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Brief Communication

THE SPS AND DIGITONIN TESTS APPLIED TO PORCINE MYCOPLASMAS

Members of the family Mycoplasmataceae, of the order Mycoplasmatales, have been found to be selectively lysed by sodium-polyanethol-sulfonate (SPS) and digitonin, whereas members of the family Acholeplasmataceae, of the same order, are resistant. A growth inhibition test based on this phenomenon has been developed for use on solid medium. The method is recommended (*Freundt et al.* 1973) for differentiation purposes in routine mycoplasma work.

In the present study the applicability of the SPS and digitonin tests for differentiation of porcine mycoplasmas has been examined.

The material examined consisted of Danish isolates, viz., 100 Mycoplasma hyorhinis (M. hyor.), 40 Mycoplasma suipneumoniae (M. suip.), 20 Mycoplasma hyosynoviae (M. hyosyn.), and 9 Mycoplasma flocculare (M. floc.). All these isolates had been cloned twice on solid medium and their identity proved by serological growth inhibition test (*Friis* 1971). Acholeplasma granularum (A. gran.) was represented by 7 strains obtained from Belgium* and identified by serological growth inhibition. For comparison, some recognised type or reference strains were included.

A medium developed for cultivation of M. suip. (*Friis* 1975) was used for all strains except those of M. hyosyn., which were grown on a modified Hayflick's medium (*Hayflick* 1965) enriched with arginine and mucin: Distilled water, 475 ml; Bacto PPLO broth w/o CV, 10.3 g; autoclaving at 1 atm. for 2 min; yeast extract, 25.0 ml; 0.5 % phenol red sol., 1.8 ml; penicillin-G, 120 mg; 5.6 % thallium acetate sol., 1.2 ml; arginine-mucin sol., 7.8 ml (an autoclaved aqueous solution containing 8 % arginininium monochloride and 0.8 % Bacto mucin bacteriological (Difco)). Serum (equal parts of horse and pig serum) was added

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Table 1. Sensitivity of porcine mycoplasmas to sodium-polyanethol-sulfonate (SPS) and digitonin.

	Number of strains with zones of inhibition as indicated (in mm)																		
	SPS 5 %										Digitonin 1.5 %								
	0-1	2-4	5	6	7	8	9	10	11	12	0-1	2-4	5	6	7	8	9	10	11
M. hyorhinis																			
100 strains			38	50	11	1							3	12	41	31	12	1	
NCTC 10121*				1											1				
M. suipneumoniae																			
40 strains				1	4	6	11	15	2	1			1	3	10	16	7	3	
NCTC 10110*						1										1			
M. hyosynoviae																			
20 strains			17	3									2	11	7				
strain M60**			1										1						
M. flocculare																			
9 strains						1	3	2	2	1						1	3	3	2
NCTC 10143*							1												1
A. granularum																			
7 strains	7										7								
NCTC 10128*	1										1								

* from the Mycoplasma Reference Laboratory, Colindale, England.
** a Danish reference strain.

to a final concentration of 20 %. Adjustment of pH to 7.2. For details, see *Friis* (1971, 1974). Agar-Agar (Oxoid) 0.8 % was used for solid medium.

The technique used was essentially that described by *Freundt et al.* Filter-paper discs were wetted with 0.02 ml of a 1.5 % ethanolic (99 %) solution of digitonin (E. Merck, Darmstadt) and dried at 22°C for 2 hrs. in glass petri dishes. SPS discs were prepared with 0.02 ml of a 5 % aqueous solution of sodium polyanethol sulphonate, pure (Koch-Light Laboratories Ltd., Colnbrook Bucks., England) and dried at 37°C overnight. The test was carried out on solid medium by a flooded-plate technique, 1 drop of culture containing 10^4—10^5 metabolic units/ml being used as inoculum. After the moisture had soaked into the agar, the discs were applied and the plates incubated at 37°C, M. suip. and M. floc. in air + 8 % CO_2, M. hyor. and A. gran. in air, and M. hyosyn. in N_2 + 8 % CO_2. After 3—5 days the zones of inhibition were measured in mm.

As will appear from Table 1, the strains belonging to the family Mycoplasmataceae, i.e. M. hyor., M. suip., M. hyosyn., and M. floc., were all inhibited significantly by both 5 % SPS and 1.5 % digitonin, the zones of inhibition being at least 5 mm. On the other hand, with none of the strains belonging to the family Acholeplasmataceae, i.e. A. gran., was a zone of inhibition exceeding 1 mm observed in any of the 2 tests.

Apparently, therefore, members of the known porcine mycoplasma species behave very specifically in the SPS and digitonin tests.

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REFERENCES

Freundt, E. A., B. E. Andrews, H. Ernø, M. Kunze & F. T. Black: The sensitivity of Mycoplasmatales to sodium-polyanethol-sulfonate and digitonin. Zbl. Bakt., I. Abt. Orig. A 1973, *225*, 104—112.

Friis, N. F.: Mycoplasmas cultivated from the respiratory tract of Danish pigs. Acta vet. scand. 1971, *12*, 69—79.

Friis, N. F.: Mycoplasmas in pigs with special regard to the respiratory tract. Thesis. DSR Forlag, Copenhagen 1974.

Friis, N. F.: Some recommendations concerning primary isolation of Mycoplasma suipneumoniae and Mycoplasma flocculare. Nord. Vet.-Med. 1975, *27*, 337—339.

Hayflick, L.: Tissue cultures and mycoplasmas. Tex. Rep. Biol. Med. 1965, *23*, suppl. 1, 285—303.

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