The choice of a basal medium is not easy. If one select as a basal medium, one which is just sufficient for growth in 24 hrs, the growth is insufficient for the colonies to be marked. I have used the asparagine medium because the single substance provides the "building stones" for all the organism's growth substances. Growth is quite good in that medium.

I have used a surface culture rather than a pour plate since the organism is strongly aerobic and the colonies do not develop very well in the agar.

This, I think, briefly sets out the techniques I have been using without success. I cannot understand why I have had no success and am wondering if you could make any suggestions as to what mistakes I may be making.

I am sorry to trouble you in this way but feel that you could help me.

Yours Faithfully
John S. Loutit

First fold here

basal medium of Asparagine + basal salts
I have used this medium because growth
is more satisfactory on it. However I have
used other media - one containing xalanin
+ basal salts and another containing glucose
+ an ammonium salt + basic salts.

After growth on this 1st medium
for 24 hours or longer all the colonies
which have grown are marked + small
glass cylinders sealed to the agar surface
These are then filled with broth and
the plates reincubated for 24 hrs. A
certain number of additional colonies
appear and these are picked off + suspended
in 10 mls of sterile distilled water +
then a loopful of this suspension is
plated out on another basal medium
agar plate.

On this second plating on the
basal medium the organisms selected
always grew readily within 24 hours.
even though they did not grow on this
time on the first plating out.

I have examined many
thousands of colonies and replated a fairly
large no of them without any success
at all.

I used the harder rays in an
attempt to see ~~what~~ whether the soft
rays were not producing a chemical
change within the cell

University of Adelaide,
South Australia.
Bacteriology Dept.
10/1/51

Dear Dr. Ledenberg,

By way of introduction I am a science graduate of the Adelaide University and I have been engaged for some time on work on the action of X rays on a strain of Pseudomonas pyocyaneus in an endeavour to produce some biochemically deficient mutants. I have been singularly unsuccessful and am at a loss to understand this and I was wondering if you could give me any assistance.

To give briefly the experimental data. The X ray beam completely unfiltered except by the glass of the X ray tube and of the culture tube which consists of a test tube with a glass bulb. The contents (20 mls) are vigorously stirred throughout the irradiation. I have worked at 40 kV and 4 ma and have been able to get doses of approx 2,500 r/min. In addition I have done some work using a tube at 110 kV + 5 ma giving a dose of 400 r/minute.

The culture contains 100×10^6 orgs/ml, 2 mls are irradiated and a total dose of 20,000 r is sufficient to reduce the no of organisms to approximately 1000/ml. The organisms are collected in the logarithmic phase and washed and suspended in normal physiological saline.

For detection of mutants I have been applying approximately this dose + then plating out the survivors on agar plates containing a

To open cut at top

BY AIR MAIL

G.P.O. ADELAIDE S.A. AUST.

AIRLETTER

COMMONWEALTH

AIR MAIL POSTAGE 7d 7d AUSTRALIA

DR. J. LEDERBERG.
Dept of Genetics,
University of Wisconsin,
Madison,
Wisconsin.
U.S.A

Third fold here

Second fold here

If anything is enclosed, letter will be sent by ordinary mail.