came bright yellow within 18 to 24 hr after inoculation. The surrounding medium remained opaque and blue. The $CaCO_3$ prevented changes in the color of the indicator in the medium surrounding the colonies, so that acid-producing sectors could be distinguished in a background of non-acid-producing colonial growth. After 48 hr incubation, colonies capable of reducing the dye were surrounded by a colorless zone. The concentrations of peptone and yeast extract should be minimal. Staphylococci are able to utilize amino acids as the energy source and if sufficient ammonia is liberated from the nitrogen source, greenish colonies result, thereby blurring the clear-cut difference between acid-producing colonies and non-acid-producing colonies.

IMPROVED MEDIUM FOR SELECTIVE ISOLATION OF VEILLONELLA

M. ROGOSA, R. J. FITZGERALD, M. ELIZABETH MacKINTOSH, AND ALFRED J. BEAMAN

National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland

Received for publication July 7, 1958

A selective medium for the isolation and enumeration of the Veillonella from the mouth has been described previously by Rogosa (J. Bacteriol., **72,** 533, 1956). Considerable experience has now shown that in addition to the Veillonella, often a large background of small extraneous colonies, principally streptococci and diphtheroids, appears. Thus, it may be difficult to obtain pure isolates on first trial and also recognition of Veillonella colonies is sometimes uncertain. Lackey and Fitzgerald (J. Dental Research, **34,** 705, 1955 and Proc. Internat. Assoc. for Dental Research, 43, 1956) found that this genus was relatively insensitive to streptomycin and the antibiotic was therefore incorporated into the original medium at 5 μg/ml.

Since then, we have studied a newer antibiotic, vancomycin (obtained through the generosity of Eli Lilly and Company). Titration experiments with pure cultures of Veillonella showed that these organisms were resistant to 50 to 500 μg/ml of vancomycin. A wide spectrum of 44 strains of the genus *Veillonella*, comprising what is believed to be probably all the serological groups and subtypes,[1] has been tested for growth in the presence of vancomycin (20 μg/ml), or streptomycin (5 μg/ml). The level of streptomycin is that employed originally (Rogosa, J. Bacteriol., **72,** 533, 1956). Growth in the presence of vancomycin compared favorably with that on either streptomycin or the basal medium. Indeed, streptomycin may often be more inhibitory than vancomycin for certain strains of Veillonella, particularly in relatively early growth (24 hr or less). A concentration of 1 μg/ml or less of vancomycin has been found to be inhibitory for diphtheroids. With rare exceptions, the oral streptococci and micrococci are inhibited by 3 μg/ml or less.

[1] This serological study is to be described elsewhere.

TABLE 1

Comparative efficiency of isolation media containing streptomycin and vancomycin

Additive to Basal Medium	Amt	No. of Samples	Avg Plate Counts	
			Veillonella	Others
	μg/ml		*no.* × 10^6	*no.* × 10^6
Streptomycin	5	15	41	144
Vancomycin	5	15	66	7
Vancomycin	7.5	15	67	7
Vancomycin	10	15	67	7
Vancomycin	20	25	69	8

Salivary samples, collected by paraffin stimulation, were plated with basal medium containing either streptomycin (5 μg/ml) or vancomycin at 5, 7.5, 10, and 20 μg/ml. The results in table 1 clearly demonstrate that Veillonella are recoverable from saliva more readily in media containing vancomycin than streptomycin, and that extraneous organisms, principally streptococci and diphtheroids, are significantly reduced, to such an extent that they no longer interfere

seriously in the enumeration and isolation of the Veillonella.

On the basis of these results, a level of 7.5 μg/ml of vancomycin was chosen for subsequent use in the routine isolation medium. The composition of the proposed basal medium and the sampling procedures are the same as described previously (Rogosa, J. Bacteriol., **72**, 533, 1956). Streptomycin is omitted, however, and vancomycin from sterile vials is diluted in H_2O and added aseptically to a concentration of 7.5 μg/ml of medium just before pouring plates.