Biochemical Characterization of *Mycoplasma agalactiae* subsp. *agalactiae* (Wroblewski) Freundt

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Sixty-two cultures representing 59 *Mycoplasma* strains from goats and sheep were purified, biochemically characterized, and serologically identified. All of the strains were identified as belonging to *Mycoplasma agalactiae* subsp. *agalactiae* (Wroblewski) Freundt by the metabolic-inhibition test. The serological reactions of the strains were specific and reproducible, and their biochemical characteristics were identical. The antigenic relationships among the strains investigated, other caprine and ovine mycoplasmas, and *M. mycoides* subsp. *mycoides* are discussed.

Mycoplasma agalactiae (Wroblewski) Freundt, the causal organism of contagious agalactia of sheep and goats initially isolated by Bridré and Donatien (5), was the first caprine and ovine *Mycoplasma* species established (8).

At present, contagious agalactia occurs in most of the Mediterranean countries, in Portugal, Switzerland, Rumania, Yugoslavia, Sudan, Iran, Pakistan, India, and the U.S.S.R. Detailed descriptions of the disease have been reported previously (6, 12, 14-16).

The purpose of this study was to characterize biochemically and to identify serologically the large collection of caprine and ovine *Mycoplasma* strains accumulated at the Plum Island Animal Disease Center and to investigate their antigenic relationships to *M. agalactiae*, *M. mycoides* subsp. *capri*, *M. mycoides* subsp. *mycoides*, *M. arginini*, and nine distinct goat and sheep *Mycoplasma* strains.

Fifty-nine *Mycoplasma* strains, including strain PG 2, recently designated (9) as the neotype strain of *M. agalactiae* subsp. *agalactiae*, isolated from sheep and goats in various foreign countries were investigated (Table 1). In a few instances, cultures of the same strain were received from different laboratories, and all were included in the study. Some of the strains were previously identified as *M. agalactiae* by various investigators; the others were received as field isolates without having been characterized or identified. The strains used in the production of antisera are cited below.

The preparation of the culture media and the procedures used for culture purification, biochemical characterization, serological tests, and propagation of the organisms were described previously (1-3).

Biochemical characterization of the strains included carbohydrate fermentation, reduction of tetrazolium chloride and methylene blue, deamination of arginine hydrochloride, urease activity, formation of "film and spots," and hemolysis of sheep red blood cells. Other tests were: growth in medium containing 0.02% methylene blue, growth in serum-free medium, growth in bile salt medium, and growth at 25 C.

Hyperimmune sera were prepared as described previously (10) in rabbits against *M. agalactiae* subsp. *agalactiae* strain PG2 and *M. mycoides* subsp. *mycoides* strain G 230. Other strains (*Mycoplasma* and *Acholeplasma*) used for the production of antisera were Y-goat, A 1343, KS-1, goat 145, goat 189, 19-L, 14-Z, 2-D, and Y-98.

Serological Procedures. The metabolic-inhibition test (13), with tetrazolium chloride as a substrate indicator (2), was performed on all of the *Mycoplasma* strains.

The 59 strains studied were identified as *M. agalactiae* subsp. *agalactiae*. They reduced tetrazolium chloride and lysed sheep red blood cells, and all but four formed "film and spots"; they did not ferment carbohydrates, metabolize arginine hydrochloride, utilize urea, or grow at 25 C, but they did require serum for growth.

All of the strains were metabolically inhibited by specific antiserum prepared against the neotype strain (PG2) of *M. agalactiae* subsp. *agalactiae* at serum titers ranging between 1:1,280 and 1:20,480. The serological reactions were found to be specific and reproducible. In addition, the strains were typed with antisera representing 11 antigenically distinct caprine and ovine *Mycoplasma* serotypes. They were also compared with *M. mycoides* subsp.

TABLE 1. Sources of the strains of *Mycoplasma agalactiae* subsp. *agalactiae* studied

Strain designation	Animal origin	Supplied by	Strain designation	Animal origin	Supplied by
PG 2	Sheep	E. A. Freundt, Denmark	Kirminshah (20)	Sheep milk	M. Kaveh, Iran
PG 2	Sheep	P. Perreau, France	Mashad (21)	Sheep milk	M. Kaveh, Iran
PG 2	Sheep	D. G. ff. Edward, England	Shiraz (23)	Sheep milk	M. Kaveh, Iran
125/9M (2707)	Sheep ^a	A. Foggie, Turkey	Shiraz V (26)	Sheep milk	M. Kaveh, Iran
125/9M (2219)	Sheep ^a	A. Foggie, Turkey	Shiraz (30)	Sheep milk	M. Kaveh, Iran
BSV (1709)	Sheep ^a	A. Foggie, Turkey	Shiraz (32)	Sheep milk	M. Kaveh, Iran
AXZ (1391)	Sheep ^a	A. Foggie, Turkey	Karaj P 6 (33)	Sheep milk	M. Kaveh, Iran
AFH (557)	Sheep ^a	A. Foggie, Turkey	Shiraz (35)	Sheep milk	M. Kaveh, Iran
99M AIK (2511)	Sheep ^a	A. Foggie, Turkey	Talghan (36)	Sheep milk	M. Kaveh, Iran
99M AIK (2542)	Sheep ^a	A. Foggie, Turkey	Shiraz 1 (37)	Sheep milk	M. Kaveh, Iran
ASU (1706)	Sheep ^a	A. Foggie, Turkey	Chatat (38)	Sheep milk	M. Kaveh, Iran
50/7E (657)	Sheep ^a	A. Foggie, Turkey	Shiraz Natami (39)	Sheep milk	M. Kaveh, Iran
BEH (484)	Sheep ^a	A. Foggie, Turkey	Shiraz (40)	Sheep milk	M. Kaveh, Iran
BSV (440)	Sheep ^a	A. Foggie, Turkey	Chah Zebbar (41)	Sheep milk	M. Kaveh, Iran
CO 4 (1063)	Sheep ^a	A. Foggie, Turkey	Shiraz 1 (42)	Sheep milk	M. Kaveh, Iran
BFI	Sheep ^a	A. Foggie, Turkey	Shiraz Sadegi (43)	Sheep milk	M. Kaveh, Iran
AFH	Sheep ^a	A. Foggie, Turkey	Kerminshah (44)	Sheep milk	M. Kaveh, Iran
99 Turkey	Sheep ^a	A. Foggie, Turkey	Aligodar (45)	Sheep milk	M. Kaveh, Iran
H Goat	Goat ^a	A. Foggie, Turkey	Karaj 11 (46)	Sheep milk	M. Kaveh, Iran
H Goat	Goat ^a	G. S. Cottew, Australia	Baraghan (47)	Sheep milk	M. Kaveh, Iran
99M	Sheep	G. S. Cottew, Australia	Karaj (48)	Sheep milk	M. Kaveh, Iran
Sapharpoor (13)	Lamb lung	M. Kaveh, Iran	Lorestan (49)	Sheep milk	M. Kaveh, Iran
Institute P 7 (52)	Goat lung	M. Kaveh, Iran	Egherbolagh (50)	Sheep milk	M. Kaveh, Iran
Kordan (25)	Sheep lung	M. Kaveh, Iran	Shiraz (53)	Sheep milk	M. Kaveh, Iran
Esfahan (8)	Sheep milk	M. Kaveh, Iran	Tirgaran P8 (55)	Sheep milk	M. Kaveh, Iran
Jamali (12)	Sheep milk	M. Kaveh, Iran	Rimeleh P6 (58)	Sheep milk	M. Kaveh, Iran
Karaj (17)	Sheep	M. Kaveh, Iran	Groon P20 (61)	Sheep milk	M. Kaveh, Iran
Shiraz (18)	Sheep milk	M. Kaveh, Iran	Turkey 99 P3 (62)	Sheep milk	M. Kaveh, Iran
Shiraz (19)	Sheep milk	M. Kaveh, Iran	Shiraz Yedollahi (1)	Sheep milk	M. Kaveh, Iran
			Mashad (2)	Sheep milk	M. Kaveh, Iran
			Alshatar P20 (56)	Sheep milk	M. Kaveh, Iran
			Chali Morghi (16) ^a	Sheep milk	M. Kaveh, Iran
			S-5 (64) ^a	Sheep milk	M. Kaveh, Iran

^a Reference (6).^b Antigenically indistinguishable from *M. mycoides* subsp. *mycoides*.

mycoides strain G 230 and were found to have no antigenic relationship to any of the caprine and ovine *Mycoplasma* serotypes or to *M. mycoides* subsp. *mycoides* strain G 230.

Two of the strains studied, Ghali Morghi (16) and S-5 (64), fermented glucose, reduced tetrazolium chloride, grew at 25 C, reduced methylene blue, and grew in a medium containing 0.02% methylene blue. They did not metabolize arginine hydrochloride or form "film and spots" on an agar medium supplemented with 10% egg yolk emulsion. They did not grow in serum-free medium, but they lysed sheep red blood cells. These reactions resemble those of *M. mycoides* subsp. *mycoides*. Both of these strains were inhibited by antisera which we prepared against goat *Mycoplasma* strains Y-goat and OSB42 at a serum titer of 1:1,280. Both showed an antigenic relationship to *M.*

mycoides subsp. *mycoides* strains PG1 and KH₃J when tested by the metabolic-inhibition test.

Fifty-nine purified *Mycoplasma* strains from goats and sheep were identified as belonging to *M. agalactiae* subsp. *agalactiae* by the metabolic-inhibition test. The serological reactions of the strains were specific and reproducible for each strain. The biochemical reactions of these strains were identical with one exception: growth in medium containing 0.02% methylene blue varied. This variability is not uncommon, as is true with strains of most caprine and ovine *Mycoplasma* serotypes and with bovine *Mycoplasma* serotypes (2). Thus *M. agalactiae* subsp. *agalactiae* appears to constitute a biochemically and serologically homogeneous taxon.

The biochemical characteristics of the strains of *M. agalactiae* subsp. *agalactiae* investigated in

this study closely resembled those of *M. bovis genitalium* (4). Both of these organisms reduce tetrazolium chloride, form "film and spots," lyse sheep red blood cells, and require cholesterol for growth; neither ferments carbohydrates, metabolizes arginine hydrochloride, nor grows at 25 C.

It has been reported (6) that colony appearance, morphology, growth in fluid medium, reaction in glucose broth, and the change on media containing blood enabled three types, A (*M. agalactiae*), N, and C, to be separated with certainty. The limited array of biochemical characteristics reported in this study should help greatly to differentiate *Mycoplasma* strains into groups, but not necessarily into species. Serological tests still remain the primary tools for specific identification of mycoplasmas. Several *Mycoplasma* strains of Turkish types C and N (7) were obtained from G. S. Cottew of Australia. These were identified as *M. mycoides* subsp. *capri* and *M. arginini*, respectively.

M. agalactiae, *M. arginini*, and *M. mycoides* subsp. *capri* differ considerably in their biochemical reactions. Arginine is metabolized by strains of *M. arginini* but not by those of the other two organisms; tetrazolium chloride is reduced under aerobic conditions by strains of *M. agalactiae* and *M. mycoides* subsp. *capri* but not by those of *M. arginini*; glucose is fermented by strains of *M. mycoides* subsp. *capri* but not by those of the other two organisms; "film and spots" are formed by strains of *M. agalactiae* but not by those of the other two organisms.

This study also confirms the report by Hudson et al. (10) that the H goat strain is serologically and biochemically indistinguishable from *M. agalactiae*.

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REPRINT REQUESTS

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