Dear Jack,

At the time, Regen felt the phenol brand looked wonderful — we put
an experiment that was like this:

\[ \text{Survival} \]
\[ \text{Time in 0.2% phenol} \]

But then we thought that the phenol brand was due to the fact that
we happened to be using mutant strains in complete agar, and wild-type
in minimal. Thus, wild-type E. coli from the following:

\[ \text{Survived} \]
\[ \text{counted on complete} \]
\[ \text{or} \]
\[ \text{Survived} \]
\[ \text{counted on minimal} \]
\[ \text{Time in 0.2% phenol} \]

It seems a "phenol-killed" bug is dead only if for an
adriamycin on minimal is averaged — something is complete agar
"reactivating" these. (There is a small 2% of the population that is
"invariably killed")

On rechecking mutants for killing in phenol: minimal
with and without growth-factor, we found just the reverse of what
Hobb's, Wez's, Claphane stated (they claimed phenol acted like penicillin).
In our hands, an E. coli mutant survived better with its growth factor
than without.

We thought the difference might be due to their usage
at organism, so we tried a E. subtilis mutant. In that run, we
got no differentiated at 0.2%, growth in 0.12%, but the named
differentiated (both survived without growth-factor) at 0.15%! Then we
couldn't repeat it. We have dropped it here, too.
quite proud, and in time I'm sure you'll be interested. We are using
penicillin to select for auxotrophs without selecting the clones arising during
intermediate cultures; i.e., we get each original mutation as a single colony.
Here is:

1) Grow wild-type 2 x cul (7632) in minimal, dilute and inoculate in minimal
   killing from 10^6 to 10^3/ml. (See log-phase cells)

2) Immediately dilute to 1:10, and plate over minimal between
   protective layers, at about 2 x 10^4 survivors per plate.

3) Incubate at 32º for 7-9 hours. Each wild-type survives becomes a
   micro-colony of about 100 cells, while each mutant (roughly 50 per plate)
   divides until autolysis is observed. Typically at least 10 cells
   are formed per mutant colony.

4) We now layer with penicillin-minimal solution, and incubate 24 hrs.

5) Layer with penicillinase solution (Schuley, 1 unit/100 units penicillin,
   about 50 units/plate). Place at 37º for 12 hours to
   allow enzyme.

6) Incubate at 37º for 48 hours. Mark wild type colonies
   (in pet about 30-40/plate)

7) Layer with complete agar. 4 hours later mutant colonies are up.
   At a perfectly reproducible 24-327 survivals per ml, i.e.,
   about 50/plate if you plate 2 x 10^8 survivors, wild-type
   are completely absent from the 48-72 hour cultures.

We have picked and picked, and are getting a variety of types. The main
Ding is that each colony is an original mutation.

If you don't begin with complete, time in a slow development
of mutant colonies anyway, due to cross-feeding or healing from pen-killed cell.
But if you work with a single growth factor, you could pick fairly
efficiently. We are working on minimizing the number of "undesired"
mutations that come up in minimal, with the goal of having to pick only
a desired class of mutants.

We'll be publishing shortly, I hope, and will be
interested to hear the results in case you decide to try it. What is
your impression?

Please send your 1951 reprints, and keep up
the wonderful work - hope to hear from you soon.

Best & Other Ed