NOTES

DIFFERENTIATION OF SMOOTH AND NONSMOOTH COLONIES OF BRUCELLAE[1]

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In studies of variation of *Brucella* species, the differentiation between smooth and nonsmooth colonies has presented difficulties to inexperienced workers. The methods usually employed are those first described by Henry (J. Infectious Diseases, **52,** 374, 1933) and more recently reviewed by Braun (Bact. Revs., **11,** 75, 1947). These techniques require considerable skill in recognizing different colony types. It is, moreover, a time-consuming task to search entire plates for a few nonsmooth types among a preponderance of smooth colonies. In work on metabolism and on vaccines of brucellae it is essential to know whether pure smooth, pure nonsmooth, or mixed smooth and nonsmooth cultures are used. A simple method for detecting various colonial types has been devised.

Albimi *Brucella* agar modified to contain 2.5 per cent agar, 1 per cent glucose, and 5 per cent glycerol was used. Plates were poured and dried for 24 hours or longer to reduce surface moisture. Cultures were streaked on the plates, which were then incubated for 96 hours at 37 C. After incubation the plates were flooded with 1:2,000 aqueous solution of crystal violet for 15 seconds. The excess dye was decanted into a disinfectant and the colonies were then examined under a low-power dissecting microscope with the use of obliquely transmitted light. Smooth colonies appeared light blue-green contrasted to a light violet background, but nonsmooth colonies appeared red to blue-red. Upon standing, smooth colonies took a progressively deeper blue-violet color peripherally, but differentiation from nonsmooth was distinct for as long as 96 hours after the plates were flooded. The test did not interfere with subsequent acriflavine tests (Braun and Bonestell: Am. J. Vet. Research, **8,** 386, 1947). Viable cells were obtained from all colony types 96 hours after the plate was flooded with dye.

Among the nonsmooths, three distinct color reactions were observed. The typical color for rough colonies was a deep violet-red, which was classified 10.0 P 4/10 on the Munsell charts (*Munsell Book of Color, Revised,* Munsell Color Co., Inc., Baltimore, Md., 1942). Most mucoid cultures studied thus far appeared lighter blue-red (5.0 P 5/10 on the Munsell charts), and one strain, Huddleson's vaccine strain, which is a mucoid *Brucella suis,* appeared deep purple-blue (Munsell: 10.0 PB 3/10). The latter strain was also singularly characterized by a cracked appearance of the colony surface following flooding with the dye.

Several other dyes were studied, with safranine O offering a good differentiation.

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Smooth colonies remained colorless or appeared faintly pink, while rough colonies appeared bright red-orange. Safranine O did not, however, afford distinction between rough and mucoid variants. Eosin and brilliant green offered slight differentiations between smooths and nonsmooths, whereas acid fuchsin, methyl green, congo red, bismark brown, malachite green, and sudan black B gave no color distinctions. Incorporation of the dyes into the medium offered no successful differentiation.

Differentiation was possible using unmodified Albimi *Brucella* agar, but the addition of 1 per cent glucose and 5 per cent glycerol markedly enhanced the color distinctions between rough and mucoid colonies. The color reactions were dependent upon the dye concentration and length of exposure: more concentrated dye solutions used for short periods (e.g., 1:400 crystal violet for 4 seconds) allowed good differentiation between smooths and nonsmooths but did not allow differentiation between roughs and mucoids. Colonies did not wash from the agar surface if thoroughly dry surfaces were streaked and if agitation of the plate during flooding with dye was avoided.

This test permits rapid surveillance of a plate, and affords an easily recognized distinction between colonies that may appear identical when observed by the usual methods.

THE PYRUVATE OXIDATION FACTOR REQUIREMENT OF STREPTOCOCCUS CREMORIS[1]

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Streptococcus cremoris has been reported to require acetate, sorbitan monooleate, or reticulogen for growth in a synthetic medium (Colins *et al.*: J. Bact., **59,** 69, 1950). These observations suggested that the pyruvate oxidation factor (POF) of O'Kane and Gunsalus (J. Bact., **56,** 499, 1948) might be the active fraction of reticulogen since Snell and Broquist (Arch. Biochem., **23,** 326, 1949) had shown that POF and the factor that replaces oleate or acetate in the nutrition of certain lactic acid bacteria are probably identical. Furthermore, reticulogen is known to be a good source of POF.

Through the courtesy of Drs. Collins, Nelson, and Parmelee, we were able to study one of their strains of *S. cremoris*. The methods of maintaining the culture, preparing the inoculum, etc., have been described (O'Kane and Gunsalus, *loc. cit.*). The semisynthetic medium was modified in that sulfuric rather than acetic acid was used in the strepogenin preparation.

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