CHAPTER VII

Attempts at co-synthesis of mycobacillin by mixed culture of mycobacillin-negative mutants

Attempts were made to detect co-synthesis of mycobacillin by growing mixtures of mutants in all possible pairwise combinations in liquid medium. Co-synthesis was achieved when the mutant pairs with non-identical genetic lesion (and so producing different peptides) were used in mixed culture. No other instances of co-synthesis were observed.
McCormick (199) studied co-synthesis of tetracyclines by pairs of *Streptomyces aureofaciens* mutants. In order to determine whether such mutants might show biological cooperation in the synthesis of tetracycline antibiotics, they were then grown as pairs in mixed fermentation and the resulting fermented mashes examined for tetracyclines. They found that in many cases substantial quantities of one or more tetracyclines were produced in the mixed fermentations even when each mutant grown alone produced no appreciable amount. Doull *et al.* (112) isolated twelve mutants of *Streptomyces venezuelae* that were blocked in chloramphenicol biosynthesis. They have studied co-synthesis of chloramphenicol by growing mixtures of mutants in all possible pairwise combinations on agar or in liquid medium. Mutants Cm 1-4, Cm 1-5 and Cm 1-8 which accumulated p-amino-phenylalanine promoted chloramphenicol synthesis when grown with mutants Cm 1-1 and Cm 1-12 (blocked in the conversion of chorismic acid to p-aminophenylalanine). Banerjee and Bose (174) isolated eleven non-producer or extremely feeble producer mutants of *Bacillus subtilis* by ultraviolet irradiation. These mutants when cultured alone or in all possible combinations, did not produce mycobacillin (200). Culture filtrates of any of these strains failed to supplement the other non-producer mutant cells to complete synthesis of mycobacillin.

In this chapter the experiments were planned to indicate whether or not (1) the simultaneous growth of any two My mutants results in the synthesis of mycobacillin or (2) the 48 hours...
cells of one My\textsuperscript{−} mutant and 48 hours old-culture fluid (free from cells) of another My\textsuperscript{−} mutant results in mycobacillin synthesis when they are incubated together.

EXPERIMENTAL

Materials

All the medium constituents were of bacteriological grade. All other chemicals were of reagent grade.

Organisms

Ten (Asp\textsuperscript{−}My\textsuperscript{−}) mutants (N-10, N-16, N-1111, N-9, N-20, N-6, N-54, N-4, N-14 and N-7) and nine (Arg\textsuperscript{−}My\textsuperscript{−}) mutants (N-2, N-3, N-32, N-25, N-23, N-40, N-55, N-51 and N-48) were used.

Medium

A complete medium was used as described in Chapter I.

Methods

Mixed culture experiment: In one set of experiments mutants pairs in all possible combinations were grown on 100 ml nutrient liquid medium for 6 days under stationary condition at 30\textdegree C. The culture broth was then assayed against sensitive organism \textit{Aspergillus niger G3} for mycobacillin.

In another set of experiments My\textsuperscript{−} mutants were grown separately for 48 hours in shake culture in nutrient liquid medium. Cells were then separated from the culture medium by centrifugation at