SECTION H, MICROBIOLOGY

The Growth Inhibitory Effect of Mechanical Agitation of Cultures of Nocardia Corallina

J. B. CLARK, University of Oklahoma, Norman

Ginsberg and Jagger (1962) reported large cell losses resulted from bubbling buffered cell suspensions with fritted glass filter tubes. By using cells labeled with P^{32}, they found little evidence of lysis of the cells, and most of the radioactivity was found in the frit after aeration. They concluded that frit-aeration causes cell loss almost entirely by trapping of bacterial substance in the frit and that this substance probably consists of whole cells. During the past several years, similar observations have been made in my laboratory.

A 15-liter quantity of nutrient broth was inoculated with *Nocardia corallina* ATCC 4273 and aerated by forcing air through a carborundum aerator. After several days' incubation, no turbidity had appeared in the broth, but a small amount of slimy growth was observed on the aerator. This growth was checked microscopically and found to resemble *N. corallina*. After the aeration was stopped, turbidity appeared in the broth within 24 hours. The turbidity was found to be due to growth of *N. corallina*. The fact that growth did occur after aeration was stopped rules out any possible toxic effect from the aerator itself. Aeration with large bubbles through glass tubing was found to cause vigorous growth of the organism within 36 hours as did bubbling pure oxyen through the substrate. Thus, the failure to grow could not be attributed to adverse oxygen tension. It is possible that the carborundum aerator acted as a trap similar to that found for fritted glass by Ginsberg and Jagger (1962). It is also possible that the mechanical agitation caused by the small bubbles prevented growth.

A second observation on a similar effect was made when growing *N. corallina* in a Delmar chemostat. In this apparatus, aeration is accomplished by introducing air through a single opening in the bottom of the growth chamber. When a vigorous stream of air bubbles was passed through this opening, no growth of the inoculum occurred in the growth chamber. However, when the rate of aeration was reduced to about two bubbles per second, heavy growth occurred. There is no pore space in this instrument to trap oganisms as with the fritted glass filter tubes, so this lack of growth apparently was due entirely to mechanical agitation.

A third observation was made on the effect of stirring on a culture. In irradiation inactivation experiments, cell suspensions are normally stirred to assure a more uniform exposure of the cells to the irradiation. For this purpose, a magnetic stirrer is very convenient. In determining the variables associated with such irradiation experiments, plate counts were run at periodic intervals on a stirred, buffered suspension of *N. corallina*. The rate of stirring was not measured, but it was a moderate speed. The magnetic stirring bar was teflon covered. After 10 minutes stirring, there was a reduction of 10 per cent in the number of viable organisms in the suspension. A control suspension under identical conditions except for the stirring gave no decrease in viability.

The primary physical factor in common in these three observations is mechanical agitation. The exact effect on the cell is unknown. Such an effect can greatly influence quantification of microbiological experiments.

TABLE 1. A COMPARISON OF EXTRACTABLE FAT BETWEEN CELLS OF *Nocardia corallina* CONTAINING NO STAINABLE FAT INCLUSIONS AND CELLS CONTAINING STAINABLE FAT BODIES.

Solvents (In order used)	Cells containing no stainable fat (0.6834 gm)		Cells containing stainable fat (0.5315 gm)	
	extract weight	per cent	extract weight	per cent
petroleum ether	6.9 mg	1.1	14.1 mg	2.7
absolute ethanol	13.9 mg	2.0	12.2 mg	2.3
choloroform	16.1 mg	2.3	17.3 mg	3.3
acetone	3.6 mg	0.6	17.2 mg	3.3
acidified ethanol	16.6 mg	2.3	18.4 mg	3.4
Totals	57.1 mg	8.3	79.2 mg	15.0

LITERATURE CITED

Adams, J. N. and N. M. McClung. 1962. On the nature of cytoplasmic inclusions of *Nocardia rubra*. J. Gen. Microbiol. 28: 230-241.

Clark, J. B. and C. Aldridge. 1960. Fat bodies in *Nocardia corallina*. J. Bacteriol. 79: 756-757.

Prince, H. N. 1960. Total lipid of *Trichophyton mentagrophytes*. J. Bacteriol. 79: 154.