E. D. [illegible]
Dept of [illegible]
U of Ch[illegible]

Cultures

CG-1	A. tumefaciens	A6 - virulent		Riker
CG-2	A. tumefaciens	A6-6 - attenuated		Riker
	" "	A6 PEN	penicillin resist.	Klemmer (see pA1a)
	" "	A6 P5a	polymyxin resid.	Klemmer (see pA1a)
	" "	A6 C5	chloramphenicol resist.	Klemmer (see pA1a)
CG-3	" "	A6-S^r	probably identical	pA5 ff
CG-4	" "	A6-S^r	probably identical	pA5 ff
CG-5	" "	A6-6-S^r	probably identical	pA5 ff
CG-6	" "	A6-6-S^r	probably identical	pA5 ff
	A. radiobacter	1001		Riker
	" "	1005		Riker
	" "	1007		Riker
CG-7	A. tumefaciens	[illegible] auxotrophs from CG-2		
CG-8	A. tumefaciens	[illegible] requiring (?). Prob. identical		
CG-9	"	auxotrophs from CG-1-S. UV.	grows on YNA	
CG-10-15	A. tumefaciens	from CG-1-S, UV.	~~[illegible]~~ Auxos, not run down	
CG-16	A. tumefaciens	" " "	Biotin -	

A1a

H. W. Klemmer

Antibiotic resistant cultures of Agrobacterium tumefaciens

Resistant cultures were developed to five antibiotics from a sub-culture (A6K1a) of a single-celled culture of the virulent A6 strain of A. tumefaciens. Three of these cultures are still in stock (on antibiotic-free, yeast extract-mannitol (medium 79) slants), the others have been lost. Those remaining have not been tested for resistance for at least 2 months. All five cultures are listed below, along with the level of resistance obtained and the % loss of virulence as compared with the parent A6 strain.

The parent A6 strain has been assayed against several antibiotics by De Ropp [Phytopath., 39, (1949)] using a 100% inhibition in broth, 24 hour endpoint, and by Klemmer [method of Josyln & Gaulbraith, J. Bact., 59 (1950)], using a 50% inhibition in broth, approx. 8 hour endpoint. The 50% inhibition levels (ID50's) of the A6 strain, and, where known, the 100% levels are given below in the table

Culture resistant to	Level of resistance µg/ml	% loss of virulence	Parent A6 culture - inhibited at (µg/ml) 50%	100%
Penicillin A6PEN	1000	80	8.0	1000
Streptomycin lost	800	20	3.5	50
Polymyxin A6P5a	150	80-90	5.6	
Chloromycetin A6C5	40	50	.24	2
Terramycin lost	15	20-30	.04	

The cultures still in stock; resistant to penicillin [A6(Pen.)], to polymyxin [A6(P5a)] and chloramphenicol [A6(C5)]

If you want more information phone 2318 for Howard Klemmer

Media

I. To maintain stock cultures. Adaptation of "Medium 79" of Fred and Waksman

AGI 1

Medium 79		Modification I (DCG)	
Mannitol	5.0 g	Sucrose	5.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g (anhyd. 0.1 g)	$MgSO_4 \cdot 7H_2O$	0.2
K_2HPO_4	0.2 g	K_2HPO_4	0.2
$CaCO_3$	1.0 g	$CaCO_3$	1.0
2% starch-free yeast extract	100 ml	Difco YE	5.0
Agar	15 g	Agar	16.0
Water to 1000 ml		Water	1000 ml
pH 6.8 (adjust)		pH 6.8 (adjust)	

In making mix, $CaCO_3$ & agar could be omitted. Then medium $\bar{s}$ agar or $CaCO_3$ could be used as complete broth; $\bar{c}$ agar but $\bar{s}$ $CaCO_3$ as complete medium for plating. Also omit sugar from mix (Sucrose OK for crown gall org. according to Dr. Riker; mannitol used for Rhizobium.)

II. Minimal medium used in glycine attenuation experiments (van Dienen, Baldwin, & Riker, J. Bact. 63, 715-721 (1952)).

SM

ST

Aniline blue

AG II

Mannitol	5.0 g	
KNO_3	5.0 g	According to Dr. Riker, sucrose can be substituted for mannitol in media for Agrobacterium
$CaCl_2$	0.19 reduce to 0.1	
NaCl	0.2	
K_2HPO_4	0.2	
KH_2PO_4	0.1	
$MgSO_4 \cdot 7H_2O$	0.2	Sterilize singly separately?

Distilled water to 1 l.

Adjust pH to 6.8.

see p 33

McIntire, Riker, and Peterson (1941) found traces of Mn. Fe+++ + Zn helpful in a synthetic medium (Mn as sulfate at 0.1 µg/ml; Zn as sulfate at 0.5 µg/ml; Fe+++ as ferric alum at 5.0 µg/ml.)

3-23-54

Made up mix for complete medium (Modification I of Medium 79 = AGI). Made up 200 ml for slants. Tested pH of mix + 0.5% sucrose (before adding $CaCO_3$ or agar). pH = 6.78 ∓ adjustment.

Possible approaches: —

1. Glycine
   A. Repeat glycine attenuation using A6. This would at least serve to give a second avirulent strain.
   B. Plate on glycine to get glycine-resistant col. (Could this be used as a marker?). Van Lamen, Baldwin, & Silver (1952, p 717) indicate that glycine-resistant clones are of normal virulence — This argues against "glycine attenuation" being a selection of cells which are glycine-resistant and avirulent. [But see Tryptophane paper — virulent more sensitive than avirulent]
2. Markers other than virulence
   A. Antibiotic resistance. Cultures on hand resistant to penicillin, chloramphenicol, and polymyxin. Try to get an S^r; also check others.
   B. Nutritional — Penicillin runs?

3. Actual repetition of Klein's experiments, especially the U-tube experiments.
4. If markers are obtained, test for recombination of markers in mixture and U-tube experiments.

3-24-54

Transfer all A. tumefaciens cultures to fresh AGI medium.

Try A6 & A6-6 on some routine E. coli media to see whether they can be used at all.

Penicillin method as used for E. coli

1. Grow up culture
2. Inoc. ca 1 ml grown culture into complete medium (Benassay for coli.)
   Add antifoam, aerate 3-3½ hrs.
3. Cfg 10 minutes, wash & saline.
   Resuspend in 10 ml saline, cfg 20 min.
   Wash pellet, resuspend in 10 ml saline
4. Add 0.1 ml suspension to each of 2 DO tubes
   Add 0.2 ml penicillin soln to one tube (other untreated)
5. Incubate at least 4 hours.
6. Spread on complete plates (0.1 ml culture and 0.1 ml of a 1:10 dilution). Replicate to minimal.

[illegible] run, [illegible] pen. [illegible] giving survival of 10^{-6} or more (Meth Med Res)

Variables to check:

Pen. conc.

Age of inoculum

Inc. time in penicillin medium

Complete medium

Minimal medium

Temp; aeration?

3-26-54

Test of growth on coli media -

A6 inoculum evidently poor - no growth in any medium tested.

A6-6 - growth in all liquid media tested (D-O, NSB, penassay)

Very scanty growth on EMB media and on D-O; none on EMS.

A6-6 growing well in stab; A6, little or no growth. (Re-inoculate A6 stab from fresh slant).

3-29-54

A6 & A6-6 well-grown in NSA & Penassay - growth scanty in D-O. Good growth on EMB media;

poor on D-O.

Try one of the regular Agrobact. minimals

Growth more uniform in NSB than in Penassay.

More clumping in latter.

Penicillin run

1. Incc. fresh Penassays from those incc. 3-24. (1 ml incc/10 ml Penassay tube). (Using Penassay rather than NSB because it is a more complete medium).

Streptomycin

Spread 2 drops Penassay culture of A6 & of A6-6 on B. lac SM plates to look for S^r's

Sealed stabs of A6 & A6-6

Made up 20x salts for AG II. (minimal)

3-30-54

Made up AG II minimal liquid
Regulated [illegible] 213 to 26° and
moved streptomycin plates [illegible]

Pen run

2. Penassay tubes inoc. 3-29 fairly [illegible]; aerated at [illegible] (hour [illegible] 9:00 AM) to see if it would help growth.

Try yeast broth for next pen run (AG I liquid)

Make up AG I and AG II plates.

Inoculated 1 ml old cultures (yesterday) of A6 + A6-6 into 10 ml tubes AG I. Inc. at 26°.

Pen run

3. 1:30 pm. [illegible] cultures, though they are not as well grown as [illegible] at corresponding step. A6 fairly uniformly turbid; A6-6 stringy [illegible].

3-31-54

Pen run: Control tubes barely turbid, penicillin tubes clear. (Used AG II as minimal). Plate out at ca 48 hrs?

How get heaviest & most rapid growth in complete medium?

Try: 1) AG-I broth at 26°

2) AG I broth aerated at [illegible] temp.

3) Penassay at 26°

Inoculate each tube c̄ 0.1 ml old Penassay culture. Inoc. 10:00 AM 3-31.

Streptomycin

Plates inoc c̄ broth culture 3-29:

papillae, probably S^r, against background of very scanty growth. Number per plate ranges from ca 6 to ca 20. [illegible] 50-200 per plate

Picked 12 papillae each of A6 & A6-6; streaked from small waters onto Bacal SM.

If these appear to be S^r, pick single colonies, streak on complete s̄ SM; replicate to SM medium.

4-1-54

Penicillin run

Both penicillin and control tubes turbid. ∴ In next run plate at 24 hours even if growth in control tube is very scanty.

Comparison of complete media - 24 hr growth
Penassay at 26° - poor
AGI at 26° - a little better than Penassay
AGI aerated at room temp - very good growth, especially of A6. Considerably more turbid than AGI tubes incubated at 26° ∃ aeration for 48 hrs

Use aerated tubes to start second penicillin run

4-2-54

Second pen run

Good growth in control and pen tubes in 24 hrs.
1) For next run, use at least 1000 U per ml
2) Spread dilutions from old penassay tubes directly on AGI plates & replicate to AGI. These old cultures have accumulated many S^r cells and probably many pen. resistant; perhaps there are auxotrophs which could be picked up directly.

0.1 to 10 ml

$\downarrow$ 0.1

10 ml = 10^{-4} $\xrightarrow{1\text{ ml}}$ 10^{-5} $\xrightarrow{0.1\text{ to plate}}$ 10^{-6}

$\downarrow$ 0.1

10 ml = 10^{-6} $\xrightarrow{0.1\text{ to plate}}$ 10^{-7}

On basis of 10^8 - 10^9 cells per ml plate 10^{-6} & 10^{-7} dilutions.

4-3-54

Replicate spread plates to AG II.
(May have to wait until Sunday.)

Pick S^{N} colonies, streak on AG I $\bar{5}$ SM.

Spread plates - wait for rep. [illegible] minima until Sun or Mon. - no visible growth at 24 hrs.

Streptomycin -

A6 - Streaks from all 12 [illegible] now well grown on K real SM.

A6-6 Seven of 12 well grown; 4 now (3 days) beginning to show scanty growth in [illegible] part of streak (S^{N} mutations?)

Pick colonies from 2 streaks from A6 & A6-6; streak on AG I plates $\bar{5}$ SM.

4-5-54 (Monday)

Streptomycin

Replicated S^r cultures streaked on AG I to AG I SM.

Spread plates from old Renassay cultures (2-4 [illegible] 10^{-7})

A6 - Only 15-20 col per each 10^{-6} plate. Plates moldy. Pick all individual colonies, spot on AG I & AG II. (Tho'd take too long to spot on complete, then replicate to minimal - 2-3 days required at each step)

A6-6 Replicate to minimal. (Ca 15 col/plate in 10^{-7} dilution; 100-150/plate in 10^{-6})

Penicillin run

Inoculate AG I broths from old Renassay cultures. Also inoculate broths from slants to use as [illegible] "stocks".

Note: Replica plating may not be very satisfactory c̄ this bug - Colonies either don't stick to the velvet at all, or else are lifted off the plate completely.

How number any mutant stocks? Using prefix A would lead to duplication. ∴ Will use CG for crown gall. A6 = CG1; A6-6 = CG2. Will not

give CG numbers to Klemmer's cultures until their markers are checked.

4-6-54

Spread plates –

A6 : spots on minimal not [illegible] grown to read

A6-6 : replicas [illegible] minimal : Only 1 small colony on complete not growing on min. Looks as though it was not picked up by velvet; however, pick & test to be sure. Spot on min. & complete plates c̄ A6 cultures being tested [illegible]

Streptomycin –

Saved 2 S^r each of A6 & A6-6

CG3, CG4 CG5, CG6

AGI slants & nutrient stabs.

Pen. run

Used 1000 U/ml

4-7-54

Penicillin run

A6 - good growth in penicillin tube (1000 U/ml!)
A6-6 - pen. tube clear. Plate out orig. & 1:10 dil.

A6 - Streak out & try to get penicillin sensitive colony. (Replica to plate spread c̄ penicillin ?). Then try pen. run again.

4-8-54

Penicillin run (A6-6) - Undiluted from pen. tube too crowded; 1:10 dilution OK for replication (ca 200 col/plate)

Started broths for pen runs on CG 3 and CG 5
Auxotrophs in S^2 cultures would permit SRP type experiments.

4-9-54

A6-6 penicillin run NOTE: A second type of colony has appeared on the AG-I plates spread from pen. run. The colonies which were small and white at 24 hrs are now (48 hrs) yellowish, considerably larger, and gummy. Now more small white colonies have appeared which were not visible at all 24 hours

ago. They are about 5 times as numerous as the earlier colonies. There is not a gradation in size of colonies at 48 hours, but a sharp, clear-cut difference.

Although these later colonies were not visible when the plates were replicated to minimal, they have now [illegible] as well on minimal as on complete, while the colonies which were quite well [illegible] complete when replicated, are now barely visible on minimal. This latter behavior rather slow growth on minimal after replication from complete, is more like that observed when plates were spread c̄ diluted culture S̄ penicillin treatment. (See pp A7 ff). However, in streptomycin experiment, many colonies appeared late on SM plates, after colonies had already been picked which were saved as S.^r stocks. Will pen run c̄ CG-5 yield only the cells which grow up faster on complete? Why should a penicillin run give a large yield of colonies which grow as rapidly on min. as on complete, when the mass culture used as inoculum apparently consisted mainly of cells which grow more readily

Unfortunately, the old broth culture used to start this run has been discarded in broth inoc. 4-5.

on complete [illegible] on mineral??

Take the following steps:

1) Streak out a colony of each type on AG I and AG II, along with mass culture of A6-6. Might as well include A6 also, for comparison.

2) Test penicillin resistance of both types.

Arbitrarily,

Early growth on complete, slow on min = a

Late on complete, good on min = b

3) Same both types

4) Above all [illegible] neither [illegible] is a contaminant

S[illegible] pen run: Inoc 2nd broth tube from broths of CG 3 & CG 5 which have [illegible] been aerated 24 hrs.

Pick a large no. of the type a colonies & spot directly (no suspensions) on AG I plates. They may all be auxotrophs, and in another 24 hours the type b colonies will be so well grown up that the plates will be over crowded for picking.

4-10-54

MAKE UP AG-I plates ✓

Penicillin run on A6-6

All "type a" col. seem [illegible] yellowish-blue - [illegible] on minimal at 48 hrs.

Make up random plates; test the colonies picked yesterday (ca 50 col). Save my plates.

For randoms, am using AA supp., YE and hydrolyzed casein.

"Type b" still small and white at 72 hours.

Streaks are [illegible] & complete; (about 20 hours)

complete

A6 - very scanty - single colonies barely visible

A6-6 - slightly better growth than A6

(These were streaked from [illegible] (cultures))

"Type a" - Quite good growth - Individual colonies large enough so they could easily be picked.

"Type b" - growth very scanty - comparable with A6

Minimal

A6 - very scanty
A6-6 slightly better than A6, but very scanty
"Type a" - no growth
"Type b" - barely visible

Plated pen run c̄ CG 3 & CG 5. Used 1000 U/ml

A6 streaked for test on penicillin - not many isolated colonies - Also no plate available to which to add pen. Pick several colonies to small Penassays & test [illegible].

Save a "Type b" A6-6 [illegible] on slant

4-11-54

CG 3 OK for plating - CG 5 [illegible] in pen tube. Plated 0.1 ml of 1:10 & 1:100 CG 3

4-12-54

CG 3 plating - no growth [illegible] hrs. Try plating direct from [illegible] tube. (Use AGI; no AGISM available)
No turbidity in pen tube at [illegible] hrs.

Streaks on minimal & complete - 3 days.

Complete - A6 and A66 - both have 2 colony types; One small & dense; other larger and more translucent. Pick and save both types.

"Type a" from pen run: Turning [illegible] margins, looks more like the translucent than the dense type in A6 [illegible]. Centers dense & [illegible], more [illegible].

"Type b" - White; uniform density (quite dense), margins [illegible] & regular.

Minimal - A6 & A6-6; 2 colony types, one moderate size & translucent second dense but very small - hardly [illegible] pull size.

"Type a" - like first type [illegible]

"Type b" - virtually no growth [illegible] [illegible], but [illegible] [illegible] shadows or ghosts; [illegible] diameter [illegible] but they are transparent, not visible unless light is just right.

Auxotrophs replicated to rundowns: No growth on AA's except for 4 poor mutants or slow growers. Scanty growth on YEX and hydrolyzed casein. Try pure iso-dc and vits. From Dave Gordon's experience - also try reduced sulfur.

4-13-54
(Tuesday - Make up 2X AG-II - incl. some in tubes.

Pen. run on CG3 - Colonies present at 48 hrs. ~ in appearance to the col. appearing at 48 hrs in previous pen expt. Ca 50/plate from 1:100 dilution. This corresponds to [illegible] in A6-6 expt. Replicate to mineral. This is Sr deriv. of A6.

A6-6 run: choose 1 auxotroph & inoc YNA, Vits, & control AG II tube.

4-14-54

A6-6 auxotroph. no growth at 20 hours in minimal + YNA or minimal + vits. Incubate at least one more day.

To summarize:

Poor growth c̄ hydrolyzed casein
Trace of growth c̄ yeast extract
No growth c̄ any AA group
" " (24 hrs) c̄ YNA or vits.

Check whether present minimal medium favors growth of rough (small) colony type over mucoid (large).

CG 3 pen run -
Colonies on min. not large enough to score. No further colonies on complete beyond no. at 48 hrs. These colonies all are the small "type b" (≈ rough?)

It seems despite that each culture as received (A6 & A6-6) a mixture of a "rough," small colony type and a very gummy, "mucoid" type.

To be verified:

1). The "rough" grows more readily than the gummy on the present minimal medium (AG-II).

2) Despite this, the "rough" is selected for in a penicillin run.
3) All "mucoid" survivors of a penicillin run were either auxotrophic or very slow in their growth on minimal.
4) CG3 (A6 S~) is "rough"

Streak out all stocks on complete. Examine colony type. Where mixed, save both (or all) components.

Also: streak A6 + A6-6 both types, on AGI 14. Compare c̄ CG3 + CG5 [illegible] streaked.

4-15-54

CG3 pen run: One possible auxotroph. Inoc. tubes of minimal & complete.

A6-6 auxotroph [illegible] vits or YNA at 48 hrs. Try single omissions c̄ AA groups.

4-16-54

CG3 possible auxotroph: at ca 20 hours, growth in complete, none in minimal. Inoc. slant; continuing incubation of broths.

A6-6 auxo (slant #1) - AA single omissions of groups:
Growth in -AA4; also in hydrolyzed casein.
Test c̄: HC + AA4
AA 1, 2, 3, 5 + the individual components of AA4.
Also - to find actual requirements, test all combinations of AA groups, excluding 4.

A1 + A2	A2 + A3
A1 + A3	A2 + A5
A1 + A5	A3 + A5
	A1 + A2 + A5
	A1 + A3 + A5
	A1 + A2 + A3

Just need to do single omissions -

1 + 2 + 3	1 + 3 + 5
1 + 2 + 5	2 + 3 + 5

Check of colony types of stocks on complete medium

A6 original (CG-1): Predominantly large, dense, yellowish.
A few colonies smaller, white, translucent
A6-6 original (CG-2): Moderate size c̄ dense, yellowish centers. Seems homogeneous

A6 "R" : Small, white; mass growth looks rough
A6 "M" : Large, c̄ yellowish centers. Gummy.
A6-6 "R" : Like A6 "R"
A6-6 "M" : Like A6 "M"

Small (prototrophic) from A6-6 pen run; Small but not rough. Centers yellowish.
Large (auxotrophic) from A6-6 pen run; Colonies not very large. But more yellow & gummy than A6-6 "M".

A6 PEN (Klemmer) ; } Much like A6 "M"
A6 P5a "
A6C5 "

CG3 (A6 S^r) - Seems intermediate between A6 "R" & A6 "M"; colonies somewhat yellowish, but mass growth looks slightly rough
CG4 (A6S^r) - Both large & small colonies; large have yellowish centers, Mass growth smooth, quite gummy.
CG5 (A6-6S^r) - Like CG-3.
CG6 (A6-6S^r) - Like A6-6 "R"

Streptomycin resistance; A6 "R" & "M" and A6-6 "R" & "M" failed to grow at all on AG-I SM in 48 hrs.

CG 3 - As on AGI $\bar{5}$ SM except colonies are smaller. This could be because medium is rather old.

CG 5 - Looks rough on SM medium, colonies very small.

Cross-brushing c̄ penicillin (loop of 10,000 U/ml soln); Pen. conc. apparently insufficient for any definite effect.

Brush of A6-6 auxotroph from pen. run looks so yellow compared c̄ other cultures that it seems wise to try to make sure it really is A. tumefaciens.

4-17-54

CG-3 possible auxotroph; at 2 days, growth is minimal. So far (24 hrs) no growth in rundowns or in complete - i.e. at same time (inoculum probably very small). Streak from 48 hr complete onto complete plate & try several individual colonies

before discarding as no good.

A6-6 auxotroph:

Original single-omission tubes (now 2 days): Good growth in −AA4; poorer growth in hydrolyzed casein; now slight growth in −AA5. Still no growth in other single omissions or in minimal.

Tubes inoc. 4-16 (24 hrs)

HC	+	complete	++
HC + A4	+	AG II - O	−
1+2+3	−	AA-4 +H	+
1+2+5	−	AA-4 + T	±
1+3+5	±	AA-4+G	+
2+3+5	−	AA-4+P	+
		AA-4+A	+

!!

4-19-54

CG-3 possible auxotroph: rundowns, 3 days: Slight growth in all tubes, but good growth only in AA5. Will pick & test several col. from plate streaked 4-17 in min, complete, & AA5.

A6-6 auxo; original single omission tubes, now 4 days; There is some growth in all tubes except minimal and − AA1 (includes [illegible]). Sulfide??

Second run down test (now 3 days);

HC	++++	complete	++++
HC+A4	++++	minimal	−
1+2+3	+	AA−4+H	+++
1+2+5	+++	−4+T	+++
1+3+5	+++	−4+G	+++
2+3+5	−	−4+P	+++
		−4+A	+++

Try AA1 group & individual acids of group; [illegible] Na_2S

4-20-54

CG-3 picked [illegible] separate colonies.
At 24 hrs, no growth in [illegible] min + AS; [illegible] turbidity in complete. Continue [illegible]

A6-6 auxotrophic. Growth in A1 (Why didn't this happen on plate?) but not in individual AA of the group [illegible] Na_2S.
Try single omissions [illegible] at higher [illegible]

4-21-54

A6-6 amastiph At ca 24 hrs, no growth in A1 or gb
omissions or in A1. ~~(Control number all mod
fluid & current test of pg the end, and
acid)~~

good A1 conditions (2 days) A1 group - quite good growth,
Slight diffuse turbidity and small pellicle. Weak.
[illegible] at same day.

CG-3 "amastiph" from right eye - good growth especially
good in mix + A5. T. O.

4-23-54

A6-6 amastiph; At 3 days, moderately good
growth in AA1 - organisms - Square. Poor growth
in AA1 capsule group. No growth in AA1 - mutt
in - cap. Kill. Dry surface organisms.

4-26-54

J.L. reports that Kleiss results were due to media contam
& cannot be reported. However, they may be less
explosive on [illegible] plates - [illegible]
can be counted just as other organisms.

Try [illegible] Use A6 only, since it is not necessary to have autotrophs [illegible] A6 [illegible]. Use A6 "R" & A6 "M" to see if any difference in behavior in pen run.

Inoculate [illegible] of the [illegible]

A6 "R" } [illegible]
A6 "M" }

A. radiobacter 1001 }
" 1005 } to [illegible]
" 1007 }

4-27-54

Broths inoc. 4-26: Moderately good growth in all. The "rough" A6 has a pellicle. Growth of A. radiobacter in this medium (AG-I) very similar to "smooth" or "mucoid" A. tumefaciens.

Streak each radiobacter culture on AG-I, see whether more than one colony type.

4-29-54

Stocks of A. radiobacter (48 hrs):

1001: 2 types of colonies: ① small
② large & gummy.

1005: [illegible] uniform [illegible] & gummy.

1007: 2 types, same as 1001.

Pick stock of both types where present.
Also pick & stock 1 [illegible] of 1005 to have stock arising from a single colony.

The large type is opaque, the small [illegible] transluscent. In mass growth the resulting appearance is much like plaques.

Inoculate broths for pen run on A6 'R' & 'M'.
Aerate

4-30-54

Started pen run on A6 'R' & 'M'. 1000 U/ml penicillin

5-1-54

Both grew in pen tubes.

Hendrickson, A.A., Baldwin, I.L., and Yiker, A.S. 1954 Studies on certain physiological characters of Phytomonas tumefaciens, P. rhizogenes and Bacillus adiobacter. Part II. J. Bact. 67, 547
Absorption of aniline blue as a marker.

5-3-54

Start broth (aerated) of A6 M for irradiation.

5-4-54

Pump got turned off, so culture was not aerated over night. Has been aerated for about 6-7 of the 24 hours it was grown

Plate 10^{-5} & 10^{-6} dil. of orig culture (cfg, washed, resusp. in 10 ml saline)

Irradiate 40 sec in saline c̄ big lamp. Plate 10^{3}, 10^{4} & 10^{5} dil of irradiated culture.

(to kill [illegible] more [illegible] available)

5-6-54

Irradiation expt: 40 sec. UV [illegible] no detectable killing. All plates after irradiation too crowded to count. Duplicate control plates of 10^{-6} dilution gave average count of 328 per plate, i.e. 3.3×10^{8}. Had estimated 10^{6}.

One plate of irradiated culture has one colony which appeared in 24 hrs and is large, yellow, & gummy like previous autotrophs. Struck

out & test.

5-7-54

Streak from col picked from UV plate grew well on plate in 24 hrs. Sick one col. to mini & ex-plate broth.

5-8-54

At about 20 hrs, good growth of "auxotroph" in complete, none in minimal

Have cultures ready for plant inoculation by end of next week.

"R" and "M" cultures of A6 & A6-6

S^r from A6

Also - grow A6-6 S^r with A6 "M", recover S^r's test. Do control plating of A6 "M" to check rate of mutation to S^r.

Inoculated together A6 "M" (CG-1-M) and CG-5 from water suspension of rods.

5-10-54

Inoc AG-1 broth c̄ A6 "M" (CG-1-M) & CG-5 separately

UV auxotroph. No growth [illegible] in 3 days. [illegible] run down. A6 UV-1

Cultures to use for plant inoculation:

1) S^r from mixture expt.
2) CG-1 M
3) CG-1 R
4) CG-3
5) A6 UV-1
6) CG-2 M
7) CG-2 R
8) CG-4
~~9) A6 UV-1~~ 9) CG-5

Streaked mixed culture on AG I & AG I SM
[At the [illegible] these were streaked [illegible] about 48 hours in complete medium at room temperature without aeration.]

5-11-54

Since rundowns of A6 UV-1 (tubes)

Started [illegible] of A6 for UV killing curve

In mixture experiment, should have spread [illegible] & $\bar{S}$ SM $\bar{c}$ appropriate dilution, rather than streaking, to get estimate of proportion of S^r to S^r. Start an identical mixture, to be plated at ca 48 hrs. Also plate separate cultures on complete & complete + SM at [illegible] hrs.

5-12-54

AG UV-1 At 24 hrs slight growth only in complete and YNA.

Mixed culture - streaked on complete $\bar{c}$ & $\bar{s}$ SM -
On complete only, 2 colony types; large, gummy ("M" type); and small translucent ("R" type). The virulent sensitive culture used is an "M" type, and the avirulent, resistent, an "R" type.
On complete +SM; the "R" type only. Not yet large enough to pick readily.

UV killing curve;
Irradiated 10^{-4} dilution (in saline) of over night, aerated. broth (AG-I) culture of AG (M). Plate as control final dilution from original culture of 10^{-7} and 10^{-8}. Irradiate 30, 60, 90, 120, 180 seconds.
Through 60 sec, same dilutions as control
90-180 sec, 10^{-6} and 10^{-7}

Spread AG-I & AG I SM plates $\bar{c}$ 10^{-5} dilution of 2 day non aerated broth cultures of CG-1-M and CG-5.

5-13-54

(48 hrs)

A6UV-1 rundowns: fair growth in min. + YNA; none c̄ other additions, but slight turbidity in minimal; Rechuck. Also check purines & pyrimidines (as 2 groups)

Mixture streaked 5-10 on complete c̄ & s̄ SM:

Complete; no SM: Colonies predominately "M" type; some "R" type. Pick a number of both types, test for S character. ~~There are a~~ few of the large, gummy colonies which have always turned out to be auxotrophs ~~Pick + test.~~ Don't know whether 1-M or 5

Complete + SM: Great majority of colonies are the "R" type, like the S^r culture used. There are now a few "M" type colonies appearing. Pick a number of the "R" colonies and as many as possible of the "M" type. Use both in virulence test. The "R" type could be pooled, but the "M" type should be checked individually.

Plant inoculations to be done 5-14. Will not be able to pick & grow up separate colonies from mixture plate (except for a few "M" type).

Scrape colonies off agar (SM plate) from areas where there are no "M" type colonies; inoc mass into broth. Inoculate slant c̄ this mixture.

These are the cultures to be used;

CG-1-M	1-M+5 (M1)	"M" type from mixture streaked on SM (Each 1 colony)
CG-1-R	1-M+5 (M2)	
CG-2-M	1-M+5 (M3)	
CG-2-R	1-M+5 (M4)	
AG UV-1	1-M+5 (R1)	"R" type from mix. on SM; each pooled colonies
CG-3	1-M+5 (R2)	
CG-4		
CG-5		

Spread AG-1 & AG-1SM plates c̄ 10^{-5} dil. of 1-M+5 mixture inoc. into broth 5-11.

5-14-54

AG-UV 1 rundowns; Still no growth (3 days) except in YNA, and for some reason, minimal.

Set up Minimal, YNA, purines, pyrimidines

Plates of CG-1-M and CG-5 incc 5-12;
(Spread of 0.1 ml 10^{-4} [illegible] of 2 day, unaerated broth)

CG-1-M; low complete [illegible] of [illegible] size [illegible], glistening [illegible]. 616 col
low SA no colonies.

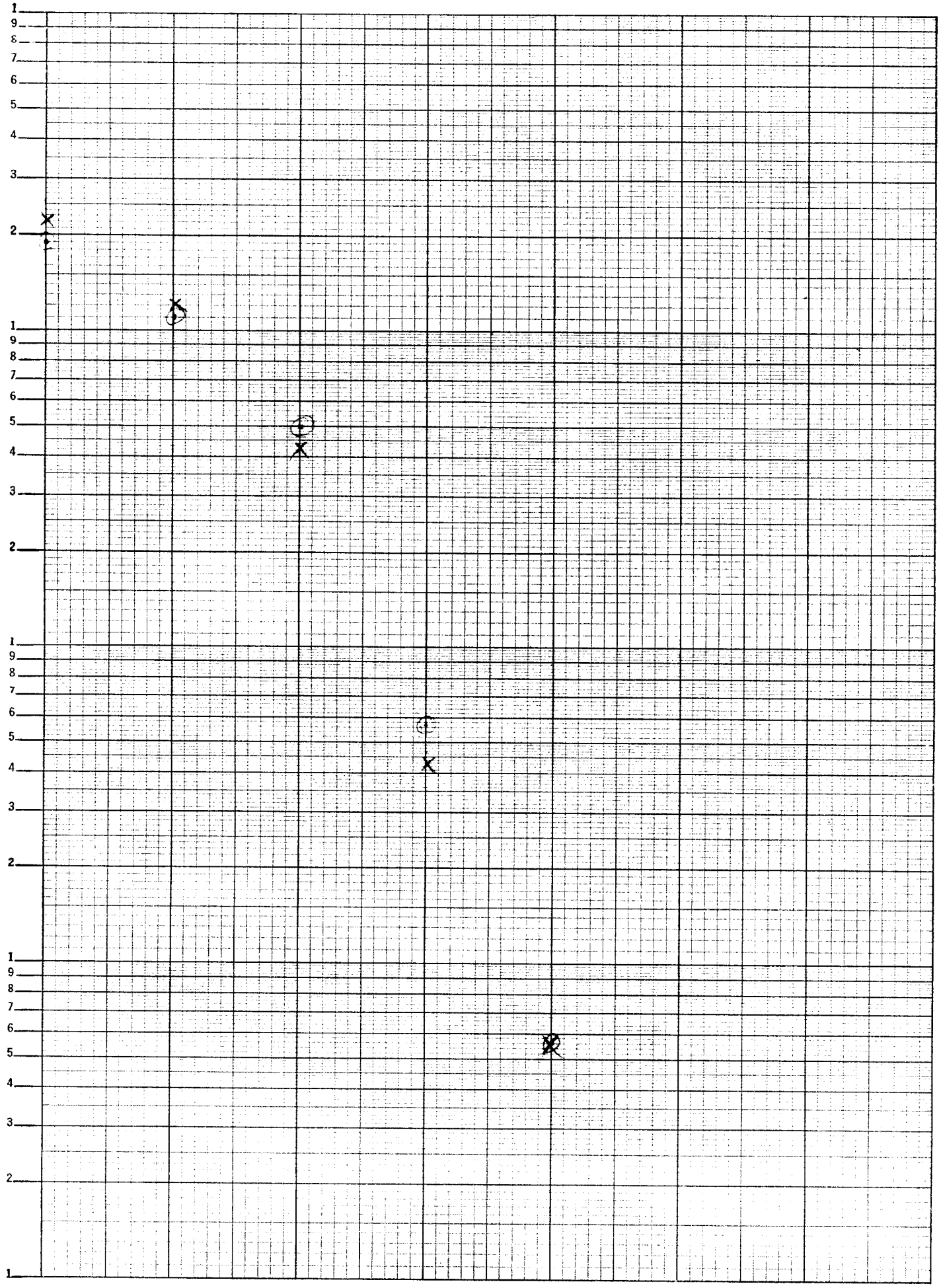
CG-5 [illegible] complete [illegible] and less [illegible] than CG-1-M. 997 col.
low [illegible] [illegible] [illegible], but much smaller. Looks [illegible] [illegible] may [illegible] too high for [illegible]

Colony types [illegible] to be quite stable; on each AG-1 plate there is one colony which looks as though it might be of the opposite colony type (1 "R" on 1-M plate; 1 "M" on 5 plate)

UV experiment

Exposure	10^{-6}	10^{-7}	10^{-8}	Exposure	10^{-6}	10^{-7}	10^{-8}
0	—	1465	219	120	54 0	2 0	—
30	—	1025	116	180	0 0	1 0	—
60	—	417	58				
90	432 165	70 20	—				

3 day [illegible] red


KEUFFEL & ESSER CO., N. Y. NO. 358-81
Semi-Logarithmic, 4 Cycles X 10 to the Inch, 5th lines accented

This is after < 2 days' incubation. The colonies on the 90 sec plates are very tiny, can hardly be counted. Continue incubation of this and the 120 & 180 sec. plates.

On the 90 sec 10^{-7} plate there are three very large gummy colonies, one of which is regular and [illegible] ○, one looks as though it arose from 4 cells stuck together ⊞, and one which has opaque and translucent sectors ⊛. Pick and streak these.

After one more day, replicate plates c̄ appropriate counts to minimal to check for auxotrophs.

Pick large gummy possible auxos from I-M + 5 plate streaked 5/10. Test for auxotrophy also whether $S^{\sim}$ to determine [illegible].

Plant inoculations: Growth of the following very poor; [illegible] inoculum small: 1-R 3 5 XM2 XM4
[illegible] plates [illegible] 2-R 4 XM1 XM3 XR1 XR2

5-15-54

UV killing curve expt.

More colonies have come up (3 days) on 90 sec plates, and some now present on 120 sec plates. Counts:

	10^{-6}	10^{-7}
90	432	70
120	56	2
180	0	1

Counts on 0-60 sec plates virtually unchanged.

Streaks from large col on 90 sec plate

One type of colony from the large, [illegible] col - gummy, [illegible] ①

Centered col. 2 types, one as along the other small & translucent. ②

Irregular col. all individual col gummy & opaque; but mass growth does not look uniform.

Same all these, test col. types for auxotrophy

Possible auxotrophs from mixture plate: very scanty growth in 24 hrs, [illegible] previous auxos on [illegible] plate.

Spread plate of 1 M + 5 mixture M-type seems predominant; difficult to tell the two types apart. Continue incubation. (85)
Dose SM plate. All col. very small; considerably lower count than on non-SM plate. (281)

5-17-54

90 sec. irradiation; streaks from large colonies; (now 3 days)
All except sectored (#2) show only one type of colony - usual large gummy type.
Streak from sectored col. - 3 types
1) large, gummy
2) smaller, translucent
3) Bright yellow, very rough, wrinkled (contaminant?)

[Many of the large, gummy colonies have narrow sectors of translucent growth.]
Pick, streak several of each type.

Possible auxotrophs from mixture plate: Pick, spot on complete for replication to minimal.

5-18-54

Possible auxotrophs from mixture expt: Replicate from AG-1 to AG-2 & AG-1-SM to check for auxotrophy and to tell whether CG-1-M or CG-5.

A6 UV-1; Attempt to run down specific growth requirement. At 24 hrs, growth only in YNA, not in purine mix nor pyrimidine mix.

Cultures from sectored colony, A6 UV plate.

1) "normal": Quite good growth for 24 hrs, no sectoring visible as yet; colonies appear uniform.

2) yellow; 2 col. were picked & streaked; one looks same as "normal"; the other shows very scanty growth at 24 hrs. No color. [EML had yellow contaminant on an EMS plate which looked similar to this yellow culture at 3-4 days]

3) translucent: Good growth in 24 hours. Colonies appear uniform. Very similar to "normal" but slightly less opaque.

Plates saved from UV run of 5/12: Replicated to AG-2.

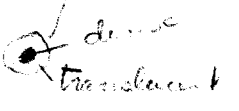
Started broths (AG-1) 3:30 pm of all A. radiobacter cultures for microscopic examination. Inc. at [illegible] temp $\bar{s}$ aeration.

5-19-54

A6UV-1 [illegible]: At 48 hrs, growth only in YNA; not in purine or pyrimidine mix. Before going into identity of requirement, recheck on AA's & on Na_2S.

Also: recheck A6-6 mutants on AA's, YNA, and Na_2S. In adding Na_2S to sterile medium, use directly from sterile stock soln and also diluted 1:10 and 1:100.

A6UV, cultures from sectored colony

1) "Normal": Quite a number of colonies (about a quarter of the well-separated colonies on plate) now show small sectors of translucent growth. There is one "pure" translucent colony and several colonies, instead of sectored, are of this [illegible] form. 

Pick: (1) translucent
(2) sectored (several)
(3) "pure" opaque ; strike out.

2) Translucent: Colonies are scarcely distinguishable from the opaque "normal". No sectors.
However, in one of the three strikes, very small translucent colonies of what [illegible] of the "R" type have now appeared (48 hrs).
From this strike, pick & strike opaque and "R" type.
The opaque colonies [illegible] normal "M" type in [illegible] cultures [illegible] rapid growth, forming colonies 1-2 mm in diameter in 24 hrs.

3) Yellow: The yellow [illegible] is a [illegible] contaminant - They [illegible] (orig. plate) [illegible] a peculiar sweetish [illegible] [illegible] appearing on [illegible] plate (48 hrs). The colonies present at 24 hrs [illegible] A/C, many are sectored as described under D).

Strike CG-1 M-R for [illegible] Hanson [illegible].

Possible [illegible] mixture plate. After [illegible] picked from orig. plate & striated [illegible] complete, 4 [illegible] picked from each of the 4 strikes. Of these, 2 from #1 and 1 from #4 are S^n. [illegible]

[illegible] that they oriented [illegible] CG-5. [illegible] [illegible] whether any are [illegible] so far it appears [illegible] [illegible] plate.

[illegible] seems very satisfactory, [illegible] liquid

Try: 1) [illegible] acetate
2) [illegible] ferric [illegible] ([illegible] manganese
([illegible], [illegible] - [illegible], 1941)
Fe^{+++} 5 γ/ml
Mn^{++} 0.1 γ/ml
[illegible] whether these conc. refer to
the [illegible] the salt ([illegible] and $MnSO_4$)
[illegible] $MnCl_2 \cdot 4H_2O$ [illegible] 1.24 mg/ml [illegible] 1:200
Fe_2 [illegible] stock 12.0 mg/ml ([illegible] [illegible] [illegible])

5-20-54 [illegible]

A6 IV-1 [illegible] 3 days Still [illegible] only [illegible] YNA.

[illegible] A [illegible] no growth [illegible] 20 [illegible] except in complete.

[illegible] from [illegible] plate. [illegible]

A6 [illegible] [illegible] [illegible]
Streak [illegible] 5/19 [illegible] [illegible]
agt. (24 hrs).

From "translucent" streak on 5/17 plate, what I called "R" type [illegible] yesterday are actually the yellow contaminant.

CG-1-R&M streaked for [illegible]
No visible growth on AG-1 in 24 hrs.
All the derivatives from the sectored colony (except the contaminant) grow up well in [illegible] hrs.

Replication of UV plates (Exp 2) to [illegible]
Found possible auxotrophs on only [illegible]
Six suspected auxos; 3 are the large colonies which appeared at 24 hrs.
∴ → Check all derivs. from sectored col. see if all are auxos. Pick up other 2 col.
Other 3 possible auxos were [illegible] being of different colonial appearance [illegible] small, less dense than "normal".
Numbering:
2UV-1 (#1 from 2nd irradiation):
Opaque, non-sectored from sectored col.
2UV-2 } Other 2 large col on 90 sec plate
2UV-3 }
2UV-4 } Small col from 90 sec plate
-5
-6 }

[illegible] of A. radiobacter with phase scope; [illegible] broth [illegible], [illegible], diluted 1:20, [illegible]

↑ 1001 S 1R 5 7S 7R

None [illegible] formation. Except for 1007R, [illegible] of any kind; 1007R had [illegible] clumps. Motility poor.

(100 U/ml)

Streptothricin resistant strains were obtained in 1947; could be useful marker. Also proflavin, 1008/ml. Try [illegible], SM, ST resistants in A6-6 auxos; A6UV-1; new auxos (2 UV series).
Try cross [illegible] to see whether it can be scored readily.

5/21/54

Hundreds of A6 [illegible] and A6UV-1: No growth in any supplement (48 hrs). [illegible]

2UV1 - 2UV6: No growth or only a trace one minimal. 1, 2, & 3 well grown at 24 hrs on complete, 4, 5, & 6 scanty.

Derivatives from sectored colony (≅ 2UV-1):
Sectored col. in streaks done 5/19, both from sectored and non sectored colonies. This culture is being saved as 2UV-1, so can be checked again later for continued suitability of colony type

Inoculated soft agar tubes c̄ [illegible] 1005, 1007S, and 1007R to see if they [illegible]; if so, can get more highly [illegible] cultures.

Gave stock numbers to first auxotrophs
A6-6 (1) = CG-7 } sulfide?
A6-6 (2) = CG-8 }
A6UV-1 = CG-9 (YNA)

5/22/54

Rundowns: (4 days; 1 day since Mn & Fe added)
CG-7 & CG-8: faint growth in YNA, possibly in highest Na_2S conc.
CG-9: good growth in YNA; possibly slight in highest Na_2S conc.

[illegible]/54

Rundowns (6 day [illegible] Mn & Fe added)
CG-7 [illegible] Fair growth in AA-1; [illegible] in YNA; slight in Na_2S orig. conc. and 1:10.
CG-9 Possibly slight growth in YNA [illegible] anything else.
(The [illegible] was added before autoclaving, & after autoclaving had a flocc[illegible] 1 ppt.)

5/28
Plant inoculations (now 2 weeks):
Galls on all plants inoc. ā CG-1-M, CG-1-R, CG-3, CG-4. S[illegible] from A6 show little or no loss of virulence.

Spotted CG 7, 8, & 9 and 2UV-1 through 6 on AG-1 for replication to suppl. plates.
(Note: Mineral plates now contain Mn & Fe)

Started aerated broths of CG-7 & CG-9 to be spread on SM & ST media.

5-29-54
Spreaded CG 7, 8, & 9 & 2UV1-6 to supplemented plates

Spread aerated over night cultures of CG 7 & 9 on SM and ST plates (SM 1/4 the conc. used [illegible]; ST 100 U/ml.)

5/31/54

Rumbows of auxos (plates)

	O	A1	A2	A3	A4	A5	HC	VITS	YNA	Sulfide
CG 7	−	−	−	−	−	−	−	−	−	−
8	−	−	−	−	−	−	−	−	−	−
9	+	+	+	+	±	+	−	−	+	+
2UV 1	+	+	+	±	±	+	+	−	+	+
2	−	−	−	+	+	±	+	−	−	−
3	+	+	+	+	+	+	+	−	+	+
4	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−

All [illegible] replication to complete.

Can [illegible] some of these results by assuming insufficient inoculum for 11 plates from one original.

Continue incubation. For 2UV 4, 5, & 6 try AA [illegible] omissions, AA + [illegible] yeast extract.

Recheck CG 9 and 2UV 1 & 3 in liquid [illegible] may [illegible] to something in agar. Also check in minimal [illegible] [illegible]

ST and SM expts:

CG 7: SM [illegible] too low; background growth quite heavy; no resistant [illegible] visible.