ST: Almost confluent growth of very small colonies; perhaps enough ST^r present so inoculum should have been smaller.

CG 9: SM: Light background growth + ca 800-1000 resistant papillae per plate. Papillae are of both the smooth + rough (or dense and translucent) colony types.

ST: Very light background; no papillae as yet.

6/11/54

Rundown plates: Only change over night: response of 2UV2 more clear cut

A3 +
A4 +
HC ++
All others −

Would have expected CG 7+8 to respond to A1 as they do in liquid media.

CG 9 S^r: All 4 col. picked as possible S^r growing well on AG2SM.

Other antibiotic plates (now 3 days)
CG 9 ST: No change: no papillae - background very light

CG7 SM: Growth so heavy, suggests this culture may already be S^r. Check slant

CG7 ST: Papillae almost confluent; picked a few of largest papillae & streaked on St medium. However, if this represents normal rate of mutation to St^r, this marker won't be very useful. Check slant - streak out & test individual colonies; if St^s, grow in broth & spread plates.

6/2/54

Rundown plates; CG7&8 now well grown on H.C.; faint growth of these & of 2UV3, 4, & 5 on YNA.

CG9 S^r: Pick 1 col.; streak on AG-1 $\bar{s}$ SM; pick & test several of resulting col. for S character; save 1 pure S^r.

Tried testing SM & St resistance of all auxotrophs by cross-brushing on AG1 $\bar{s}$ SM or St.

CG7 2UV1
8 2
9 3
4
5
6

6/3/54

Final readings on reindrow plates (5 days)

	0	A1	A2	A3	A4	A5	HC	VITS	YNA	Na_2S
CG 7	−	−	−	−	−	−	+	−	−	−
8	−	−	−	−	−	−	+	−	−	−
9	+	+	+	+	+	±	(−)	(−)	+	+
2UV1	+	+	+	+	+	+	+	(−)	+	+
2	−	−	−	±	±	+	+	−	−	−
3	+	+	+	+	+	±	+	(−)	+	+
4	−	−	−	−	−	−	±	−	−	−
5	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−

6/4/54

Tests of auxos for S & St sensitivity by cross-brushing

	S	St
CG 7	R	R
8	R	R
9	R	S

	S	St
2UV1	S	S
2	S	R
3	R	S & R
4	R	R
5	R	R
6	R	R

However, speed plates indicate that CG7 is not uniformly St^R nor CG9 uniformly S^R. CG7 did appear to be uniformly S^R.

CG7 [illegible] streaked out on AG-1; pick & test at least

10 col. for St reaction.

6/5/54

Picked col. of CG7, cross brushed c̄ St.

CG9; 2UV1 and 3; Check for auxotrophy.
Try 1) spotting on minimal
2) Growing in minimal tubes,
c̄ & s̄ aeration.

Checked motility of auxotrophs (microscopically from rather old plate). Under these circumstances only CG7 and 8 motile. All should be checked in soft agar.

6/7/54

CG7 single colonies/St; all 10 St^R.

CG9, 2UV1 + 3; Failed to grow in aerated or non-aerated min. tubes (2 days). On min. plate, growth very scanty. Unfortunately failed to spot w.t. as control on plate.

6/8/54

Made rundown plates - single omissions of AA groups - to check 2UV3, 4, & 5. Will also include other

auxos, since none of the requirements is completely determined

Spotted auxos on AG 1 for repl. to rundowns

6/9/54

Auxos on AG-1 (24 hrs); Good growth of CG 7, 8 + 9; 2UV1, 2, + 3; fair growth of 4, 5, + 6. Replicated to AA group single omission rundown

6/10/54
Streaked all CG and radiobacter cultures on AG-1 c̄ aniline blue added (1:10,000).

6/11/54

Examined galls (4 weeks). See results on next page. Saved plants for recovery of organisms from galls.
In plants in 1-M+5 series, where one +, for example is indicated, there were usually one or 2 good galls and very little hypertrophy at the other inoculation sites.
In other cases (purified cultures - A6 derivatives) galls were quite uniform at all sites.

Doubtful auxotrophs spotted on AG-2 June 5.
Moderate growth (CG9, 2UV1 #3) Should have used prototroph control. Still no growth in minimal tubes inoculated at the same time.

Random ones replicated 6/9.

	0	-A1	-A2	-A3	-A4	-A5	AA+	HC	Y.EX	complete
CG 7	−	±	+	+	+	+	+	+	+	+++
8	−	±	+	+	+	+	+	+	+	+++
9	±	±	+	+	+	+	+	−	++	+++
2UV1	±	−	+	+	+	+	±	+	+	+++
2	−	+	+	+	+	+	±	+	+	+++
3	±	±	+	+	+	±	+	+	+	+++
4	−	−	±	±	±	±	−	−	+	++
5	−	−	±	±	±	±	−	−	+	++
6	−	−	±	±	±	±	−	−	+	++

Note: this is the second time CG9 has shown anywhere from a trace to good growth on minimal and a variety of supplements, but no growth on minimal + HC.

Attempt isolations from (circled) plants.
Macerate galls in sterile H_2O, streak heavily on B glu and AG-1 + an. ll.

Plant readings
6/11/54

control 0
0
0

CG5 (+)
" +
" ±

CG2M ±
" ±
" ±

CG4 +++
+++
+++

CG3 (+++)
+++
++

CG1-R + (poor growth of plan
++
(++)

CG1M (+++)
+++
+++

AGUV-1 0
(±)
0

1M+5 R1 ± (one good gall)
1M+5 R1 +
" " R4 +
" M3 +
M3 +
(M3 ++)
M2 +
M1 +
M1 ++
M2 ±
M2 +
M1 +

1M+5 (R2) +
R2 ±
R2 +
M4 ±
M4 ±
M4 +

When through c̄ plants, cut tops off & throw in garbage can; dirt in large cans near door.

6/12/54

At 2 days, no absorption of aniline blue c̄ any cultures used.

6/14/54

Made attempts to recover bacteria from galls, as listed on previous page. Macerated slice of gall tissue in 2 ml saline, streaked on 3 AG-1 at

Rundowns – final reading (replicated 6/9)

	0	–A1	–A2	–A3	–A4	–A5	~~AA+~~	HC	YEX	Complete
CG 7	–	±	++	+++	+++	++	++	++	+	++++
8	–	±	++	+++	+++	++	++	++	+	++++
9	±	+	+	++	+	+	+	–	+	+++
2UV1	±	±	(++)	++	+	+	+	+	+	+++
2	–	++	++	+++	+	+	++	+	+	+++
3	±	++	++	++	+	+	+	+	+	+++
4	–	(–	+	+	+	+	+	+	+	++
5	–	–	+	+	+	+	+	±	+	++
6	–	–)	+	+	+	+	+	–	+	++

Being fed at one edge by mold colony

Note that most cultures grew best in the absence of A3, and CG 7+8 in the absence of A4.

2UV 4, 5, + 6 Try in A1 alone + c̄ various other ~~~ ~~~. Also try this c̄ CG 7+8. In this expt,

CG 9 & 2 UV 1 & 3 grew little if any on unsupplemented minimal, in contrast to previous experiment.

6/16/54

Isolations from galls: After 2 days, all plates have colonies predominating that look like Agrobacterium. Some col. from galls inoc. c̄ S^R (CG 4 + CG 5) absorb aniline blue strongly.

Pick at least 10 col. each. Test for prototrophy/auxotrophy and SM. Save for tests of virulence. CG-1-R as received is still rough

Save actinomycete on 1-M + 5 (M3) plate for J. L.

6/17/54

At 3 days, the col. from CG-4 that absorbed aniline blue are very large, gummy, and yellow. Test one of these and one ordinary, colorless colony for SM resistance and auxotrophy. The blue-absorbing col. from CG 4 did not become yellowish or very gummy.

Now, on 3rd day, more contaminants are showing up in streaks; most are highly pigmented, yellow, orange, and pink.

R2 from mixture experiment came out smooth, unlike CG-1-R, which was rough when recovered. This probably indicates that the organisms causing the gall were from the CG-1-M used in the mixture, carried over as a contaminant when S^R growth [illegible] was picked, or perhaps a mutation to S^R in CG-1-M

For consistency, CG-1-M and CG-2-M should be called smooth, rather than mucoid, because they are not unusually gummy for this organism. As of now, CG-1-M = CG-1-S; CG-2-M = CG-2-S.

Made up a batch of AG-1 agar using 0.5% mannitol instead of 0.5% sucrose to see whether a different carbohydrate would make any difference in aniline blue absorption.

Made up 20X salts solution for AG-2, incorporating Fe+ & Mn in soln (from conc stock [illegible]).

6/18/54

Some streaks from gall isolations not yet grown up enough to pick. Continue inc. for another day.

6/19/54

Spotted all auxos on complete for replication to AA's + AA1. Stored supplemented plates in frig.

Picked 1 col from each streak from 6/15/ plates of gall isolates.

CG-1-S: some streaks have a few R-type col.

CG-1-R: all R type col, except 1 streak mixed S & R.

CG-4: 3 of the ten streaks are yellowish, with intense blue color in the medium. The remainder are ordinary white S colonies.

CG-5: One streak - no growth; of remaining 9, one has blue colonies & intense blue coloring in medium. Blue colonies are small and not gummy.

CG-9: White, S-type col. Some streaks have a very few R.

X M3: 1 yellow, very gummy; 2 moderately gummy remainder less gummy and rather translucent.

X R2: All colonies moderately smooth; not so smooth CG-1-S

Spotted each suspension on complete for replication to minimal & SM.

6/21/54

Replicated gall re-isolates to AG1, AG1 (SM) & AG2

(used half the conc. used for E. coli (0.1 mg/ml))

(3 of the isolates from CG4 & 2 from XM3 are very yellow & gummy; check identity)

Replicated auxotrophs to complete, mini, and A1 + other AA groups singly.

6/22/54

Put in stabs:

CG-1-S
CG-1-R
CG-2-S
CG-2-R

} Have stabs only of A6 and A6-6 as received (mixed S & R)

CG-7	2UV-1	2UV-4
CG-8	2	5
CG 9	3	6

In plant experiment, inocula taken from mixtures were put on slants as well as inoculated into plants. Streak out R2 & M3, pick 10 or more colonies, test for purity $\bar{c}$ respect to SM.

6/23/54

Soil isolates

	Type of growth	SM		Type of growth	SM
CG-1-S	10 S	10 S^{s} (see note)	CG 9	10 S	10 S^{R}
CG-1R	2 S, 8 R	10 S^{s}	X M3	10 S	2 S^{S}, 8 S^{R}
CG-4	3 S yellow, 7 S white	10 S^{R}	X R2	10 S	10 S^{R}
CG-5	8 S, 1 R	9 S^{R}			

S^{S} more gummy than S^{R} though all are smooth type of growth.

CG-1-S ; all S^{S}, but all spots have a few S^{R} colonies (2 days) . CG-1-R ; no growth at all on SM.

All isolates grew very well on minimal.

The three yellow isolates from CG 4 are bright yellow and extremely gumming ; check microscopically

Randoms inoc. 6/21

	0	A1	A1+2	A1+3	A1+4	A1+5	A1+HC
CG 7	–	–	–	–	–	–	+
8	–	–	–	–	–	–	+
9	–	–	–	–	–	±	±
2 UV-1	±	±	±	±	±	±	+
2	±	±	±	±	+	±	+
3	±	±	±	±	±	±	+
4	–	–	–	–	–	–	±
5	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–

Might be able to try some SRP (or St^R P) experiments, using the following cultures. All are apparently stable auxotrophs, although their requirements are elusive.

	S	St	Source	Arrangement on test plates	
CG 7	R	R	CG-2		
CG 8	R	R	CG-2	1 S	2UV1
				1 R	3
2UV-2	S	R	CG-1	2 S	4
2UV-4	R	R	"	2 R	5
2UV-5	R	R	"	7	6
				8	
2UV-6	R	R	"		

Check these and CG-1 S & R and CG-2 S & R by cross brushing $\bar{c}$ SM & St. If the prototroph used is $S^S St^S$, one of these markers could be used, possibly along $\bar{c}$ virulence, as an unselected marker.

In testing SM & St status, include 2UV1, previously recorded as $S^S St^S$, as control.

Stabs - CG 7 stab appears to be contaminated (yellow). Inoc. new stab from slant.

6/24/54

Pick colonies from streaks of XR2 and XM3 from slants; restreak.

Auxotrons inoc. 6/21:

	0	A1	A1+2	A1+3	A1+4	A1+5	A1+HC
CG 7	±	±	±	±	±	±	++
8	±	±	±	±	±	±	++
9	−	−	−	−	−	−	(+)
2UV1	±	±	±	±	±	±	+
2	±	±	±	±	+	±	++
3	±	±	±	±	±	±	+
4	−	−	−	−	−	−	±
5	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−

CG9 has always failed to show even a faint trace of growth on HC alone.

Needed: a better minimal medium.

Try medium based on Hodgson, Peterson, & Riker, 1951

$(NH_4)_2SO_4$	1.0
NaCl	0.2
$CaCl_2 \cdot 2H_2O$	0.1
$MgSO_4 \cdot 7H_2O$	0.2
$ZnSO_4 \cdot 7H_2O$	8.8 mg
ferric alum	43 mg
$Mn_2SO_4 \cdot 4H_2O$	0.8 mg

Use K_2HPO_4 0.2 g/l, KH_2PO_4 0.1

per liter

+ sucrose 20 g/l for liquid, 5 g/l for agar

+ 40 ml pH 7.0 phosphate buffer per liter.

Use Mn + Fe solns as made up - Use 1:200

Make up $ZnSO_4$ soln to be used 1:1000

0.176 g / 20 ml stock soln.

This differs from present medium chiefly in N source ($(NH_4)_2SO_4$ instead of KNO_3); also has less $CaCl_2$ and includes zinc.

Transferred to slants an example of each kind of isolate from galls - 1 per gall where all appeared identical; an example of each type when there were 2 or more types from one gall.

6/25/54

6/21 rundowns:

	0	A1	1+2	1+3	1+4	1+5	1+HC
CG 7	±	±	±	+	+	±	++
8	±	±	±	+	+	±	++
9	±	—	—	—	±	—	±
2UV 1	±	±	±	±	±	±	+
2	±	±	mold	±	(+)	±	+
3	±	±	±	±	±	±	+
4	—	—	—	—	±	±	+
5	—	—	—	—	±	±	±
6	—	—	—	—	±	±	—

Checks of streptomycin and streptothricin resistance:

	S	St		S	St
CG-1-S	S	R	2UV1	S	S
1-R	S	R	3(?)	S	R
2-S	S	R	4	S	R
2-R	S	R	5	R	R
7	R	R	6	R	R
8	R	R			

Hence St not useful to distinguish between proto. & auxos. {2UV-4 had been scored S^R on previous test}

2UV6 looks like a good auxo to try in SRP test.
Problem: How make sure it is really *A. tumefaciens*?
1) Microscopic
2) Growth in complete broth, aerated and unaerated.
3) Growth at room temp + at 37°
(For (2) + (3), use CG-1-S & R for comparison).
4) - Eventually - plant inoculation.

6/26/54

2UV6, CG1-S, + CG-1-R: Aerated; Pump was turned off sometime during evening, so don't know how long cultures were actually aerated. CG-1-S aerated in

very much heavier than unaerated; slight difference between 1-R aerated and aerated; and little or no difference between 2UV6 aerated and unaerated. (ca 20 hrs)
Growth at 37°: CG1 S&R - slight growth; 2UV6 very little growth. Growth of all unaerated cultures at rm temp much better than at 37°.

Yesterday made up 5 plates AG1 c̄ 1:10,000 aniline blue (Coleman-Bell). Have been using National Aniline, same conc. Natl Aniline → pale greenish blue medium; Coleman-Bell → grayish purple.
Streaked CG1 S&R on plate made c̄ Coleman Bell dye.

Motility check on 2UV6: Motility about the same as CG-1-R; higher proportion of motile cells than CG-1-S. (Observations on 20-2½ hr cultures, AG-1 broth, rm temp.

Inoc. broth cultures of 2UV6 and CG-2-S, together and separately, to be inc. at rm temp c̄ + s̄ aeration.

XM3 & XR2 from slants ((I-S) + 5 mixture expt.)
Picked 1 col from each streak (12 from each culture),
spotted on AG-1 for replication to SM.

6/28/54

Test of [illegible]-Bell aniline Blue: Neither CG 1-S or 1-R colored on AG-1 with dye 1:10,000.

2UV-6 identity. Have reservations about this culture being Cycloclasticum because:

1) cells appear slightly smaller than CG-1
2) CG-1 grows slightly at 37°; 2UV-6 not at all
3) CG-1 grows poorly in AG-1 broth s̄ aeration
2UV-6 grows almost as well s̄ as c̄ aeration
(Final opacity of culture about the same, but requires 48, rather than 24 hrs. CG-1 never does grow very well in unaerated broth).

XM3 & X.R2 from slants: Replicate to AG-1-SM.

Plated CG2-S / 2UV-6 crosses on AG2-SM.

Grown Together - aerated   Grown separately - aerated
Grown Together - not aerated   Grown separately - not aerated

2UV-6 – In further check on identity, streak on EMB sugar media, see if it ferments any sugars.

6/29/54

XR2 & XM3 from slants: All 12 colonies tested from streak of each are S^R – no evidence of mixture. (Though of course S^R could come from mutation in CG-1-S, rather than from CG-5.

SRP plates (now only ca 20 hrs)

Controls: 2 UV6 aerated & unaerated: no growth.

CG-2-S unaerated: moderate background film, a few papillae (S^R mutants?)

CG-2-S aerated: moderately heavy background growth, no S^R papillae.

"Cross" plates

1) Grown together, aerated: Moderately heavy background film, possibly some papillae.

2) Grown together, not aerated: Less heavy background film, many papillae.

3) Grown separately, aerated: Moderately heavy background, no papillae

4) Grown separately, not aerated: moderate background, many (?) papillae.

When papillae better grown, pick, test for autotrophy. Probably auxotrophs being fed by background of CG-1-S. If prototrophic - !!!

Too early to try reaching any conclusions. Results observed so far could be explained by feeding of 2UV6 by background growth of CG-2-S; the aerated cultures, the background growth of CG-2- may be heavy enough to obscure papillae. This growth of CG-2-S seems to be due to too low SM conc, rather than confluent growth of S^R mutants, since papillae are readily seen on plates spread c̄ unaerated cultures. SM conc critical; at twice the conc. used, growth of S^R cultures is inhibited.

Next - Try "cross" using S^R auxo from CG-2, so a "normal" CG-1 can be used, and virulence of any resulting colonies can be tested. (Quite likely that S^R auxo from CG-1 may have lost its virulence - this apparently happened c̄ CG-9).

Use CG7 (from CG-2) and CG-1-S.

Grow as before, mixed & separately, aerated & unaerated. Also inoc extra mixture tube, inc. c̄ aeration for several days, rather than only 48 hrs.

Again - identity of auxo. as Agrobacterium is not definite.

[illegible] 1:30 pm 6/29

Picked 4 col. each 2-S / 2UV6 "crosses" grown together & grown separately & plated together. Streaked on AG 2SM.

6/30/54

SRP plates:

Controls:

2UV6 aerated and unaerated: no growth

CG-2-S aerated; Moderate background growth; one large colony; many small papillae (thousands).

CG-2S unaerated: Heavy background growth (confluent growth corresponding to small papillae on plate from aerated culture?) ca 100 large papillae, of which 10 are as large as normal colonies.

"Cross" plates

1) Grown together, aerated: Rather heavy confluent growth. About 100 large papillae per plate (4 plates). About 10 per plate are the size of normal colonies. On 2 of the plates the background is not quite so heavy as on the other two, and there are thousands of small papillae.

One plate of the 4 has what appear to be

phage plaques in the area of heaviest growth. The plaques are present only in the portion of the plate near two large mold contaminants. (Molds supplying factor needed for phage growth??)

2) Grown together, not aerated: Moderately heavy confluent background growth. Many more large & moderate-size papillae than on plates from aerated culture. ("Colony-size" papilla on 4 plates 25, 30, 60, & 70).

3) Grown separately, aerated: Light to moderate background; thousands of tiny papillae; on 4 plates, total of only 3 large papillae.

4) Grown separately, not aerated: Moderate background; a few tiny papillae; rather large number of medium and large papillae ("Colony-size" papillae on 4 plates: 44; 29; 12; 58).

Colonies picked from "cross" plates & streaked on AG2SM no growth in 24 hrs. Continue incubation. Also: check SM level of new batch of AG2SM by streaks c̄ CG2S and CG5 (or CG-2-S & CG 3 or 4)

Pick more papillae from "cross" plates, streak on AG1 (no SM). Pick colonies from streaks, spot on complete, repl. to minimal & SM.

Phage: Scrape up growth and plaques, add to AG1 broth. Also add to cultures of CG-2-S and 2UV6 in broth. 10:10 AM. Started aerating cultures of 2UV6 and CG-2-S in AG1; also growth & plaques scraped from plate. 1:15: added 0.1 ml broth from culture from plate to each of other two tubes.

Cultures for CG-1-S / CG7 experiment: CG-1-S growing rather poorly, both in aerated and unaerated culture. However, should be OK to use at 48 hrs.

7/1/54

2S / 2UV6 SR expt.: papillae which were tiny yesterday now full-size colonies; evidently S^R mutants of CG2S, since nos. are about the same on 'cross' plates & CG2S control plates. About 200-300 per plate from unaerated inocula; several thousand from aerated inocula, which gave heavier suspensions for plating.
Discarded plates. (Most were moldy)

Phage
✓ 1) Aerated cultures: a) virus direct from plate; b) 2UV6 + virus from plate; c) CG2S + virus from plate. None show evidence of lysis. Cross-brush each on complete med. c̄ 2UV6 & CG2S.
2) EMB plates streaked c̄ 2UV6 (6/28); 2 kinds

2 colonies, one very translucent, one more dense. Could this difference be due to phage? Pick, streak out (on EMB), save 1 col. of each type.

✓3) Save mold from plate on which plaques were found, in case cannot get plaques on ordinary complete medium c̄ mold present.

4) Spread plates of complete medium c̄ mixture of CG2S and 2UV6.

CG-1-S/CG7 SRP expt: Cfg, wash, plate. SRP's picked from previous SRP experiment are growing rather poorly on present batch of AG2SM; probably had the usual E. coli con of SM added.

7/2/54

Papillae picked from 2S/2UV6 SRP plates: Streaked on complete — 2 kinds of colonies; small translucent, a larger, opaque. Check both for auxotrophy & S resistance (Pick to complete; repl. to min. + SM)

"Phage" This may not be a phage at all. The plates spread with mixture c̄ CG2S + 2UV6 have what look like tiny plaques in rather large numbers, but the plate

streaked the previous day with material scraped directly from "plaque" area on original plate has two kinds of colonies:

1) Rather large, opaque (These were visible yesterday).
2) Tiny, transparent. These little, water-clear colonies are not overgrown by the larger colonies, but when surrounded by a developing opaque colony, show up as round clear areas which one would interpret as plaques were it not for the other small transparent colonies on the plate.

Make further attempts to run down requirements of auxotrophs. Find out smallest conc. of YE which will permit good growth when added to minimal tubes; then check for inhibition by AA groups, vits, HC, etc.

Try some "crosses" of various auxotrophs to see whether they will grow on minimal either by recombination to form prototrophs or by syntrophism.

Add sterile 5% yeast extract to 10 ml tubes of AG 2, 0.1, 0.2, 0.4 & 1.0 ml per tube. Inoc. c̄ CG9 and 20VL [illegible] because it has shown a better

specific in relation to YE and YNA, and latter because it is S^s, the only one among the auxotrophs, and thus has an additional marker for crosses.

Also inoculated AG1 broths of CG9 and 2UV1 together & separately, for crossing attempt. To be plated on minimal. Did not start aerated cultures this time.

7/3/54

CG-1-S / CG-7 SRP; (2 days)

Controls:

7, not aerated: Thin film, no papillae
7, aerated: " "
1-S, not aerated: fairly heavy background, 16 large papillae
1-S, aerated: Heavy background, no papillae

Grown together, not aerated: fairly heavy background, ca 50 - 100 papillae per plate. Tiny clear areas, either plaques or transparent colonies.
Grown together, aerated: Heavy background, few papillae no "plaques"
Grown separately, not aerated: Moderate background, 50 - 100 large papillae per plate
Grown separately, aerated: Moderately heavy background,

only 2 - 4 papillae per plate.

On checking back to plate on which "phage" appeared - the "plaques" on that plate do not look like the effect produced by the small transparent colonies. They ~~have~~ (the "plaques") have rather ragged edges and turbid centers. Save plate, try again to get a phage from it.

7/5/54

Plate spotted c̄ two col. types from 25/2UV6 (7/2) — Moldy; spot on fresh AG 1 plate for replication ~~to~~ min & S/4.

~~Original "phage" plate; "plaques" still visible; Try picking from individual clear areas, see whether → small, clear colonies; also mix in broth c̄ 2UV6 + CG25, see if any phage.~~ (look very similar to CG-1-5 plate - see below)

CG9 & 2UV1 in min. + yeast extract.

CG9: Good growth in 10 ml min + 0.1 ml 5% YEX. (In recent runs this expo was recorded as responding to YNA).

2UV1: Some growth c̄ 0.1, 0.2, & 0.4 ml YEX; quite good growth c̄ 1.0 ml, but still not as

good as in complete. ($\bar{c}$ CG 9, growth $\bar{c}$ 0.1 ml YEX is about as good as in complete). Growth in min + 0.4 ml YEX probably OK for testing for inhibition.

Add regular conc of vits & AA groups to min + necessary amt of YEX.

CG-1-S / CG 7 "SRP" expt; (plated 7/1)

Controls: 7 aerated & unaerated; several hundred colonies; evidently this single assay not too satisfactory for "crossing" expts.

1-S, not aerated; moderated background which has "plaqued" appearance - apparently due, again, to small, transparent colonies. Also ca 100 large papillae.

1-S, aerated; moderate background, several hundred papillae.

"Cross" plates; very similar to CG 1S controls.

2011 / CG 9: C/g, wash, plate on min. (grown together for [illegible]). Also plate on min 5M mixture of CG 1S and CG 7 incubated 5 days. Not too promising

in view of behavior of controls.

1/7 Result about the same as those incubated only 2 days, Many [illegible] [illegible] + numerous large population

7/6/54

Cultures from 2S/2UV6 expt. Replicated to AG2 + AG1SM.

CG9 + 2UV1 - in min + YE + randoms (24 hrs)

	CG.9 (48 hrs)		2UV1			CG9 (48 hrs)		2UV1	
0	−	−	±	−	YE+AA4	−	±	±	−
YE only	+	++	+	++	AA5	+	++	+	++
YE + AA1	+	++	+	++	HC	−	−	±	−
AA2	+	++	±	++	VITS	−	+	−	−
AA3	+	++	+	+	Na_2S	+	++	++	+++

(++ circled:) better growth in YE + Na_2S than in YE alone.

Incubate at least one more day, then try individual members of any AA groups which are inhibitory.
Try vitamin mix for inhibition of prototrophic Agrobacterium and other growth factors.
Start this type of experiment c̄ other auxotrophs

7/7/54
Test of "papillae" from 2S/2UV6 SRP experiment. Papillae on minimal SM picked
| streaked on complete

small, translucent — larger, opaque
6 col. of each tested

all S^R, auxotrophic — all S^S, prototrophic (but inhibition on SM not complete — hence gave background which permitted auxotroph S^R (2UV6) to grow).

2UV1/CG9 "cross"; No colonies on controls or cross plates. (2 days). Had to discard most of cross plates because of mold.

Check on inhibition of prototrophs by growth factor solution 24 hrs: growth poor; detectable only in complete, AA3, and sulfide.
Perhaps would be a good idea to add Na_2S routinely to minimal medium;
Also: try using $(NH_4)_2SO_4$ instead of KNO_3 as N source. See medium listed p. A33

7/8/54

Make up 20x salts for minimal, using only
0.2 g $CaCl_2 \cdot 2H_2O$ per 100 ml 20x soln
(see p A33)

Then make up 2 media
1) 0.5% sucrose, 0.5% KNO_3 ; Fe & Mn
(usual A_g 2, except for less $CaCl_2$)
2) 0.5% sucrose, 0.1% $(NH_4)_2SO_4$; Fe, Mn, Zn
(~ Hodgson, Peterson, & Riker 1951)

<u>**ADD Na_2S to portion of each**</u>

CG9 and 2UV1 in min. + YE + rundowns (3 days)

	CG9	2UV1		CG9	2UV1
O	—	—	YE + A4	++	—
YE only	+++	+++	A5	+++	+++
YE + A1	+++	+++	HC	—	—
A2	+++	+++	VITS	+++	—
A3	+++	++	Na_2S	+++	+++

CG15 and CG25 in min. + rundowns (2 days)

	CG15	2nd	CG25			CG15		CG25	
O	+	++	+	+++	A5	—	++	—	++
A1	+	+++	+	+++	HC	—	—	—	—
A2	+	+++	+	+++	VITS	—	—	—	—
A3	++	++	++	+++	Na_2S	+++	+++	+++	+++
A4	—	—	—	—	complete	++	+++	++	+++

Inoculated CG1S into tubes as follows:

1 Complete 3 A 3 and components

2 Minimal 4 A 4 and components.

5 A 5 and components

7/9/54

Discarded remaining plates of 2UV1/CG9 "crossing" experiment. 1 colony on 2UV1 control; 1 colony on a cross plate. Both near molds. No other colonies

CG1S in min + AA groups & components;

	1 day	2 days	3 days		1 day	2 days	3 days
O	−	++	++++	glutamine	−	−	−
complete	++	+++	++++	aspart	−	−	contaminated mold; unable to score
A3	+	+++	++++	prol	+	++	++++ *
tryph	±	++	++++	A5	−	−	++
tyros.	+	+++	++++ *	alan	+	+++	++++
ϕ al	+	+++	++++ *	glyc	+	+++	++++
A4	−	−	−	serine	−	±	+++
hist	−	++	++++	OHprol	±	+++	++++
threo	±	+++	++++				

* Tubes marked * there was very heavy growth in the upper 1/4 to 1/3 of the tube. Other tubes in which growth occurred were more uniform.

Prepared tubes of AG 2 (low $CaCl_2$) c̄ & s̄ sulfide; also Tris $(NH_4)_2SO_4$ − Zn^{++} medium (AG 3) c̄ & s̄ sulfide.

Inoc. c̄ CG1S

Attempted crosses of auxotrophs

	CG7	8	9	2UV1	2	3	4	5	6
CG7		7/15/54 1 on 8 control 1 on cross plate	7/15/54 0	7/26/54 ✓ 61; controls 0					
8			7/15/54 0						
9				7/9/54 0					
2UV1									
2									
3									
4									
5									
6									

Start cultures of CG7, 8, 9, 7+8, 7+9, & 8+9 for crossing expt

Inoc. c̄ CG15 tubes of minimal + each vitamin available in solution.

7/~~10~~ 9/54

Test CG1~~B~~R c̄ individual acids of AA groups 1 and 2.
(Used CG1R by mistake — shouldn't make any difference in eventual result.)

7/10/54

CG1S and CG2S in min + growth factors.

	CG1S	CG2S		CG1S	CG2S
0	+++	+++	A5	+++	+++
A1	+++	+++	HC	—	—
A2	+++	+++	VITS	—	—
A3	+++	+++	Na_2S	++++	++++
A4	—	—	complete	++++	++++

Discard

See on page 43 results of adding individual AA's. McIntire, Peterson, and Riker (1941) used glutamic acid at 2.5 gm per liter in their minimal medium!!! This was for the same strain (A6) Van Lanen, Baldwin, & Riker (1940) reported mono-amino-dicarboxylic acids inactive in attenuation (and presumably inhibition) up to M/20

CG1R in groups AA1 & AA2 and components of these groups. No growth at 20 hrs in min. or any supplement.

CG1S in Ag 2 & Ag 3 c̄ + s̄ sulfide - no growth at 24 hrs.

CG1S in min + individual vits - no growth 24 hrs.

7/11/54

CGIR in A1 + 2 + components:

	2 days	3 days		2 days	3 days
0	+	+++	A2	++*	+++
A1	++*	+++	leu	++*	+++
lys	++*	+++	Isoleu	++*	+++
arg	++*	+++	val	++*	+++
meth	++*	+++			
cyst	+	+++			

3rd day; all have heavier growth near top of tube.

* Growth heavy in upper portion of tube (not a pellicle)

CG-1-S in AG 2 + AG 3 c̄ + s̄ sulfide

	2 days	3 days
AG 2	− ?	− (?)
2 + Na_2S	++ (uniform turbidity)	+++
A3 3	−	−
3 + Na_2S	+ (bottom ½ of tube turbid)	+ (now uniform turbidity)

REPEAT

CG1S in individual vits.

	2 days	3 days		2 days	3 days
0	++	+++	Nic	+++*	+++
B6	++	+++	B_1	+++	+++
Choline	++	+++	B_2	++	+++
Menadione	++	+++	paba	+++*	+++
Pantothenate	++	+++	inositol	+++	+++
[illegible]	+++*	+++	B12	+++	+++

7/12/54

Cf₃ + plate crosses of CG 7, 8, & 9, pairs grown together & separately

Repeat of CG15 in AG2 + AG3 c̄ + s̄ sulfide inoc. (May have been an error in conc. of AG 2 + 3 salts soln. If no growth in AG 2 this time make up new 20x stock)

7/13/54

Repeat on AG 2 + AG 3 ± sulfide.

	1 day	2 days	3 days
2	−	−	−
2 + sulfide	−	−	−
3	−	−	−
3 + sulfide	+	+++	+++

Inoc. AG 2 & AG 2 + folic acid (pteroylglutamic) c̄ CG15.

7, 8, & 9 crosses – Nothing at 24 hrs.

[Try CG15 in min + glutamic or aspartic + sulfide]

7/14/54

CG 15 in AG 2 ± PGA: nothing at 20 hrs.

Crosses involving 7, 8, & 9: nothing at 2 days except good growth around molds. A few plates appear uniformly seeded (in the agar) with old friend yellow contaminant.

7/15/54

CG 15 in AG 2 ± folic acid (PGA): equally good growth $\bar{c}$ & $\bar{s}$ PGA.

Crosses involving 7, 8, & 9: (3 days) - nothing definite. 1 possible prototroph on (7+8) plate. Plate contaminated $\bar{c}$ yellow bug, but this colony buff & looks like typical CG. Also one buff colony on CG 8 control plate, but aside from color it looks just like yellow contaminant.

Pick: 1) col from cross plate

2) buff col from control plate

3) yellow colony.

Streak out; test whether inhibited by glutamic acid.

7/16/54

(Could try sterile vits mix dated 12/15/52) also try repeat (w [illegible])

Start repeat rundowns of all auxotrophs, avoiding any inhibitory supplements. (Do vits individually).

Make up new 20x Ag 2 salts; previous batch gave no growth $\bar{s}$ sulfide

CG1S in AG2 ± glutamine ± Na_2S (2 days)

AG 2	−	AG 2 + Na_2S	++
AG 2 + glutamine	−	AG 2 + g.a. + Na_2S	++

Try CG2S in min. This was earlier batch than that which gave no growth in previous expt.

Try rundowns of auxos $\bar{c}$ Na_2S added as detoxifyer.

For some reason – new batch of AG2 salts → no ppt in 2x concentration.

NOTE: There is a large bottle of d-glutamic acid in the AA box, and much of it has been used. Could our present stock soln be d-glutamic? The aspartic acid on hand is dl, so possibly d isomer could explain inhibition by both glutamic and aspartic. Check.

7/17/54

CG1S and 2UV6 in [illegible] + sulfide

	CG2S 1 day	2 days	3 days	2UV6 1 day	2 days	3 days
no Na_2S	−	+	++	−	−	−
Na_2S only	−	+	+++	−	−	−
A1	±	+	+++	−	−	−
A2	±	+	+++	−	−	−
A3	+	+	+++*	−	−	−
A4	+	+	+++*	−	−	−
A5	−	+	++	−	−	−
HC	++	++	+++	+	+	++
VITS	−	±	++	−	−	−
YEX	++	++	+++	+	++	+++

7/19/54

Prototrophs from 7+8 cross & 8 control plate. Both grew very poorly on minimal (plate rather old). Colonies look identical, the one from 8 control plate does not resemble the yellow contaminant. Ino each into mini tube & mini + AA4 or glutamic acid.

Prepared the following tubes & inoc. c̄ CG1S

mini
mini + l-glutamic
mini + d-glutamic

mini + d glut. + Na_2S
mini + " + thiosulfate (1:10,000)

(as check on whether thiosulfate is as effective as Na_2S)

Started randoms + sulfide c̄ CG7 & CG8

7/20/54

CG1S in l-glutamic & in d-glutamic alone & c̄ Na_2S + ~~thiosulfate~~; no growth at 24 hrs.

"Prototrophs" from 7x8 & 8 control: No growth at 24 hrs in min or min + glut.

Randoms on CG7 & 8 (black sulfide ppt in some tubes)

	CG7				CG8			
	1 day	2 days	3 days	4	1 day	2 days	3 days	
Na_2S only	−	−	±	±	−	−	−	
A1	−	++	++	+++	−	++	+++	+
A2	−	−	±	±	−	++	+++	+
A3	−	−	±	+	−	−	−	−
A4	−	−	+	+	−	−	++	++
A5	−	−	±	±	−	−	+	+
HC	+*	+++	+++	++++	+*	+++	+++	++
VITS	~~++~~?	?(−)	?(−)	−	~~++~~?	?(−)	?(−)	−
YEX	+*	+++	+++	+++	+*	+++	+++	+

* [illegible]

7/21: Turbidity in vitamin tubes apparently was ppt in medium; has largely disappeared.

[illegible]

7/21/54

CG15 in d- & l-glut. acid (2 days)

Mold growing in 0, d-glut only; & l-glut only tubes.

0	++		
l-glut	–	d-glut + Na_2S	+++
d-glut	–	d-glut + thiosulfate	—

CG7 ~~& CG8~~ in std randoms + sulfide: Heavy ppt causing turbidity in tubes. This medium was made c̄ same salt mixture as that which gave little or no ppt in last batch of AG2!

Started broths of CG7, 8, & 9 and 2UV1 for crossing attempts. Also inoc 2UV1 in broth c̄ 7, 8, & 9.

7/23/54

CG7 in std randoms: no growth (inoc 7/20)

Plated crosses of CG7, 8, 9 c̄ 2UV1

7/24/54

Inoc CG7 into A1 randoms; CG8 into A1 & A2

7/26/54

CG 7 & 8 rundowns

CG 7	2 days	3 days	4 days
O	−	−	−
A1	+	++	++
lys	−	−	+
arg	−	±	±
meth	−	−	−
cyst	−	±	+
complete	+++	++++	++++

CG 8	2 days	3 days	4 days
O	−	±	±
A1	±	++	++
lys	−	−	±
arg	−	±	±
meth	−	−	±
cyst	−	±	+
A2	+	+	+
leuc	−	−	−
isol	±	±	+
val	−	−	−
compl	+++	++++	++++

This batch of minimal apparently not very satisfactory. Better growth in A2 at 2 days in previous test (AA rundowns)

Crossing attempts of CG 7, 8, & 9 c̄ 2UV1

Controls: negative except CG8, which is quite heavily contaminated c̄ mold and a pink bacterial contaminant. A few col. present which look like Agrobacterium.

CG 8 & 9 +2UV1 cross plates: negative

CG 7 × 2UV1 : Grown together; 2 plates; negative

Grown separately; 1 plate; 61 colonies. Look like

Agrobacterium. Plate has some mold near edge, but bacterial col. evenly distributed on plate, do not seem to be stimulated by mold.

Pick colonies (at least 20); spot on complete, replicate to minimal. Repeat cross, this time grown together & separately, aerated & unaerated.

7/27/54

CG 7 & 8 AA rundowns: (See table on previous page)

CG 8 seems rather "leaky"; this has happened before. CG 7: Might try A 1 single omissions. Also, try individual vitamins and the vitamin mix used in yeast medium, since the sulfide does not counteract very well the inhibition by the vitamin mix.

Next rundown attempt - 2UV1

Repeat l- & d-glutamic expt c̄ CG 15, since previous expt. was lost because of mold.

Start cultures for repeat crossing attempt c̄ CG 7 & 2UV1.
(together & sep; aerated & unaerated)

7/28/54

Col. picked from 7 x 2UV1 plate & repl from complete & minimal: 29 picked, all prototrophs.

CG1S in d & l glutamic: no growth in 24 hrs

7/29/54

Plated 7 x 2UV1 crosses (grown together & separately, aerated & not aerated)

[Some [illegible] c̄ non-aerated cultures that labels may have been interchanged on CG7 & CG7+2UV1

CG1S / glutamic:

	2 days	3	~~4~~
0	+±	+++	
Na_2S only	+∓	+++	
d-glut	±	++ !	
d-glut + Na_2S	++	+++	
l-glut	++	+++	
l-glut + Na_2S	++	+++	

Checking reveals the "d-glutamic" is actually l(+) glutamic (Kodak). The other glutamic is l(+) glutam[ic] (GBI).

7/30/54

CG7 rundowns

	2 days	3 days		2 days	3 days
O (Na_2SO_4 only)	+	+	A1	++	+++
B2	+	+	" - lys	++	+++
Biotin	±	mold	" - arg	++	+++
B_6	−	±	" - cyst	−	++
pantoth	−	−	" - meth	+	+++
K	−	±			
B_1	−	±	complete	++++	++++
nic	++	mold			
B_{12}	−	±			
inos	−	±			
chol	++	contaminant? +++ (pellicle)			
paba	−	±			
pga	−	−			

Streak out CG7; check several colonies on anything giving 2+ or better.

7/31/54

CG7 x 2UV1 "cross": at 48 hrs, colonies on all three plates from cultures grown together, not aerated. None on other plates.

Inos. rundowns (preliminary) of 2UV1

8/2/54

2UV1 primary rundowns;
At 2 days, growth only in YNA.
Try individual vits; also p & p?

CG7: from individual colonies: Check each in min ($\bar{c}$ sulfide) +: 0; biotin; nicotinic; choline; A1 & individuals of A1 group. Try first $\bar{c}$ plates; 2 or three in tubes

CG7 + 2UV "cross": (4 days)
Colonies on all 3 plates grown together, not aerated. (Counted 1 plate: 115 col; other 2 similar). Heavy film of background, so could be feeding. Pick a few col, streak, test for prototrophy. Col. are small.
No real control on this tube; could be mutation to prototrophy.

Repeat cross again.

8/3/54
2UV1 primary rundowns; 3 days; still growth only in YNA; possibly trace (±) in A1.

8/4/54
2UV1 primary rundowns; 4 days

0	−	A3	−	HC	−
A1	±	A4	+	VITS	+
A2	−	A5	+	VFX	+++

Try individual vits.

7×2UV1 "protos" (8/2): Very poor growth when streaked on minimal - very tiny colonies. Grew well on complete. Replicate from complete to mini.

Repeat 7×2UV1 cross: Use several cultures of each parent, plate control from each.

8/5/54

7× 2UV1 "prototrophs" - poor growth when replicated to minimal (24 hrs)

CG7 from single col. repl. to mini agar + supplements - 48 hrs. No growth except on complete.
(Suppl. used: Biotin, nic, choline, A1, & individuals of A1 group)

8/6/54

2UV1 in individual vits: no growth at 2 days.

Plated repeat of 7×2UV.1 cross.

8/9/54

Repeat of 7 X 2UVI cross

Grew up several tubes of 7 and of 2UVI, plated control & cross from each (except 7-5 & 7-6, control only). Also grew 2 tubes of 7+2UVI together.

	CG7 control	2UVI control	cross plate
1	ca 200 col	1 col.	ca 200
2	7 col may be contaminants	0	several very small
3	0	confluent	confluent
4	1 may be contaminant	0	3 + several very small
5	0	—	—
6	10 very small	—	—
7+2UVI (1)	—	—	confluent
7+2UVI (2)	—	—	0

8/10/54

Check whether filtered Ag 2 supports good growth of CG 15 (in order to get rid of confusing turbidity). Inoc. tube (ē Na_2S) c̄ water suspension.

Inoculum very small; visibly no growth in Ag 1 control until 48 hrs

8/12/54
2 days: growth in filtered medium only

8/13/54 3 days: growth in both filtered & unfiltered.

9/7/54

Made up 40 tubes Ag 2 (2x) + 40 tubes 1% sucrose. Ag 2 has no Fe, Mn, or Zn, also no sulfide. Filtered before tubing & autoclaving. Check all auxos

1) in min
2) in min + sulfide
3) + YE. Complete

before starting any expts.

9/13/54

Check of auxotrophs inoc into min ± sulfide, & complete 9/11:

No growth in any min or sulfide tubes.
Growth in all complete tubes except 2UV2.
Continue inc.

9/14/54

Still no growth in any minimal; no growth in 2UV2 complete. Check again on viability of 2UV2 stab.

Transfer stabs to have one set as reserve.

9/15/54

Growth in CG7 in min + sulfide. Could be contamin, since another tube has mold.