Lipolytic Activity by Oral Pleuropneumonia-Like (Mycoplasma) Organisms

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SUMMARY

Ninety-five strains of *Mycoplasma* were isolated from human saliva; all were micro-aerophilic; 72 were actively lipolytic and 23 were non-lipolytic and were inhibited by Tweens. A type culture of human type 4 (*Mycoplasma salivarum*) was culturally identical with the lipolytic strains, and two type cultures of *M. orale* with the non-lipolytic strains. The strains serologically identifiable as *M. orale* were non-lipolytic, whereas those identifiable as *M. salivarum* were of both types. Comparison was also made with type strains from other sources. Those of human origin were non-lipolytic and those of saprophytic or animal origin were slightly lipolytic.

INTRODUCTION

Mycoplasma (PPLO) organisms were first isolated from the human mouth and pharynx by Smith & Morton (1951) and by many workers since. Nicol & Edward (1953) regarded them as distinct from human genital and veterinary strains, and Edward (1954) classified them on cultural grounds as human type 4, the genital strains being type 1. Huijsmans-Evers & Ruys (1956) confirmed the distinction serologically, as also did Edward & Freundt (1956) who named the oral strains *Mycoplasma salivarum*; Pease (1965) showed that, although the oral and genital strains were antigenically distinct, at least four antigens were common to both.

A second antigenic type of oral *Mycoplasma* was identified and given the name of *Mycoplasma orale* by Herderschee, Ruys & van Rhijn (1963) and by Taylor-Robinson, Canchola, Fox & Chanock (1964).

Lipid metabolism in *Mycoplasma* has attracted attention: Edward (1950) described the 'film and spots' reaction which he later (Edward, 1954) showed to be associated with lipid metabolism, and specifically to the clearing of egg yolk. Nicol & Edward (1953) showed that some human type 4 strains produced this reaction, whereas others did not. The fatty-acid requirements of *Mycoplasma* strains were investigated by Razin & Rottem (1963) and lipolytic activity recorded by Rottem & Razin (1964). In the present paper, the lipolytic activity of some oral strains of *Mycoplasma* is described, and compared with that of named strains.

METHODS

Strains used. Ninety-five strains were isolated from human saliva in this laboratory; 5 strains of *Mycoplasma hominis* type 1, one each of *M. salivarum* (H 110 Edward), *M. Mycoides* (*capri*), *M. gallinarum* and *M. laidlawii* types A and B, all maintained as

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type cultures in this laboratory; two strains of *M. orale*, Ella & Hilverda, obtained from Dr A. C. Ruys. Generic and specific names are used for convenience without prejudice to the problem of their validity.

Media. The basal medium used consisted of 1% (w/v) proteose peptone (Oxoid), 0.25% (w/v) yeast extract (Oxoid), 1% (w/v) Lab-Lemco (Oxoid beef extract) and 0.5% (w/v) sodium chloride. The medium was adjusted to pH 7.8 and sterilized by autoclaving at 120° for 15 min. Any precipitate which developed was removed by filtration, and the clear medium again autoclaved. When a solid medium was required, the broth was supplemented with 1.2% (w/v) New Zealand agar or 1% (w/v) Ionagar no. 2 (Oxoid, L 12). Bovine serum, heated at 60° for 30 min. was added to a final concentration of 10% (w/v). Horse serum was used only for the initial isolations because of the reproduction of the film-and-spots reaction by some mycoplasmas, which would interfere with the tests for lipolysis. The oral mycoplasma strains were normally incubated anaerobically, but aerobic growth was also examined. The remaining strains were grown aerobically. Tests were examined after 7 days of incubation.

Egg-yolk reaction. The basal medium agar was supplemented with 10% (v/v) egg yolk suspension (Oxoid, SR 47). Inoculated plates were examined up to 7 days for lipolysis indicated by the development of a film over the growth and precipitation in the agar, and for proteolysis indicated by a wide zone of clearing of the egg-yolk emulsion.

Lipolysis. Butter fat and castor oil were added to the solid medium in the form of 10% (v/v) emulsions in distilled water, prepared by treatment with a Mullard ultrasonic drill, to give final concentrations of 0.5% (v/v). The breakdown of tributyrin was tested on Oxoid tributyrin agar. Decomposition of butter fat and castor oil was indicated by a clearing of the emulsions and a film over the surface of the growth; the breakdown of tributyrin was indicated by a clearing of the emulsion. Breakdown of Tweens 20, 40, 60 and 80 was tested on base agar containing 2% (w/v) Tween and 0.01% (w/v) calcium chloride. Decomposition was indicated by the production of a film over the growth and precipitation beneath the agar.

Serology. Gel-diffusion precipitin tests were made according to the technique of Pease & Laughton (1965). Growth-inhibition tests (Edward & Fitzgerald, 1954) were done on plates of basal medium agar with filter-paper discs soaked in undiluted rabbit antiserum. Fluorescent antigen antibody tests were observed by the use of fluorescein-linked fowl anti-rabbit serum on colony impression preparations (Chanock, Hayflick & Barile, 1962). Antisera were prepared as described by Pease & Laughton, (1965).

RESULTS

Of the 95 oral *Mycoplasma* strains isolated, 72 produced a strong reaction on egg-yolk agar and showed lysis of tributyrin, butter fat, castor oil and Tweens 20, 40 and 60, but not Tween 80; these strains grew rather poorly aerobically. The remaining 23 strains were non-lipolytic and were inhibited by Tweens; these strains grew very poorly indeed aerobically. Of the named strains, type 4 (*Mycoplasma salivarium*) was indistinguishable from the lipolytic group; *M. gallinarum*, *M. mycoides (capri)* and *M. laidlawii* (A and B) were slightly lipolytic; *M. orale* was non-lipolytic and closely resembled the non-lipolytic group (Table 1).

Twenty strains were examined serologically by gel-diffusion precipitin reactions, growth inhibition and immunofluorescence, against type antisera for type 4 (*Mycoplasma salivarium*) and *M. orale*. Type strains of *M. orale* possessed one antigen (as observed by lines of precipitation) which was absent from type strain of type 4. Five strains were serologically identifiable as *M. orale*, all were non-lipolytic; 15 were identifiable as type 4, of which 12 were lipolytic and three non-lipolytic.

Table 1. *Lipolysis by strains of Mycoplasma*

	Egg-yolk reaction	Castor oil	Butter fat	Tribu- tyrin	Tween 20	Tween 40	Tween 60	Tween 80
Oral strains (72) and <i>M. hominis</i> , type 4	+	S	+	S	+	+	+	—
Oral strains (23) and <i>M. orale</i>	—	—	—	—	I	I	I	I
<i>M. hominis</i> type 1	—	—	—	—	I	I	I	I
<i>M. gallinarum</i>	+	—	—	—	+	+	+	+
<i>M. mycoides</i> (capri)	—	—	—	+	+	+	+	S
<i>M. laidlawii</i> (A and B)	—	—	—	S	I	+	+	S

+ = positive reaction; — = negative; S = weakly positive; I = growth inhibited.

These results show that two types of oral mycoplasmas are distinguishable by their lipolytic activity. Culturally, the lipolytic strains correspond to the type strains of *Mycoplasma salivarium*, and the non-lipolytic correspond both culturally and antigenically to the type strain of *M. orale*; both lipolytic and non-lipolytic strains, however, are found among those antigenically definable as *M. salivarium*. The saprophytic strains and those of animal origin examined are slightly lipolytic, and are not inhibited by the Tweens, whereas the human vaginal strains examined are non-lipolytic and, like *M. orale* strains, are inhibited by the Tweens.

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