Induction of Mutations in *Serratia marcescens* by a Proteosynthesis Block

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ABSTRACT

The formation of colour mutations of *Serratia marcescens* by the action of chloramphenicol was studied. Pink variants were the commonest; the proportion of white variants was much smaller. Almost 100% mutations were formed in a two-day culture containing 100 μg. chloramphenicol/ml. Comparative experiments showed that the change in pigment formation was hereditary, i.e. that actual mutation, and not selection of chloramphenicol-resistant mutants, occurred.

Mutation occurred both in strain 151 and strain HY. The resultant mutations remained constant throughout ten passages on normal nutrient medium. The minimum chloramphenicol concentration which produced an increase in the mutation frequency was 5 μg./ml. The combined effect of X-ray irradiation and chloramphenicol treatment somewhat stimulated the increase in the frequency of mutation as compared with cells which were only irradiated. The increase in the frequency of mutation was much slower on solid medium containing chloramphenicol.

*Serratia marcescens* cells form colour variants both spontaneously and after exposure to ultraviolet radiation and X-rays. Bunting (1947), Shapiro (1946) and Kaplan (1947) demonstrated that the frequency of these mutations was 10⁻⁴ per generation. Bunting (1947) did not exclude the possibility of the participation of cytoplasmic factors in these mutations and the same conclusions were reached by Herčík and Pelánková (1959) and Herčík and Janovská (1960).

Preliminary experiments on the effect of chloramphenicol on cells of *Serratia marcescens* (Herčík & Janovská, 1961) showed that red variants cultivated on medium containing 5—100 μg. chloramphenicol/ml were transformed into pink or white mutants, which, when passaged on medium not containing chloramphenicol, remained stable up to the tenth generation.

It is known that chloramphenicol specifically inhibits proteosynthesis and the incorporation of radioactive amino acids into proteins (Brock, 1961). We hope that a detailed study of the effect of chloramphenicol on the production of mutants in *Serratia marcescens* will help to explain the origin of colour variants. As far as we are aware, mutations have not previously been formed in any species of bacteria by blocking proteosynthesis.

MATERIALS AND METHODS

*Serratia marcescens* strain 151 from the collection of the Department of Microbiology of the Natural Science Faculty of Purkyně University, Brno, which was employed in all previous experiments, was used. *Serratia marcescens* strain HY, A.T.C.C. 8195 (Labrum & Bunting, 1953) was also used in some experiments. In the latter strain few relatively stable pink and white types were observed among the typical red colonies in comparison with strain 151. As a rule, mottled red-white colonies also appeared in strain HY.

The bacteria were cultivated on
Kaplan's glycerin medium (Kaplan, 1948): glycerin 25 g., citric acid 5 g., \( \text{NH}_4\text{H}_2\text{PO}_4 \) 3 g., \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \) 0.3 g., \( \text{FeNH}_4(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O} \) 0.3 g., distilled water 1,000 ml. (agar 20 g.), pH 7.

Crystalline D-chloramphenicol (Spofa) was used. The effect of chloramphenicol on the formation of colour variants was tested on solid agar medium and in liquid medium.

Chloramphenicol was added to liquid glycerin medium in concentrations of 5, 20, 40 and 100 µg./ml. Flasks containing 50 ml. of this medium were inoculated with 0.5 ml. of a 24-hour *Serratia marcescens* culture and were then incubated at 30°C. The percentage of spontaneous mutations was determined in every culture. Specimens (0.1 ml.) collected after 6 hours' and 1, 2, 3, 4, 6, 8, 10 and 14 days' cultivation were suitably diluted and were reinoculated on Petri dishes containing glycerin agar. The number and pigmentation of the colonies was read after three days' cultivation at 30°C and the results were calculated for the number of cells in 1 ml. culture. Transformation of red colonies to pink and white colonies was evaluated as mutation. The protein content was determined in other specimens at the same intervals, by the method of Lowry *et al.* (1951).

The bacteria were irradiated by means of a roentgen tube (Müller) (60 kV., 4 mA., no filter, dose rate 976 r/min).

**RESULTS**

*Frequency of mutants in aging Serratia marcescens cultures after adding chloramphenicol.* The addition of chloramphenicol to *Serratia marcescens* cultures resulted in a marked increase in the percentage of mutations (Fig. 1). The number of mutations in a two-day culture containing 100 µg. chloramphenicol and inoculated with cells from a red colony was almost 100%. In the same culture 40 µg. chloramphenicol produced 90% mutations and 20 µg. 43%. Pink mutants were the commonest, white mutants were least common.