the growth medium was Lab M agar. Depending on their biochemical activities, the mycoplasmas were grown in liquid medium containing 1% (w/v) glucose (pH 7.8) or in liquid medium containing 0.2–1.0% (w/v) arginine (pH 7.3), and with 500 U penicillin ml⁻¹. Species grown in 1.0% arginine were not inhibited by this concentration. *Mycoplasma genitalium* and *Mycoplasma synoviae* were grown in SP-4 broth or agar medium (Tully *et al.*, 1977). Agar cultures were incubated at 35–37 °C, either aerobically in a humid chamber or under anaerobic conditions in either a GasPak system (BBL Microbiology Systems) or in a CO₂-plus-N₂-enriched atmosphere. Liquid cultures were stored at –70 °C in ampoules.

Morphological studies. Mycoplasma colonies grown on agar were examined microscopically at 100× magnification after 2, 7 and 14 d incubation. The colonies were transferred to slides and stained with Giemsa stain (Fallon & Whittlestone, 1969; Klieneberger-Nobel, 1962). Liquid cultures were observed several times under dark-field microscopy and the micro-organisms were stained with Giemsa stain. Organisms grown in broth were harvested by centrifugation at 30000 g for 45 min. Cell pellets prepared for electron microscopy were fixed in cacodylate-buffered paraformalin and glutaraldehyde (final concentrations of 2.5% and 2.5% at pH 7.6, respectively), with and without tannic acid (1%) for 24 h, then washed in cacodylate buffer and post-fixed for 2 h in 1% (w/v) osmium tetroxide with 1.5% ferrocyanide in 0.1 M cacodylate buffer (Karnovsky, 1971). Dehydration followed with embedding in epoxy resin glycidether 100 (Merck). Ultrathin sections were contrasted with aqueous uranyl acetate (2%, w/v) and lead citrate (1%, w/v) with a LKB ultrastainer and examined in an electron microscope (Philips EM 201/400) operating at 80 kV.

Sterol requirement. Single colonies of strain FC 097-2^T, were seeded onto serum-free solid medium supplemented with 0.05% bovine serum albumin, 0.05% glucose and 10 µg palmitic acid ml⁻¹. Cholesterol, dissolved in Tween 80, was added to give concentrations of 20, 10, 5 and 1 µg ml⁻¹. Plates containing no cholesterol were included (Edward, 1971; Edward & Fitzgerald, 1951; Razin & Tully, 1970; Tully & Razin, 1969). The micro-organisms were also subcultured onto the basal agar medium without serum. When growth occurred on any of the serum-free media, single colonies were passaged three times on similar plates to ensure that growth was not due to a carry-over of serum from the original culture, giving misleading results. The strain was tested indirectly for sterol dependence by a paper disk method (Freundt *et al.*, 1973) using either dried disks that originally contained 0.02 ml 1.5% (w/v) ethanolic solution of digitonin (Sigma) or wet disks that contained 0.02 ml 20% (w/v) aqueous solution of sodium polyanetholesulfonate (Koch-Light Laboratories) on basal growth medium containing Lab M agar. The width of zones of growth inhibition was measured in millimetres. Also, the ability of strain FC 097-2^T to maintain sustained growth in serum-free medium was tested following the serial dilution technique described earlier (Rose *et al.*, 1993).

Tests for biological and biochemical properties. Strain FC 097-2^T was examined for: metabolism of arabinose, arbutin, cellobiose, dulcitol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, sucrose and trehalose; hydrolysis of aesculin, 1.0% and 0.1% arginine, and urea; reduction of methylene blue, resazurin, tetrazolium and tellurite; and phosphatase activity (Aluotto *et al.*, 1970; Barber & Fabricant, 1971; Leach, 1976; Shepard & Howard, 1970; Tully, 1965; Williams & Wittler, 1971). Temperature requirements for growth were determined aerobically with broth cultures incubated at 25, 30, 32 and 37 °C (Williamson *et al.*, 1996). The level of sensitivity to methylene blue was determined by adding 0.002% (w/v) methylene blue to the basal solid medium, and the growth of inoculated mycoplasmas was compared with the growth of mycoplasmas cultivated on agar medium without methylene blue. Lipolytic activity was tested by inoculating mycoplasmas onto basal solid medium enriched with 10% egg yolk emulsion (Fabricant & Freundt, 1967), with plates incubated aerobically and examined after 3, 7 and 14 d for visual evidence of lipolysis (clearing) or film production. The strain was also examined for haemolytic activity, haemadsorption and haemagglutination with chick, guinea pig and sheep erythrocytes (Aluotto *et al.*, 1970; Manchec & Taylor-Robinson, 1968).

Filtration characteristics of strain FC 097-2^T were determined with a 24 h culture diluted 1:10 in liquid medium and then filtered through a series of membrane filters (Millipore) with pore diameters of 220, 300, 450, 650 and 800 nm. For each filtrate, the number of c.f.u. per ml was determined by plating onto an agar medium; the number obtained was compared with the number in the unfiltered culture dilution. The strain was also tested for possible reversion to conventional bacterial forms by examining five consecutive passages of the organism on both solid and liquid media that contained no microbial inhibitors. Agar colonies of the cloned line were also treated with Dienes stain in order to further differentiate mycoplasma colonies from bacterial L-forms (Timms, 1967).

Genomic analysis. To extract and prepare DNA, the strain was grown in Herderschée broth and pelleted by centrifugation at 20000 g. The DNA was extracted and purified by

Table 1. Strains used for the sequencing in order to establish the relatedness of *Acholeplasma vituli*

Species	Accession no.	Strain	ATCC no.
<i>Mycoplasma bovis</i>	U02968	Donetta ^T	ATCC 25523 ^T
<i>Mycoplasma fermentans</i>	M24289	PG18 ^T	ATCC19989 ^T
<i>Mycoplasma lipophilum</i>	M24581	MaBy ^T	ATCC 27104 ^T
<i>Mycoplasma hyorhinis</i>	M24658	BTS7 ^T	ATCC 17981 ^T
<i>Mycoplasma arginini</i>	M24579	G230 ^T	ATCC 23838 ^T
<i>Mycoplasma hominis</i>	M24473	PG21 ^T	ATCC 23114 ^T
<i>Mycoplasma orale</i>	M24659	CH-19299 ^T	ATCC 23714 ^T
<i>Mycoplasma salivarium</i>	M24661	PG20 ^T	ATCC 23064 ^T
<i>Mycoplasma pirum</i>	M23940	HRC 70-159 ^T	ATCC 25960 ^T
<i>Mycoplasma pneumoniae</i>	M29061	FH ^T	ATCC 15531 ^T
<i>Mycoplasma iowae</i>	M24293	695 ^T	ATCC 33552 ^T
<i>Acholeplasma laidlawii</i>	M23932	PG8 ^T	ATCC 23206 ^T
<i>Acholeplasma oculi</i>	U14904	19L ^T	ATCC 27350 ^T
<i>Phytoplasma</i> sp.	Y11933	ESFY	None
<i>Acholeplasma modicum</i>	M23933	PG49 ^T	ATCC 29102 ^T
<i>Entomoplasma ellychniae</i>	M24292	ELCN-1 ^T	ATCC 43707 ^T
<i>Mesoplasma entomophilum</i>	M23931	TAC ^T	ATCC 43706 ^T

a procedure described previously (Carle *et al.*, 1983a). The G+C content of purified DNA was determined by the buoyant density (Bd) and melting temperature (Tm) methods (Carle *et al.*, 1983b). The G+C content was calculated from the mean of four values by Tm and the mean of two Bd estimates. *Mesoplasma entomophilum* strain TAC^T, which has a previously reported G+C content of 30±1 mol% (Tully *et al.*, 1988), was used as control. The genome size was calculated from the mean of four calculations by using a previously published method (Carle *et al.*, 1995).

Serological studies. Antisera to strain FC 097-2^T were prepared by previously described methods (Hill, 1971; Morton & Roberts, 1967; Polak-Vogelzang *et al.*, 1978). The agar plate growth inhibition test (Clyde, 1964), metabolism inhibition tests (Hill, 1977; Purcell *et al.*, 1966a, 1966b; Taylor-Robinson *et al.*, 1966), an immunoperoxidase procedure (Hill, 1978; Polak-Vogelzang *et al.*, 1978) and an epifluorescence test (Gardella *et al.*, 1983) performed on agar colonies were used to compare the response of strain FC 097-2^T to hyperimmune antisera with that of previously described *Acholeplasma* and *Mycoplasma* species.

Type strains were obtained from the National Collection of Type Cultures, Colindale, UK, and from Drs H. Atobe, M. F. Barile, J. M. Bradbury, R. J. Fallon, E. A. Freundt, J. T. Heywood, P. T. W. Jordan, D. E. Jaspar, H. Kirchhoff, R. H. Leach, G. J. McGarrity, D. L. Rose, G. Smith, D. Taylor-Robinson and J. G. Tully. The following type strains were used: *Mycoplasma adleri* G145^T; *Mycoplasma agalactiae* PG2^T; *Mycoplasma alkalescens* PG51^T; *Mycoplasma alvi* IIsley^T; *Mycoplasma anatis* 1340^T; *Mycoplasma anseris* 1219^T; *Mycoplasma arginini* G230^T; *Mycoplasma arthritis* PG6^T; *Mycoplasma auris* UIA^T; *Mycoplasma bovirhinis* PG11^T; *Mycoplasma bovirhinis* PG43^T; *Mycoplasma bovis* PG45^T; *Mycoplasma bovoculi* M165/69^T; *Mycoplasma buccale* CH-20247^T; *Mycoplasma buteonis* BbT2g^T; *Mycoplasma californicum* ST-6^T; *Mycoplasma canadense* 275C^T; *Mycoplasma canis* PG14^T; *Mycoplasma capricolum* subsp. *capricolum* California kid^T; *Mycoplasma capricolum* subsp.

capripneumoniae F38^T; *Mycoplasma caviae* G122^T; *Mycoplasma cavipharyngis* 117C^T; *Mycoplasma citelli* RG-2C^T; *Mycoplasma cloacale* 383^T; *Mycoplasma collis* 58B^T; *Mycoplasma columbinale* 694^T; *Mycoplasma columbinum* MMP1^T; *Mycoplasma columborale* MMP4^T; *Mycoplasma conjunctivae* HRC581^T; *Mycoplasma corogypsi* BV1^T; *Mycoplasma cottewii* VIS^T; *Mycoplasma cricetuli* CH^T; *Mycoplasma cynos* H831^T; *Mycoplasma dispar* 462/2^T; *Mycoplasma edwardii* PG24^T; *Mycoplasma equigenitalium* T37^T; *Mycoplasma equirhinis* M432/72^T; *Mycoplasma falconis* H/T1^T; *Mycoplasma fastidiosum* 4822^T; *Mycoplasma faucium* DC333^T; *Mycoplasma felifaucium* PU^T; *Mycoplasma feliminutum* Ben^T; *Mycoplasma felis* CO^T; *Mycoplasma fermentans* PG18^T; *Mycoplasma flocculare* Ms42^T; *Mycoplasma gallinaceum* DD^T; *Mycoplasma gallinarum* PG16^T; *Mycoplasma gallisepticum* PG31^T; *Mycoplasma gallopavonis* WR1^T; *Mycoplasma gateae* CS^T; *Mycoplasma genitalium* G-37^T; *Mycoplasma glycophilum* 486^T; *Mycoplasma gypis* B1/T1^T; *Mycoplasma hominis* PG21^T; *Mycoplasma hyopharyngis* H3-6BF^T; *Mycoplasma hyopneumoniae* J^T; *Mycoplasma hyorhinis* BTS7^T; *Mycoplasma hyosynoviae* S16^T; *Mycoplasma imitans* 4229^T; *Mycoplasma indiane* 3T^T; *Mycoplasma iners* PC30^T; *Mycoplasma iowae* 695^T; *Mycoplasma lagogenitalium* 12MS^T; *Mycoplasma leonicaptivi* 3L2^T; *Mycoplasma leopharyngis* LL2^T; *Mycoplasma lipofaciens* R171^T; *Mycoplasma lipophilum* MaBy^T; *Mycoplasma maculosum* PG15^T; *Mycoplasma meleagridis* 17529^T; *Mycoplasma moatsii* MK 405^T; *Mycoplasma mobile* 163K^T; *Mycoplasma molare* H542^T; *Mycoplasma muris* RIII-4^T; *Mycoplasma mustelae* MX9^T; *Mycoplasma mycoides* subsp. *capri* PG3^T; *Mycoplasma mycoides* subsp. *mycoides* PG1^T; *Mycoplasma neurolyticum* Type A^T; *Mycoplasma opalescens* MH5408^T; *Mycoplasma orale* CH-19299^T; *Mycoplasma ovipneumoniae* Y98^T; *Mycoplasma oxoniensis* 128^T; *Mycoplasma penetrans* GTU-54-6A1^T; *Mycoplasma pirum* HRC 70-159^T; *Mycoplasma pneumoniae* FH^T; *Mycoplasma primum* HRC292^T; *Mycoplasma pullorum* CKK^T; *Mycoplasma pulmonis* PG34^T; *Mycoplasma putrefaciens* KS-1^T; *Mycoplasma salivarium* PG20^T; *Mycoplasma simbae* LX^T; *Mycoplasma spermatophilum* AH159^T; *Mycoplasma*

spumans PG13^T; *Mycoplasma sualvi* Mayfield B^T; *Mycoplasma subdolum* TB^T; *Mycoplasma synoviae* WVU 1853^T; *Mycoplasma testudinis* 01008^T; *Mycoplasma yeatsii* GIH^T; and *Mycoplasma verecundum* 107^T.

The following *Acholeplasma* species, all type strains, were used in the study: *Acholeplasma axanthum* S-743^T; *Acholeplasma brassicae* 0502^T; *Acholeplasma cavigenitalium* GP3^T; *Acholeplasma equifetale* C112^T; *Acholeplasma granularum* BTS-39^T; *Acholeplasma hippikon* C1^T; *Acholeplasma laidlawii* PG8^T; *Acholeplasma modicum* PG49^T; *Acholeplasma morum* 72-043^T; *Acholeplasma multilocale* PN525^T; *Acholeplasma oculi* 19L^T; *Acholeplasma palmae* J233^T; and *Acholeplasma parvum* H23M^T.

16S rDNA sequence analysis. The 16S rRNA gene of strain FC 097-2^T was amplified using the universal primers 16S8FE (GGA ATT CAG AGT TTG ATC MTG GYT CAG) and 16S556RB (CGG GAT CCC TTT ACG CCC ART RAW TCC G). Both strands of the PCR product were sequenced directly using fluorescent dye terminators in the cycling sequencing system (Perkin-Elmer Cetus). The sequence was compared with the sequences from the EMBL database using the FASTA program (Pearson, 1990). Previously determined mollicute sequences were retrieved from the GenBank database and truncated to fit the 16S rRNA gene sequence lacking the outer primer sites used for PCR. The sequences were compared using the CLUSTAL method in the MEGALIGN module of the DNASTAR software package. The characteristics of strains used are given in Table 1.

RESULTS AND DISCUSSION

Growth and morphological properties

Strain FC 097-2^T grew well in liquid medium under aerobic conditions or in a CO₂-enriched atmosphere. Optimal growth occurred at 37 °C after 3 d incubation, but equal amounts of growth were apparent at both 30 and 32 °C after 6 d incubation. Colonies of strain FC 097-2^T were visible on subculture after 7 d incubation under aerobic conditions and in a CO₂-plus-N₂-enriched atmosphere. The colonies on agar (Fig. 1) had a typical 'fried egg' appearance. Agar growth was inhibited by methylene blue. No film was produced on egg yolk agar and no lipolytic activity was observed.

Fig. 1. Colonies of strain FC 097-2^T grown under aerobic conditions for 5 d on Herderschée agar medium. Bar, 180 μm.

The strain was not motile and no helical forms were observed when broth cultures were examined under dark-field microscopy. Liquid cultures stained with Giemsa stain contained pleomorphic forms characteristic of mollicutes. The strain did not revert to a bacterial form when subcultured on medium without inhibitors. The colonies rapidly stained with Dienes reagent, confirming the isolate as a true member of the class *Mollicutes* rather than a bacterial L-form.

The ultrastructure of the organism was typical of the morphology of other mollicutes when thin sections were viewed by electron microscopy (Boatman, 1979). Cells were round with a mean diameter of about 700 nm, their cytoplasm was loosely or compactly filled with varying amounts of ribosomes and nuclear material, and they possessed a single trilaminar symmetrical cell membrane, lacking any evidence of a cell wall structure (Fig. 2a). After tannic acid fixation, a dense 10 nm surface layer was seen external to the cell membrane (Fig. 2b). Vacuoles were sometimes seen.

Sterol requirement

Strain FC 097-2^T did not require cholesterol for growth since it could be cultured on medium without cholesterol. Tests for sustained growth in serum-free medium established that the strain could be maintained through 24 serial tenfold dilutions in medium devoid of serum. Strain FC 097-2^T was not susceptible to digitonin or sodium polyanetholesulfonate, again confirming a characteristic typical of other *acholeplasmas* that do not require cholesterol or serum for growth.

Biochemical and biological properties

Strain FC 097-2^T fermented glucose, fructose and mannose, reduced tellurite and resazurin, but did not hydrolyse aesculin, arginine or urea, and did not reduce methylene blue and tetrazolium. This strain was haemolytic for all three types of erythrocytes tested, but did not haemadsorb these cells. The strain haemagglutinated only sheep cells. Filtration of a broth culture of the strain reduced the viable count from 2×10^5 c.f.u. per ml in the original dilution to 7×10^2 c.f.u. per ml in the 300 nm-pore-size membrane filtrate.

Serological tests

The four serological tests performed with strain FC 097-2^T and the antisera to currently described *Mycoplasma* and *Acholeplasma* species listed above revealed no cross-reaction with any of these reagents: no zones of inhibition were observed around disks saturated with mycoplasma or *acholeplasma* antisera; the titre in the metabolism inhibition test was 1:16 or lower when this new *acholeplasma* was tested against antisera to all mycoplasma or *acholeplasma*; in the indirect immunofluorescence and the immunoperoxidase tests, dilutions of 1:10 or lower of mycoplasma and *achole-*

Fig. 2. Electron micrographs of a section of strain FC 097-2^T stained with uranyl acetate and lead citrate showing: (a) the absence of a cell wall (bar, 250 nm); and (b) the presence of a trilaminar membrane (arrows) (bar, 100 nm).

Fig. 3. Unrooted phylogenetic tree showing the relatedness of the 16S rRNA gene sequence from strain FC 097-2^T with those of other mollicutes. The scale beneath the tree measures the distance between sequences. Units indicate the number of substitution events.

plasma antisera failed to stain agar colonies of strain FC 097-2^T.

Genomic characterization

The DNA G+C content of strain FC 097-2^T calculated from the mean of four T_m estimates was 37.6 ± 1 mol%, whereas the mean of two Bd estimates

was 38.3 ± 1 mol%. The mean of four estimates of the genome size of this strain was 2095 kbp.

Phylogenetic analysis

Analysis of the 16S rRNA gene sequence revealed that this component in strain FC 097-2^T comprised 1550 bp. Comparison of this sequence with those

present in the nucleotide databases revealed high similarities with mollicute sequences. The highest similarity found (88%) was with the sequence of *Phytoplasma* sp. strain ESFY. Optimized alignment and clustering showed that the closest relatives of the 16S rRNA gene sequence of strain FC 097-2^T were found in the *Acholeplasma* group. A phylogenetic tree indicating the genetic relatedness with other mollicutes is displayed in Fig. 3. The species and strains used in the nucleotide comparison and their accession numbers are given in Table 1.

Habitat

The occurrence of FC 097-2^T in foetal bovine serum would suggest that the organism is of bovine origin. However, since direct isolation of the organism from either bovines or other animals has not been established, it only remains a strong possibility that the strain is derived from cattle.

Taxonomic assignment

The properties of strain FC 097-2^T described herein fulfil recently revised criteria (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes, 1995) for new species descriptions in the class *Mollicutes*. Properties mandating assignment to this class include absence of a cell wall, filterability and resistance to penicillin. The non-helical morphology of the strain, the ability of the strain to grow over a temperature range of 30–37 °C, the inability to hydrolyse urea, a genome size of 2095 kbp and the lack of a sterol requirement for growth place the organism within the order *Acholeplasmatales*, family *Acholeplasmataceae* (Tully *et al.*, 1993). Finally, the lack of a serological relationship to other established species currently assigned to the genus *Acholeplasma* demonstrates its unique status as a new species within this genus. Accordingly, the designation *Acholeplasma vituli* is proposed for this organism. The taxonomic description below summarizes the properties of the organism.

Description of *Acholeplasma vituli* sp. nov.

Acholeplasma vituli [vi.tu'li. L. n. *vitulus* calf; L. gen. n. *vituli* of calf, referring to the provenance of the organism (foetal calf serum)].

Cells predominately coccoid in shape, with mean diameter of about 700 nm. Cells devoid of cell wall and surrounded only by a cytoplasmic membrane. Non-motile and non-helical. Colonies on solid medium demonstrate typical 'fried egg' appearance under both aerobic and anaerobic conditions. Chemo-organotroph. Acid produced from glucose, fructose and mannose. Resazurin and tellurite are reduced. Aesculin, arginine and urea are not hydrolysed. Methylene blue and tetrazolium are not reduced. Casein is not digested. Phosphatase and urease are not produced. No film produced on egg yolk agar and no

lipolytic activity observed. Sterol or serum not required for growth. Temperature range of growth is 25–37 °C. Haemolytic for guinea pig, chick and sheep erythrocytes. Sheep erythrocytes are haemagglutinated. Serologically distinct from all other *Acholeplasma* and *Mycoplasma* species. Isolated so far only from foetal bovine serum or contaminated cell cultures. The DNA G+C content is 38.3±1 mol% (Bd method) or 37.6±1 mol% (Tm method). Genome size is 2095 kbp. Type strain is FC 097-2^T (= ATCC 700667^T).

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