Vancomycin Induction of L Colonies of *Staphylococcus aureus*

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L colonies of *Staphylococcus aureus* were induced with vancomycin and propagated on agar. The yield of L colonies induced by vancomycin was very low in comparison with other inducers.

Antimicrobial agents which inhibit bacterial cell wall synthesis such as penicillin, methicillin, and cycloserine have been found to be good inducers of L colonies of *Staphylococcus aureus* (2, 9, 11). On the contrary, it has been mentioned that vancomycin, which prevents the polymerization of the phospho-disaccharide-pentapeptide lipid complex during the second stage of cell wall synthesis (1), does not induce L colonies readily (6-8). Williams has failed to induce L colonies of *S. aureus* by vancomycin (11). The present report concerns the successful induction of L colonies of *S. aureus* by vancomycin and the propagation of these L colonies.

The August Harmon strain of *S. aureus* which had previously been used in our laboratory for the induction of L colonies and wall-defective staphylococci by lysostaphin, methicillin, and cycloserine (2, 9, 10) was used in this study. The minimal inhibitory concentrations of methicillin and vancomycin for this strain of *S. aureus* were 1.56 $\mu\text{g/ml}$ and 0.39 $\mu\text{g/ml}$, respectively. Serum-salt broth, which was the liquid medium used, is composed of Brain Heart Infusion broth (Difco) with added NaCl, MgSO_4 , and heat-inactivated human serum to final concentrations of 5, 0.2, and 5%, respectively. When the inactivated human serum is omitted, it is referred to as salt broth. Serum-salt agar is composed of serum-salt broth and 1% Noble agar.

A 0.1-ml amount of the appropriate dilution of an 18- to 24-hr culture of *S. aureus* was spread over serum-salt agar incorporated with different amounts of vancomycin or methicillin. Plates were then sealed with cellophane tape and incubated at 37 C. Colonies were counted after 5 days of incubation. Table 1 shows the yields of L colonies from four experiments. The L colonies induced by vancomycin had the typical "fried egg" appearance and were indistinguishable from those induced by methicillin. Methicillin

over a wide range of concentrations (2.5 to 100 $\mu\text{g/ml}$) induced large numbers of L colonies. Very low yields of L colonies were obtained with vancomycin at 5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$, but no L colonies were induced outside these concentrations.

L colonies from a 5 $\mu\text{g/ml}$ vancomycin agar plate were transferred onto serum-salt agar containing vancomycin (2.5 $\mu\text{g/ml}$). L colonies which grew on this agar plate were transferred to a 1.25 $\mu\text{g/ml}$ vancomycin agar plate. After this transfer, L colonies were maintained on serum-salt agar without vancomycin. They have since been transferred more than 100 times over a period of 2 years and have remained stable. During this period of time, many attempts to propagate these L colonies in serum-salt broth or salt broth without vancomycin were unsuccessful. When L colonies were transferred into the liquid medium, some reverted to the parent staphylococci without further transfers, whereas others grew as wall-defective forms in many consecutive transfers but eventually reverted. Two L colonies grew as wall-defective forms in 23 consecutive transfers in liquid medium before reversion. The morphology of these wall-defective *S. aureus* before reversion occurred, as determined by phase microscopy and Gram stain, was similar to that of wall-defective *S. aureus* induced by lysostaphin, methicillin, or cycloserine. In previous experiments of induction of L colonies with lysostaphin and cycloserine, some stable L colonies also reverted after they were transferred into liquid medium, but stable wall-defective staphylococci ultimately grew and propagated in liquid medium (9, 10).

The results of this study indicate that vancomycin is able to induce L colonies of *S. aureus*; the yield was, however, very low in comparison with methicillin, cycloserine, and lysostaphin induction (9, 10). The low yield was probably

TABLE 1. Comparison of the yields of L colonies of *Staphylococcus aureus* induced by different concentrations of vancomycin and methicillin

Inoculum	Concn of antimicrobial agent (μg/ml)	Vancomycin agar		Methicillin agar	
		Vegetative colonies	L colonies	Vegetative colonies	L colonies
2×10^7 – 7×10^7	100	0	0	0	> 500
2×10^6 – 7×10^6	100	0	0	0	172
2×10^5 – 7×10^5	100	0	0	0	2
2×10^7 – 7×10^7	50	0	0	0	> 500
2×10^6 – 7×10^6	50	0	0	0	1
2×10^5 – 7×10^5	50	0	0	0	8
2×10^7 – 7×10^7	10	0	0–3	0	> 500
2×10^6 – 7×10^6	10	0	0	0	49–> 500
2×10^5 – 7×10^5	10	0	0	0	> 500
2×10^7 – 7×10^7	5	35–> 500	0–36	35–> 500	> 500
2×10^6 – 7×10^6	5	0–9	0–5	23–117	76–> 500
2×10^5 – 7×10^5	5	1	0	29	> 500
2×10^4 – 7×10^4	2.5	17	0	91	95
2×10^3 – 7×10^3	2.5	2	0	15	7
2×10^2 – 7×10^2	2.5	0	0	1	0

related to the additional mode of action of vancomycin (3–5). Once L colonies were induced by vancomycin, they were easily propagated and maintained on agar without vancomycin, but attempts to propagate them in liquid medium were unsuccessful.

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