THE LILLY RESEARCH LABORATORIES

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Dr. Joshua Lederberg
Dept. of Genetics
College of Agriculture
University of Wisconsin
Madison 6, Wisconsin

Dear Dr. Lederberg:

On behalf of our staff I want to tell you that we enjoyed your visit here and are hoping you will want to drop in again when you have time to meet more of our research people.

On the attached table you will find a list of media which we have found useful in cultivation of streptomycetes. Three are chemically defined or contain only starch as an ingredient of questionable purity. The other three are complex. The numbers in parentheses have the following significance: Streptomycetes, in our experience, rather frequently sporulate well on only one of the two general classes of media included here. Strains preferring inorganic nitrogen may often do best on numbers 1 and 2, less well on 3, and sporulate inversely as the medium number rises. Conversely, some (relatively few) prefer a very rich medium such as Emerson's (6) and sporulate progressively less on the lower-numbered substrates of the series. Of course, all variations may occur. It might be mentioned that starch is often the best carbon source for sporulation. Medium 1 with starch (weight for weight) in place of sucrose is one of the best "synthetic" media we have used.

The above considerations might have application in the handling of mutants. I have isolated spontaneous variants (in cultural characteristics) which differed greatly from the parent and from each other on such a series of media. You may face the same problem.

Max Stark agrees that we have no information concerning sporulation "run-down" in cultures used here. In a few experiments with different streptomycetes, production "run-down" has uniformly occurred in a dozen transfers or less, but sporulation was not significantly affected. The work of Williams and McCoy is the best in this area so far as I know. One or two tricks may help: Sporulation of certain strains is depressed by water on the agar surface. They will do much better if a dry transfer is made (avoid suspensions) and well dried slants are inoculated. If sporulation "run-down" should occur in spite of precautions, you might plate out on several media

and select a more vigorous colony. As an alternative or supplementary measure, we frequently pass the strain through a culture generation in sterile soil (witchcraft!). Simply cover the bottom of an Erlenmeyer flask with soil, sterilize and inoculate with a suspension of the organism. Enough water should be added to moisten the soil thoroughly, and I usually spread the "mud" with a sterile glass rod so as to form a fairly thin layer on the inner wall of the lower part of the flask. Incubate at 25-30°C for one to four weeks and look for a fine white film covering the soil surface. When this is observed, recover the culture by direct transfer or by plating.

Incidentally, you will get best plating results by the "surface-inoculum" method, for submerged actinomycete colonies don't tell you much. If many plates must be inoculated with a single dilution, we usually make the dilution in melted and cooled 0.25 per cent agar held at 42-45°C. One or two ml of such a dilution can quickly be spread over a plate by vigorous tipping or swirling, thus avoiding the use of glass spreaders. This works best if two people cooperate. The 0.25 per cent agar dries out quickly during incubation.

My apologies for such a long-winded letter. Where I have included trivial details already known to you, please ignore them. I hope at least a little of this will be valuable. If we can help in any other way, please let us know.

Sincerely

R. C. Pittenger

R. C. Pittenger
Department of
Microbiological Research

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Attachment