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NOTES

Use of Malachite Green for Staining Flagella in Bacteria

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Preparations are prepared, using completely clean slides and aqueous suspensions of young bacterial agar cultures. A drop of the suspension is placed on one end of the slide; the slide is then tipped, making the suspension drain down to the opposite end (Society of American Bacteriologists, *Manual of Microbiological Methods*, McGraw-Hill Book Co., New York, 1957). Both the mordanting and the staining of the flagella are done in the same operation at 37 C. As a mordant, the three components of the A solution from P. H. H. Gray's procedures (J. Bacteriol. **12**:273, 1926) are used. The stain is malachite green.

The following solutions are needed: (1) saturated aqueous solution of potassium alum; (2) saturated aqueous solution of mercuric chloride; (3) 20% aqueous solution of tannic acid; and (4) 0.5% filtered aqueous solution of malachite green.

The four solutions are placed in small flasks, in a volume sufficient to stain the required number of preparations, and are left stoppered in the incubator at 37 C for 1.5 hr. The prepared slides and all materials used, such as pipettes, Erlenmeyer flasks, funnels, and filtering paper, are also incubated.

All subsequent operations are done in the incubator. Solution 1 (5 ml) is placed in an Erlenmeyer flask, and 2 ml of solution 2 and 2 ml of solution 3 are added; the resultant mixture is gently stirred, and 1 ml of solution 4 is quickly added. This mixture is stirred discontinuously for exactly 2 min and is then filtered through folded Prat Dumas paper (no. 40). The filtrate is briefly stirred again exactly 2 min after the filtration started, and each smear is covered with 2.5 ml of the filtrate. The slides are left 6 to 9 min for staining. The smear is then rinsed with distilled water, drained, and dried.

To determine the optimal staining time, we recommend trials of 6, 7.5, and 9 min. If the temperature does not reach exactly 37 C, the volume of solution 4 should be increased to 1.5 ml.

In cases in which the bacterial cell is not stained, we suggest that the following procedure be followed: 8 ml of distilled water is put into an Erlenmeyer flask, 1 ml of 0.075% NaOH is added, and the solution is stirred intermittently for 45 sec.

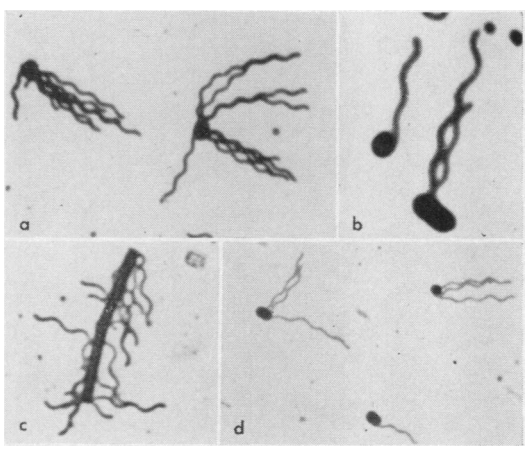

FIG. 1. *(a) Proteus vulgaris.* × *1,400. (b) Serratia marcescens.* × *2,300. (c) Bacillus megaterium.* × *1,250. (d) Serratia marcescens.* × *1,150.*

Then, still stirring, 1 ml of Loeffler's alkaline methylene blue is added (*Manual of Microbiological Methods*), and intermittent stirring is continued for another 45 sec. The smears are immediately covered with 3 ml of diluted Loeffler's alkaline methylene blue, and stained for 6 to 12 min at room temperature. Flagella are bright green and bacterial bodies deep blue. Representative species, stained in this manner, are shown in Fig. 1.