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Szybalski, W.

Directed "Induction" of Streptomycin Resistance

Three bacterial species (Escherichia coli, Micrococcus pyogenes and Mycobacterium tuberculosis) were reported to become streptomycin resistant when incubated 7-30 days in phosphate buffer containing streptomycin. (T. Akiba—discussion at the Symposium on Bacterial resistance—Washington, D. C., 1954).

Present studies have confirmed these data and in addition provide tentative evidence that streptomycin resistance acquired by the majority of the cells in the absence of bacterial multiplication (i.e. eliminating selection) is of nuclear origin.

The experiments may be summarized as follows: Suspensions of E. coli strains B/r, K-12 and of Salmonella typhimurium (6-18 hrs. old) were centrifuged and washed with phosphate buffer 3-5 times. Cells were resuspended in buffer (10^6-10^7/ml). These suspensions were incubated overnight at 36°C with aeration, to exhaust all possible nutrients within the cells and in the buffer. The suspensions of starved cells were assayed and divided into equal 2 ml portions. Streptomycin was added to half of these samples, up to a final concentration of 50-100 mcg/ml. All samples were incubated for two weeks at 36°C. After this period they were again assayed on nutrient agar with and without streptomycin (100 mcg/ml).

In the successful experiments 1-70% of the washed and starved bacteria survived, both in the absence and in the presence of streptomycin. In several experiments, however, all the bacteria were killed in the presence of streptomycin, probably due to incomplete washing and starving of the cells (E. coli was more susceptible than Salmonella). Where the bacteria survived in the presence of streptomycin, the majority of them acquired complete and stable (for at least 40 transfers) resistance to this antibiotic.

Lack of nutrients, the small size of the tested population, the rarity of completely streptomycin-resistant mutants, and the fact that almost all streptomycin treated cells become highly resistant indicates that a selective mechanism is highly improbable. Experiments were, however, performed to exclude the role of selection where a few, non-specifically induced, resistant mutants may multiply at the expense of dying, sensitive cells. Streptomycin-sensitive cells (10^7/ml) were mixed with a small proportion (10^2/ml) of differently marked, streptomycin-resistant E. coli and incubated in streptomycin-containing buffer. The resulting resistant population was proven to be derived from the sensitive strain.

Recombination analysis show consistant segregations and linkage relationships for the "induced" streptomycin resistance indicating its nuclear character.

The studied example seems to represent a case of the nuclear inheritance of a single, purposefully acquired character. Surprising as it seems, this may be due to rather specific chromosomal damage associated with the loss of a dominant character of streptomycin sensitivity.

Institute of Microbiology
Rutgers University
New Brunswick, N. J.