

Mycoplasma phocarhinis sp. nov. and *Mycoplasma phocacerebrale* sp. nov., Two New Species from Harbor Seals (*Phoca vitulina* L.)

J. GIEBEL, J. MEIER, A. BINDER, J. FLOSSDORF, J. B. POVEDA, R. SCHMIDT, AND H. KIRCHHOFF*

*Institut für Mikrobiologie und Tierseuchen, Tierärztliche Hochschule Hannover,
Bischofsholer Damm 15, D-3000 Hannover, Federal Republic of Germany*

A total of 120 mycoplasma strains were recovered from 97 of 265 diseased seals investigated during the seal epidemic in the North Sea and in the Baltic Sea in 1988. Mycoplasmas were isolated from the respiratory tracts (including lungs), hearts, brains, and eyes of the seals. Thirty strains were filter cloned and investigated for their morphological, biochemical, and serological characteristics compared with the characteristics of previously described species. The results of an indirect immunofluorescence test, a growth inhibition test, and an immunobinding assay showed that these strains belong to two new species, for which the names *Mycoplasma phocarhinis* and *Mycoplasma phocacerebrale* are proposed. *M. phocarhinis* (17 strains) did not ferment glucose or hydrolyze arginine but did reduce tetrazolium chloride and potassium tellurite and produced films and spots. *M. phocacerebrale* (13 strains) metabolized arginine but not glucose and produced phosphatase but did not reduce tetrazolium chloride and potassium tellurite. Both species lysed sheep erythrocytes but did not absorb sheep or guinea pig erythrocytes. The type strain of *M. phocarhinis* is strain 852 (= ATCC 49639), and the type strain of *M. phocacerebrale* is strain 1049 (= ATCC 49640).

In 1988 mass mortality spread among the harbor seals (*Phoca vitulina* L.) of the Baltic Sea and the North Sea. More than 18,000 seals died because of an acute disease characterized by pneumonia, skin lesions, diarrhoea, polyarthritis, nervous signs, and abortions in pregnant females (9, 10, 12, 16, 17, 19–21). In addition to several viruses (9, 10, 19–21), mycoplasmas were isolated from a large number of the diseased seals (12). Altogether 122 mycoplasma strains were recovered from 265 diseased seals investigated.

In this paper we report the isolation details of these strains, describe our taxonomic examination of 30 selected strains compared with previously described species of *Mollicutes*, and discuss our findings in relation to the requirements for description of new species.

MATERIALS AND METHODS

Seals. Investigations for mycoplasmas were performed on 265 seals found moribund or dead along the coasts of Schleswig-Holstein and Lower Saxony (10, 12, 19). Organ materials from seals found dead were taken by M. Stede (Staatliches Veterinäruntersuchungsamt, Cuxhaven, Federal Republic of Germany). Samples from living animals were obtained from the Institute for Pathology, Tierärztliche Hochschule, Hannover, Federal Republic of Germany, where moribund seals were sacrificed for histological investigations, or from the seal orphanage at Norddeich, Federal Republic of Germany, where diseased seals were kept for treatment. Swabs were taken from noses, eyes, and throats of living animals. Samples of tracheae, lungs, hearts, livers, brains, kidneys, and spleens were obtained from dead or sacrificed animals.

Cultivation of mycoplasmas. Homogenized organ materials and swabs were suspended in 2 ml of modified Friis medium (13). Portions (0.2 ml) of these suspensions were transferred to 2-ml portions of modified Friis medium, modified Hayflick medium and modified SP4 medium (13). After incubation at 37°C in an atmosphere containing 5% CO₂ for 7 days,

subcultures from all broth cultures were made on the corresponding solid media. The plates were incubated in the same way as broth cultures and checked for growth daily by using a stereomicroscope (Leitz).

Morphological studies. The colonies of the mycoplasmas were examined with a stereomicroscope (Leitz). The cellular morphology of the organisms was assessed by dark-field microscopy, light microscopy after Gram staining and Giemsa staining, and transmission electron microscopy of sectioned organisms. Ultrathin sections of organisms were prepared as described previously (11).

Filtration studies. The numbers of viable cells per milliliter in young (24- to 48-h) unfiltered broth cultures and in filtrates of these cultures after passage through membrane filters (pore size, 220 or 450 nm; Millipore Corp.) were determined by using the method of Albers and Fletcher (1).

Reversion experiments. Mycoplasmas were subcultured five consecutive times by using liquid or solid growth medium devoid of penicillin or any other antimicrobial agent. Agar plates and fluid cultures of all passages were examined for alterations in the morphology of colonies and cells, respectively.

Sterol requirement. Inhibition of growth was tested by the standard method (6), using paper disks previously wetted with 5, 10, or 20% sodium polyanetholsulfonate (Liquoid; Roche Diagnostics) and with 1.5% digitonin (Serva). The sterol requirement was determined by using a broth culture technique described previously (11).

Biochemical tests. The tests for breakdown of glucose, arginine, and urea, reduction of 2,3,5-triphenyltetrazolium chloride and potassium tellurite (aerobically and anaerobically), liquefaction of coagulated serum, phosphatase activity, and film and spot production were performed as described by Aluotto et al. (2).

Hemolysis and hemadsorption. Hemolysis was tested by the overlay technique (2). Hemadsorption was investigated by using the method of Sobeslavsky et al. (22). Both tests were performed with guinea pig and sheep erythrocytes.

Serological tests. Antisera against selected and filter-cloned seal mycoplasma strains were produced in rabbits as described previously (18). A serological comparison of the

* Corresponding author.

TABLE 1. *Mycoplasma* and *Acholeplasma* strains and antisera used in comparative serological tests with the seal mycoplasmas

Strain	Source(s) ^a	
	Mycoplasma	Antiserum
<i>Mycoplasma agalactiae</i> PG2 ^T	IRC	IRC
<i>M. alkalescens</i> DBS80	NIH	NIH
<i>M. alvi</i> IIsley ^T	IRC	IRC
<i>M. anatis</i> 1340 ^T	NIH	NIH, IMT
<i>M. anseris</i> 1219 ^T	VMT	VMT
<i>M. arginini</i> G230 ^T	NIH	NIH, IMT
<i>M. arthritis</i> PG6 ^T	NIH	NIH, IMT
<i>M. bovinegenitalium</i> PG11 ^T	NIH	NIH, IMT
<i>M. bovirhinis</i> PG43 ^T	NIH	NIH, IMT
<i>M. bovis</i> Donetta ^T	IRC	IRC, IMT
<i>M. bovovulvi</i> M165/69 ^T	IRC	IRC
<i>M. buccale</i> CH2024 ^T	NIH	NIH
<i>M. californicum</i> ST6 ^T	IRC	IRC
<i>M. canadense</i> 275C ^T	IRC	IRC, IMT
<i>M. canis</i> PG14 ^T	NIH	NIH, IMT
<i>M. capricolum</i> California Kid ^T	IRC	IRC
<i>M. caviae</i> G122 ^T	IRC	NIH, IMT
<i>M. cavipharyngis</i> 117C ^T	FCR	FCR
<i>M. citelli</i> RG-2C ^T	IRC	IRC
<i>M. cloacale</i> 383 ^T	FCR	FCR
<i>M. collis</i> 58B ^T	FCR	FCR
<i>M. columbinasale</i> 694 ^T	IRC	IRC
<i>M. columbinum</i> MMP-1 ^T	IRC	IRC
<i>M. columborale</i> MMP-4 ^T	IRC	IRC
<i>M. conjunctivae</i> HRC 581 ^T	IRC	NIH, IMT
<i>M. cricetuli</i> CH ^T	FCR	FCR
<i>M. cynos</i> H831 ^T	IRC	IRC, IMT
<i>M. dispar</i> 462/2 ^T	IRC	IRC, IMT
<i>M. elychniae</i> ELCN-1 ^T	FCR	FCR
<i>M. edwardii</i> PG24 ^T	IRC	IRC, IMT
<i>M. equigenitalium</i> T37 ^T	IMT	IMT
<i>M. equirhinis</i> M432/72 ^T	LIRA	LIRA, IMT
<i>M. fastidiosum</i> 4822 ^T	IRC	IRC
<i>M. faucium</i> DC333 ^T	NIH	NIH
<i>M. felifaucium</i> PU ^T	MRC	MRC
<i>M. feliminutum</i> Ben ^T	IRC	IRC
<i>M. felis</i> CO ^T	IRC	NIH, IMT
<i>M. fermentans</i> PG18 ^T	NIH	NIH, IMT
<i>M. flocculare</i> Ms42 ^T	SVS	SVS
<i>M. gallinaceum</i> DD ^T	NIH	NIH, IMT
<i>M. gallinarum</i> PG16 ^T	NIH	NIH
<i>M. gallisepticum</i> PG31 ^T	NIH	NIH, IMT
<i>M. gallopavonis</i> WR1 ^T	IRC	IRC
<i>M. gateae</i> CS ^T	IRC	IRC, IMT
<i>M. genitalium</i> G37 ^T	FCR	FCR
<i>M. glycophilum</i> 486 ^T	FCR	FCR
<i>M. hominis</i> PG21 ^T	NIH	NIH
<i>M. hyopharyngis</i> H3-6BF ^T	FCR	FCR
<i>M. hyopneumoniae</i> J ^T	SVS	SVS, IMT
<i>M. hyorhinis</i> BTS7 ^T	NIH	NIH, CVM
<i>M. hyosynoviae</i> S16 ^T	CVM	CVM, IMT
<i>M. iners</i> PG30 ^T	NIH	NIH
<i>M. iowae</i> 695 ^T	IRC	IRC
<i>M. lactuca</i> 831-C4T	FCR	FCR
<i>M. lipofaciens</i> R171 ^T	FCR	FCR
<i>M. lipophilum</i> MaBy ^T	NIH	NIH
<i>M. lucivorax</i> PIPN-2 ^T	FCR	FCR
<i>M. luminosum</i> PIMN-1T	FCR	FCR
<i>M. maculosum</i> PG15 ^T	NIH	NIH
<i>M. melaleuca</i> M1 ^T	FCR	FCR
<i>M. meleagridis</i> 17529 ^T	IRC	IRC
<i>M. moatsii</i> MK405 ^T	IRC	IRC, IMT
<i>M. mobile</i> 163K ^T	IMT	IMT
<i>M. molar</i> H542 ^T	IRC	IRC, IMT
<i>M. muris</i> RIII4 ^T	FCR	FCR

Continued

TABLE 1—Continued

Strain	Source(s) ^a	
	Mycoplasma	Antiserum
<i>M. mustelae</i> MX9 ^T	FCR	FCR
<i>M. mycoides</i> subsp. <i>mycoides</i> PG1 ^T	IRC	IRC
<i>M. mycoides</i> subsp. <i>capri</i> PG3 ^T	IRC	IRC
<i>M. neurolyticum</i> type A ^T	NIH	NIH, IMT
<i>M. opalescens</i> MH5408 ^T	IRC	IRC, IMT
<i>M. orale</i> CH19299 ^T	NIH	NIH
<i>M. ovipneumoniae</i> Y98 ^T	IRC	IRC, IMT
<i>M. pirum</i> HRC 70-159 ^T	FCR	FCR
<i>M. pneumoniae</i> FH ^T	NIH	NIH, IMT
<i>M. primatum</i> HRC 292 ^T	NIH	NIH
<i>M. pullorum</i> CKK ^T	IRC	IRC
<i>M. pulmonis</i> PG34 ^T	NIH	NIH, IMT
<i>M. putrefaciens</i> KS1 ^T	IRC	IRC, IMT
<i>M. salivarium</i> PG20 ^T	NIH	NIH
<i>M. somnilux</i> PYAN-1 ^T	FCR	FCR
<i>M. spumans</i> PG13 ^T	NIH	NIH, IMT
<i>M. sualvi</i> Mayfield B ^T	GIRA	GIRA
<i>M. subdolum</i> TB ^T	MIT	MIT
<i>M. synoviae</i> WVU 1853 ^T	IRC	IRC
<i>M. testudinis</i> 01008 ^T	MRC	MRC
<i>M. verecundum</i> 107 ^T	IRC	IRC, IMT
<i>Acholeplasma axanthum</i> H86N	IRC	IRC, IMT
<i>A. entomophilum</i> TAC ^T	FCR	FCR
<i>A. equifetale</i> C112 ^T	IMT	IMT
<i>A. florum</i> L1 ^T	FCR	FCR
<i>A. granularum</i> ^T	NIH	NIH, IMT
<i>A. hippikon</i> BTS39 ^T	IMT	IMT
<i>A. laidlawii</i> PG8 ^T	NIH	NIH, IMT
<i>A. modicum</i> PG49 ^T	IRC	IRC, IMT
<i>A. morum</i> 72-043	FCR	FCR
<i>A. oculi</i> 19L ^T	IRC	IRC, IMT
<i>A. parvum</i> H23M ^T	FCR, VFFV	FCR, VFFV
Seal mycoplasma strain 1627	IRC	IRC
Seal mycoplasma strain 1628	IRC	IRC
Seal mycoplasma strain 4359	MAFC	MAFC
Seal mycoplasma strain 33657	ATCC	ATCC
Bovine serogroup strain 7PG50	IRC	IRC

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CVM, R. F. Ross, College of Veterinary Medicine, Iowa State University, Ames; FCR, J. G. Tully, Mycoplasma Section, Frederick Cancer Research Facility, Frederick, Md.; GIRA, R. M. Gourlay, Institute for Research on Animal Diseases, Compton Newbury, Berkshire, England; IMT, H. Kirchhoff, Institut für Mikrobiologie und Tierseuchen, Tierärztliche Hochschule Hannover, Hannover, Federal Republic of Germany; IRC, E. A. Freundt, Food and Agricultural Organization, World Health Organization International Reference Centre for Animal Mycoplasmas, Aarhus Denmark; LIRA, R. Lemcke, Institute for Research Animal Diseases, Compton, Newbury, Berkshire, England; MAFC, H. L. Ruhnke, Ministry of Agriculture and Food, Veterinary Laboratory Services, Guelph, Ontario, Canada; MRC, A. Hill, Medical Research Council Laboratories, Carshalton, Surrey, England; NIH, M. F. Barile (Food and Drug Administration) and J. G. Tully, National Institutes of Health, Bethesda, Md.; SVS, N. F. Friis, Statens Veterinære Serum Laboratorium, Copenhagen, Denmark; VFFV, M. Ogata, Department of Veterinary Public Health, Azabu University, Fuchinobe Sagami-hara, Kanagawa, Japan; VMR, L. Stipkovits, Veterinary Medical Research Institute, Hungarian Academy of Science, Budapest, Hungary.

seal mycoplasmas with each other and with the previously described mycoplasma species was performed by using a growth inhibition test (GIT) (3), an indirect immunofluorescence test (IIFT) (4), and an immunobinding assay (IBA) (14). Antisera were used undiluted for the GIT and were diluted 1:100, 1:200, or 1:300 for the IIFT and IBA. The

TABLE 2. Distribution of the mycoplasmas isolated from 93 of 265 diseased or moribund seals examined

Organ	No. investigated	No. (%) yielding mycoplasmas
Nose	176	39 (22)
Eye	58	11 (19)
Throat	86	40 (46)
Trachea	8	5 (62)
Lung	81	14 (17)
Heart	66	2 (03)
Brain	34	10 (29)
Spleen	22	0
Kidney	36	0
Liver	36	0
Skin	1	1 (100)

antisera and type strains used for serological investigations are listed in Table 1.

DNA. The guanine-plus-cytosine (G+C) content of DNA was determined by isopycnic centrifugation of three DNA batches (5).

RESULTS AND DISCUSSION

A total of 122 mycoplasma strains were isolated from 97 of the 265 seals investigated. Mycoplasmas were found mainly in the respiratory tracts (i.e., lungs, tracheae, noses, and throats) but were isolated frequently also from eyes, hearts, and brains. They were not detected in samples of spleens, kidneys, and livers (Table 2). All mycoplasmas isolated grew well in the three media used at 37°C in an atmosphere containing 5% CO₂. They did not grow at room temperature. Thirty of the mycoplasma strains were filter cloned three times and were examined biochemically and by using serological tests. Two strains (strain 852^T [T = type strain], isolated from pus of a seal lung, and strain 1049^T, obtained from the brain of a necropsied seal) were selected as representative strains.

Morphology and ultrastructure. All of the seal mycoplasmas produced typical fried egg colonies as shown for strains 852^T and 1049^T in Fig. 1a and b. Pleomorphic cells were observed in broth cultures by using dark-field microscopy and after staining by the Gram or Giemsa technique. Ul-

trathin sections demonstrated the trilaminar structure of the cell membrane and the absence of a cell wall (Fig. 2). In these preparations cells of strain 852^T appeared to be mainly coccoid or round, whereas many cells of strain 1049^T exhibited a typical dumbbell shape (Fig. 2); Fig. 2d shows the binary fission of a cell of strain 1049^T.

Reversion studies. None of the phocine mycoplasma strains reverted to a bacterial form after serial subcultivation in broth or on agar medium devoid of bacterial inhibitors.

Filtration characteristics. The cells of strain 1049^T passed through 450-nm-pore-size membrane filters with a reduction in viable counts from 4.4×10^{10} to 4.8×10^9 CFU/ml and through 220-nm-pore-size filters with a reduction in viable counts from 3.3×10^{10} to 1.3×10^8 CFU/ml. For strain 852^T cells a decrease in viable counts from 3.2×10^8 to 5.2×10^7 CFU/ml was observed after the organism passed through the 450-nm-pore-size filters. However, no growth was detected after filtration through 220-nm-pore-size filters.

Sterol requirement. Both representative strains were sensitive to the three concentrations of sodium polyanetholsulfonate tested and to 1.5% digitonin. In the cultural tests for sterol requirement, there was a clear growth response to different concentrations of cholesterol (Table 3). No growth occurred in medium without serum, in the base medium, and in the base medium containing 1% cholesterol. However, there were increasing levels of growth when higher concentrations of cholesterol (5, 10, or 20 µg/ml) were added to the base medium.

Biochemical characteristics. The results of the biochemical tests are shown in Table 4. The 30 strains examined fell into two chemical types, represented by strains 852^T and 1049^T.

Serological investigations. Of the 30 seal mycoplasma strains investigated, 17 were identical to strain 852^T and 13 were identical to strain 1049^T as determined by the GIT, IIFT, and IBA. The distribution of these strains in the seals is shown in Table 5. There are still 90 seal mycoplasma isolates left which have to be typed.

In the serological comparisons no reaction was observed in the GIT and IBA between strain 852^T or 1049^T and any of the previously described *Mycoplasma* and *Acholeplasma* species or the four New England seal mycoplasma strains (strains 1627, 1628, 4359, and ATCC 33657) isolated by Madoff et al. (15) and Geraci et al. (7, 8) during the seal epidemic along the New England coast in 1979 and 1980.

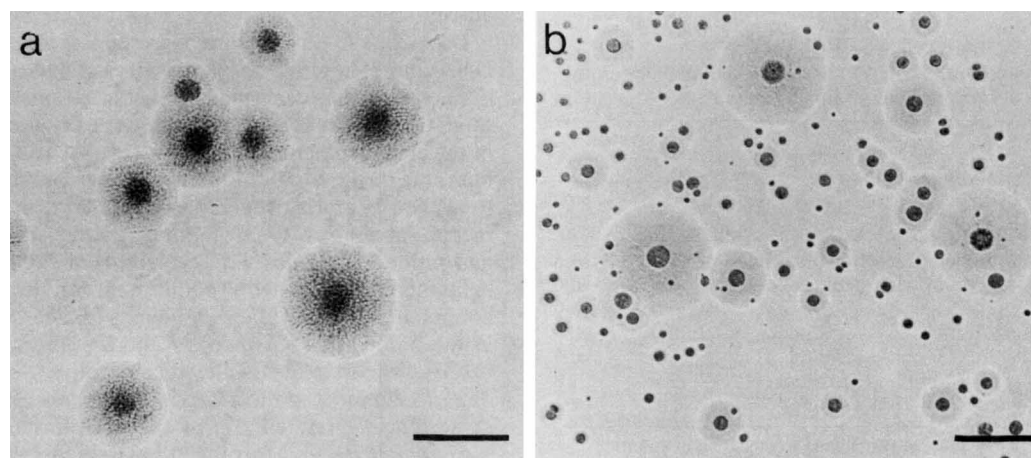


FIG. 1. Typical fried egg morphology of colonies of seal mycoplasma strains 852^T (a) and 1049^T (b) on modified Friis medium. Bars = 200 µm.

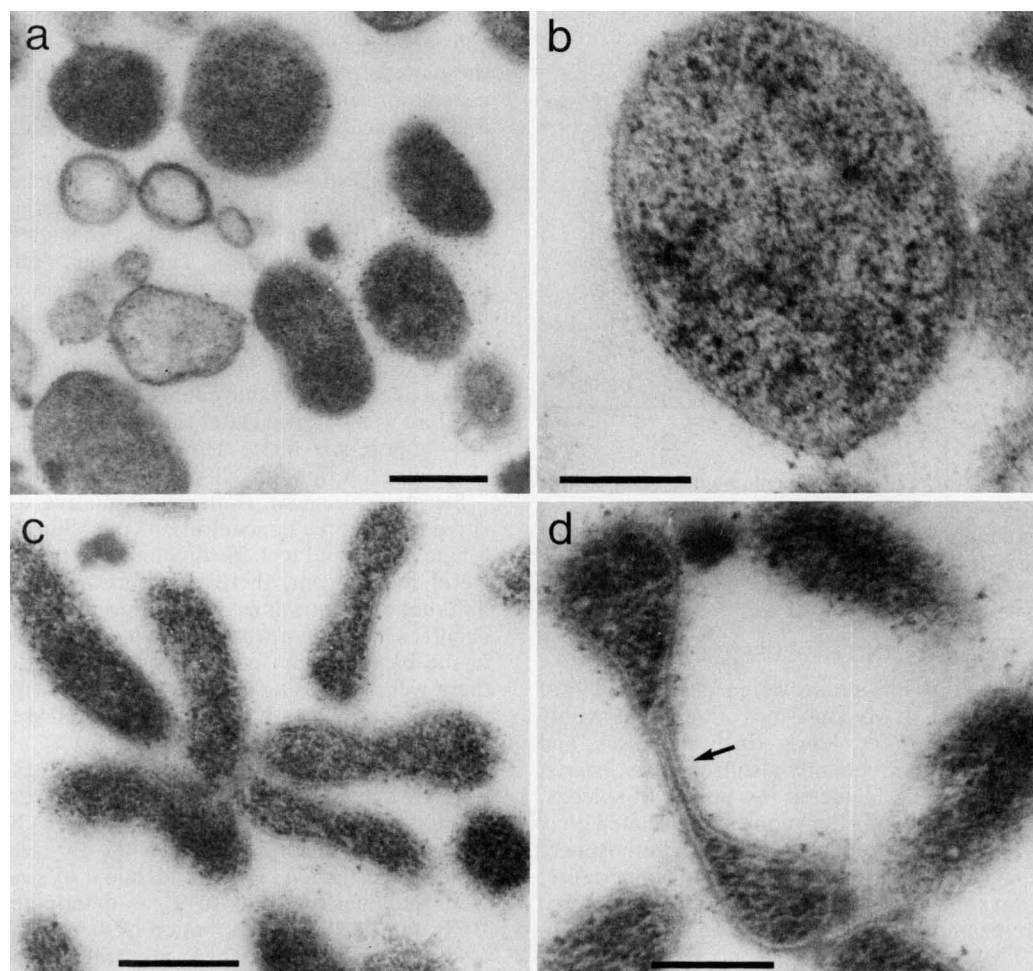


FIG. 2. Electron micrographs of ultrathin sections of seal mycoplasma strains 852^T (a and b) and 1049^T (c and d), demonstrating the absence of a cell wall and the presence of a trilaminar membrane. (d) The arrow indicates binary fission of a cell of strain 1049^T. Bars (a, c, and d) = 200 nm. (b) Bar = 100 nm.

Because of its strong autofluorescence (even very young colonies) strain 852^T repeatedly gave equivocal results in the IIFT. However, there were no positive reactions in the GIT and IBA except with the homologous antiserum. Strain

1049^T showed weak cross-reactions in the IIFT with antisera against *Mycoplasma arginini*, *Mycoplasma cavipharyngis*, *Mycoplasma cloacale*, and *Mycoplasma hyosynoviae*, but in the GIT and IBA, it reacted only with the homologous antiserum.

DNA. The G+C contents were 26.5 mol% for strain 852^T DNA and 25.9 mol% for strain 1049^T DNA.

The properties described above for strains 852^T and 1049^T fulfill the criteria (24, 25) for species descriptions of members of the class *Mollicutes*. A cell wall is absent, and the cells are filterable through 450-nm-pore-size membranes, fail to revert to walled bacteria when they are grown in antibiotic-free media, have a low G+C content, are resistant to penicillin, and produce colonies with typical fried-egg morphology on solid media. The growth requirement for sterol or serum, in conjunction with the lack of helicity, place these organisms in the order *Mycoplasmatales* and the family *Mycoplasmataceae*. The inability of the strains to hydrolyze urea mandates assignment to the genus *Mycoplasma*. Finally, the lack of serological relatedness of these strains to other *Mycoplasma* species and to other unclassified strains that could represent putative species in the genus demonstrates that they represent previously unrecognized species. We propose

TABLE 3. Effect of cholesterol content of the medium on the growth responses of strains 852^T and 1049^T

Medium composition	Protein content (mg) ^a	
	Strain 852 ^T	Strain 1049 ^T
Modified Hayflick medium (10% horse serum)	3.4	3.6
Modified Hayflick medium without serum	0.0	0.0
Base medium ^b	0.0	0.0
Base medium plus 1 µg of cholesterol/ml	0.0	0.0
Base medium plus 5 µg of cholesterol/ml	0.7	0.5
Base medium plus 10 µg of cholesterol/ml	1.9	1.8
Base medium plus 20 µg of cholesterol/ml	2.8	2.4

^a Final protein content in a pellet obtained from 100 ml of culture.

^b Base medium consisted of modified Hayflick medium without serum enriched with 0.5% bovine serum albumin (fraction V) and 10 µg of palmitic acid per ml.

TABLE 4. Biochemical properties of the two seal mycoplasma groups represented by strains 852^T and 1049^T

Strains	Fermen- tation of glucose	Hydrolysis of:		Phos- phatase activity	Reduction of:				Liquefaction of coagu- lated serum	Film and spot pro- duction	Hemolysis of sheep erythrocytes	Hemadsorption (guinea pig and sheep erythro- cytes)
		Argi- nine	Urea		2,3,5-Triphenyl- tetrazolium chloride		Potassium tellurite					
					Aero- bically	Anaero- bically	Aerobi- cally	Anaero- bically				
852 ^T and 16 other strains	—	—	—	+	+	+	—	+	—	+	+	—
1049 ^T and 12 other strains	—	+	—	+	—	—	—	—	—	+	+	—

the following names: *Mycoplasma phocarhinis* for strain 852^T and *Mycoplasma phocacerebrale* for strain 1049^T. The taxonomic descriptions below summarize the properties of the two strains.

***Mycoplasma phocarhinis* sp. nov.** *Mycoplasma phocarhinis* (pho. ca' rhi. nis. L. n. *phoca*, seal; Gr. n. *rhis*, nose; N. L. gen. n. *phocarhinis*, of the nose of a seal). Cells lack true cell walls and are coccoid or round. Colonies on solid medium containing 0.8% agar (Agar No. 1; Oxoid) usually have a typical fried egg appearance. Chemoorganotroph. Does not ferment glucose. Arginine and urea are not hydrolyzed. Reduces tetrazolium chloride and potassium tellurite. Films and spots are produced on 1.1% agar medium containing 20% horse serum. Lyses sheep erythrocytes. Does not hemadsorb sheep or guinea pig erythrocytes. Cholesterol or serum is required for growth. The optimum growth temperature is 37°C. Serologically distinct from other *Mycoplasma* species. Isolated mainly from noses but also from throats, tracheae, lungs, and hearts of seals (*P. vitulina* L.). The G+C content of the DNA is 26.5 mol% as determined by isopycnic centrifugation. The type strain is strain 852 (= ATCC 49639).

***Mycoplasma phocacerebrale* sp. nov.** *Mycoplasma phocacerebrale* (pho. ca' ce. re. bra. le. L. n. *phoca*, seal; N. L. neut. adj. *cerebrale*, of the brain; *phocacerebrale*, pertaining to brain of a seal). Cells lack true cell walls and are coccoid or round or exhibit a typical dumbbell shape. Colonies on solid medium containing 0.8% agar usually have a fried egg appearance. Chemoorganotroph. Does not ferment glucose. Hydrolyzes arginine but not urea. Does not reduce tetrazolium chloride or potassium tellurite. Film and spot production negative. Lyses sheep erythrocytes. Does not adsorb sheep or guinea pig erythrocytes. Cholesterol or serum is required for growth. The optimum growth temperature is 37°C. Serological distinct from other *Mycoplasma* species. Isolated from brains and also from noses, throats, lungs, and hearts of seals (*P. vitulina* L.). The G+C content of the DNA is 25.9 mol% as determined by isopycnic centrifugation. The type strain is strain 1049 (= ATCC 49640).

The role that these phocine mycoplasmas played in the mass mortality of seals in 1988 has not been elucidated. The

fact that they were isolated in large numbers from several pathologically altered internal organs of the diseased seals (i.e., from lungs, hearts, and brains) suggests that even if they are not the primary cause, they might have been involved in the production of these pathological changes and in the general disease, leading to the death of the seals. This probability is enhanced by other observations which showed that the seal mycoplasmas displayed strong cytotoxic capacities, as observed in investigations with tracheal organ cultures in which the organisms caused inhibition of the ciliary activity and extensive damage to the multilayered epithelium (23). Further studies on the pathogenicity of the seal mycoplasmas are in progress in our laboratory.

ACKNOWLEDGMENTS

We thank all of the colleagues listed in Table 1 for providing mycoplasma strains and antisera. We are grateful to M. Stede (Staatliches Veterinäruntersuchungsamt, Cuxhaven, Federal Republic of Germany), T. Willhaus (Seehundaufzuchtstation, Norddeich, Federal Republic of Germany), and J. Pohlenz (Institute for Pathology, Tierärztliche Hochschule Hannover, Hannover, Federal Republic of Germany) for supplying swabs and organ samples from diseased or sacrificed seals.

This investigation was supported by the Minister of Science and Arts of Lower Saxony.

REFERENCES

- Albers, A. C., and R. D. Fletcher. 1982. Simple method for quantification of viable mycoplasmas. *Appl. Environ. Microbiol.* **43**:958-960.
- Aluotto, B. B., R. G. Wittler, C. O. Williams, and J. E. Faber. 1970. Standardized bacteriologic techniques for the characterization of *Mycoplasma* species. *Int. J. Syst. Bacteriol.* **20**:35-58.
- Clyde, W. A. 1964. *Mycoplasma* species identification based upon growth inhibition by specific antisera. *J. Immunol.* **92**:958-965.
- Del Giudice, R. A., F. Robillard, and T. R. Carski. 1967. Immunofluorescence identification of mycoplasma on agar by use of incident illumination. *J. Bacteriol.* **93**:1205-1209.
- Flossdorf, J. 1983. A rapid method for the determination of the base composition of bacterial DNA. *J. Microbiol. Methods* **1**:305-311.
- Freundt, E. A., B. E. Andrews, H. Erno, M. Kunze, and F. T. Black. 1973. The sensitivity of *Mycoplasma* to sodium-poly-anetholsulfonate and digitonin. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1. Orig. Reihe A* **225**:104-112.
- Geraci, J. R., D. J. St. Aubin, I. K. Barker, V. S. Hinshaw, R. G. Webster, and H. L. Ruhnke. 1984. Susceptibility of grey (*Halichoerus grypus*) and harp (*Phoca groenlandica*) seals to the influenza virus and mycoplasma of epizootic pneumonia of harbour seals (*Phoca vitulina*). *Can. J. Fish. Aquat. Sci.* **41**:151-156.
- Geraci, J. R., D. J. St. Aubin, I. K. Barker, R. G. Webster, V. S. Hinshaw, W. J. Bean, H. L. Ruhnke, J. H. Prescott, G. Early, A. S. Baker, S. Madoff, and R. T. Schooley. 1982. Mass mortal-

TABLE 5. Distribution of *M. phocarhinis* (represented by strain 852^T) and *M. phocacerebrale* (represented by strain 1049^T) in seals

Species	No. of strains isolated from:						
	Noses	Eyes	Throats	Tracheae	Lungs	Hearts	Brains
<i>M. phocarhinis</i>	8	0	3	3	2	1	0
<i>M. phocacerebrale</i>	4	0	1	0	1	1	6

- ity of harbour seals: pneumonia associated with influenza A virus. *Science* (Washington, D.C.) **215**:1129–1131.
9. Kennedy, S., J. A. Smyth, P. F. Cush, S. J. McCullough, G. M. Allan, and S. McQuaid. 1988. Viral distemper now found in porpoises. *Nature* (London) **336**:21.
 10. Kennedy, S., J. A. Smyth, S. J. McCullough, G. M. Allan, and F. McNeilly. 1988. Confirmation of cause of recent seal deaths. *Nature* (London) **335**:804.
 11. Kirchhoff, H., P. Beyene, M. Fischer, J. Flossdorf, J. Heitmann, B. Khattab, D. Lopatta, R. Rosengarten, G. Seidel, and C. Yousef. 1987. *Mycoplasma mobile* sp. nov., a new species from fish. *Int. J. Syst. Bacteriol.* **37**:192–197.
 12. Kirchhoff, H., A. Binder, B. Liess, K. T. Friedhoff, J. Pohlenz, M. Stede, and T. Willhaus. 1989. Isolation of mycoplasmas from diseased seals. *Vet. Rec.* **124**:513–514.
 13. Kirchhoff, H., and R. Rosengarten. 1984. Isolation of a motile mycoplasma from fish. *J. Gen. Microbiol.* **130**:2439–2445.
 14. Kotani, H., and G. J. McGarrity. 1986. Identification of mycoplasma colonies by immunobinding. *J. Clin. Microbiol.* **23**:783–785.
 15. Madoff, S., R. T. Schooley, H. C. Ruhnke, R. A. Del Giudice, J. K. Barker, J. Geraci, and A. S. Baker. 1982. Mycoplasmal pneumonia in phocid (harbour) seals. *Rev. Infect. Dis.* **4**:1982.
 16. Mahy, B. W. J., T. Barrett, S. Evans, E. C. Anderson, and C. J. Bostock. 1988. Characterization of a seal morbillivirus. *Nature* (London) **336**:115.
 17. McCourty, C. 1988. Seal epidemic still spreading. *Nature* (London) **334**:553.
 18. Morton, H. E., and R. J. Roberts. 1966. Production of antimycoplasma (PPLO) antibodies in rabbits. *Proc. Soc. Exp. Biol. Med.* **125**:538–542.
 19. Osterhaus, A. D. M. E. 1988. Seal death. *Nature* (London) **334**:301–302.
 20. Osterhaus, A. D. M. E., J. Groen, P. De Vries, F. G. C. M. Uytde Haag, B. Klingeborn, and R. Zarnke. 1988. Canine distemper virus in seals. *Nature* (London) **335**:403–404.
 21. Osterhaus, A. D. M. E., and E. J. Vedder. 1988. Identification of a virus causing seal death. *Nature* (London) **335**:20.
 22. Sobeslavsky, O., B. Prescott, and R. M. Chanock. 1968. Absorption of *Mycoplasma pneumoniae* to neuraminic acid receptors of various cells and possible role in virulence. *J. Bacteriol.* **96**:695–705.
 23. Stadtländer, C., D. Hartmann, A. Binder, and H. Kirchhoff. 1989. Investigations of seal mycoplasmas for their cytotoxic potential on tracheal organ cultures of SPF and gnotobiotic rats. *Zentralbl. Bakterirol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.* **272**:216–224.
 24. Subcommittee on the Taxonomy of *Mollicutes*. 1979. Proposal of minimal standards for description of new species of the class *Mollicutes*. *Int. J. Syst. Bacteriol.* **29**:172–180.
 25. Subcommittee on the Taxonomy of *Mollicutes*. 1988. Minutes of the interim meeting, 25 and 28 August 1986, Birmingham, Alabama. *Int. J. Syst. Bacteriol.* **38**:226–230.