1/11/55

Irradiation effected on WCG-1-S. Used overnight aerated culture of grown in Ag 1 broth, rm temp.
Irradiated 10^{-4} dilution; cfgd, washed, resuspended in saline, then diluted.
90 sec irradiation on big lamp.
Diluted further to give 10^{-7} and 5×10^{-7} dilutions of original culture on plates.
Inc. 25° incubator

1/13/55

9:00 AM: Irradiated culture plated 1/11: Dilutions used too high: 5×10^{-7} ⇒ about 20/plate. Colonies still very small; inc. another day before replicating.

Started culture for second irradiation: 0.1 ml Ag 1 broth culture (not aerated) → Ag 1 broth aerated, rm temp.

1/14/55

Replicated 5 plates of 5×10^{-7} dilution to mimeod. 6th plate contaminated, incl molds; picked colonies, spot to complete for later replication. Also picked the few colonies from 10^{-7} dilution to complete.

1/15/55

Expmtd plates refl. to mm from 1/11 irradiation: 9 possible auxotrophs from 245 colonies. Picked, spotted on complete. Some colonies very rough; use caution in identity. If possible, concentrate on isolated colonies.

Second UV run, WCG-1-S:

Culture: Aerated at rm temp overnight; stored s aeration at rm temp another 24 hrs.

Cfgd; pellet washed c saline; diluted to 10 ml in saline. 10^{-4} dilution irradiated 90 sec.

Plating: 0.1 ml and 0.2 ml 5×10^{-6} dilution.

1/17/55

Second UV run. Very few colonies visible at 48 hrs!

Continue incubation at 25°. By 2 30 pm colonies on [illegible] 5-12 per plate

"Autos" from first run: Of the 9 spotted on complete, three are gummy, look like typical Agrobacterium growth. Other six rough & rather leathery. Replicate all to minimal.

Of colo. picked from 1st run plates (plates which were contaminated or too dilute for replication) spotted, then repl to min, 1 of 66 appears to be an auxotroph. Test along c other 9.

Made up 2 minimal tubes. To check medium, inoc 1 drop water suspension from WCG 1-S stab into 1) complete, not aerated 2) min + $NaNO_2$ aerated 3) " " " not aerated

Used 1 drop 1% sterile $NaNO_2$/tube (10 ml)

1/18/55

"Autos" from first UV run: 4 of 10 grew well on min. in 24 hrs. These included all the 3 "typical" gummy growers. 2 of the rough type show poor

growth on ~~replication~~ replica plates on both to mini & complete at 24 hrs. Remaining 2 replicated show good growth on complete, poor growth on mini. The 10th "auxo", brushed on plates to which others were replicated, shows fair growth on compl at 24 hrs, no growth on mini. Continue incubation of these plates until early pm.

~~Several~~ Two of the "rough" spots ⇒ mixed growth when replicated to mini & complete; center of spot rough sparse growth; outer ring of heavier, gummy growth. Especially marked on complete. So it appears these rough colonies may be Lysobacter.

Colo picked from 2nd UV: Overnight growth poor; continue incubation until early pm before replicating to mini.

More colonies now visible (3 days) on 5×10^{-4} & on 10^{-7} plates from 2nd UV run. Pick; spot on complete for repl. to mini.

Check on Ag 2 minimal: No growth overnight in mini tubes 5 or 5 aeration. Unaerated Ag 1 tube (compl) slightly turbid. Room temp probably quite low during night.

1/19/55

Check of mineral liquid (see 1/17): At 2 days, very good growth of WC&-1-S in min (+ Na_2S) aerated. About the same turbidity in unaerated mineral as in unaerated complete.

Why Na_2S & aeration needed for best growth? Seems contradictory. Effect of aeration may be largely agitation rather than O_2 availability; but at pH slightly below 7 should think sulfide would be rapidly lost.

Set up: min + Na_2S } aerated
min + Na_2S }
min + Na_2S } unaerated
min + Na_2S }

1/20/55

Possible auxos from 1st UV run: The rough type grew up eventually on mineral. Check these on a couple of other sugars; could poor growth & lack of green be due to mutation involving utilization of sucrose?

#10 picked from first run appears to be auxotroph. Stab; prepare renderon tubes. Growth is rather scant even on complete. How be sure of identity ???

Second UV run: Of 53 col. picked, 10 are poorly-growing, rough type. If this is a contaminant, it certainly appears

consistently.

One possible aspo from 2nd run. Stab, streak on complete.

1/21/55

Effect of aeration & $5\ Na_2S$: (48 hrs)

Not aerated: Little difference & $5\ Na_2S$. Poor growth.

Aerated, no Na_2S: better growth than 5 aeration.

Aerated, $\bar{c}$ Na_2S: Very good growth; est. 10^9/ml.

1/22/55

Inoc Ag 2 roundrums $\bar{c}$ UV1-10, UV2-1, and WCG1S from water suspensions from slabs.

UV2-1 grows very poorly when streaked on complete.

"Rough" strains streaked on compl. $\bar{c}$ glucose & mannitol look the same as on sucrose.

1/24/55

Roundrums (2days)

	WCG1S	UV1-10	UV2-1
O	++	−	−
A1	++	−	−
A2	++	−	−
A3	++	−	−
A4	++	−	−
A5	++	−	−

	WCG1S	UV1-10	UV2-1
HC	++	+	−
YNA	+	±	−
VITS	++	−	−
compl	++	++	++

1/25/55

Lundrums (3 days)

	WCGIS	1-10	2-1		WCGIS	1-10	2-1
0	+++	−	−	AA5	+++	−	−
AA1	+++	−	−	HC	+++	+++	++
2	+++	−	−	YNA	++	−	+
3	+++	−	−	VITS	++++	−	−
4	+++	−	−	compl	++++	+++	+++

Try amino acid single omissions. [The HC used is contaminated, so growth in HC tubes not necessarily due to HC components].

UV runs (#3)

24 hr aerated culture (Ag 1, rm temp)

90 sec. . Pl. 0.1 & 0.2 ml 10^{-5} dil. (Irradiated 10^{-4})

1/28/55

UV 3: Replicated to minimal from plates c >10 col. Picked from plate c < 10 col & spotted on compl.

One yellow (or creamy) colony. Picked to stab, labelled UV 3-1. Colony was same size, shape, height, & texture as others on plate. Check motility; growth c & $\bar{c}$ sulfide, c & $\bar{c}$ aeration.

1/28/55

Rundowns, single omissions of AA groups (2 days)

	UV1-10	2-1		1-10	2-1	
0	0	0	-5	+	+	
-1	+	+	all	+	+	
-2	+	+	all + vits	+	+	
-3	+	+	HC	-	+	Fresh HC used (taken from freezer)
-4	+	+	compl	++	++	

Very similar to behavior of previous mutants. New vit mix not inhibitory. Try various carbon sources & N sources

1/29/55

Rundowns, single omissions, 3 days

	UV1-10	2-1		1-10	2-1
0	0	0	-5	+++	+
-1	++	+	all	+++	+
-2	++	+	all + vits	++++	++
-3	++	+	HC	±	+++
-4	++	+	compl	++++	+++

3rd UV run: Replicated to mini. col. picked to complete 1/28.
Direct replications: 2 possible auxos out of ca 80 col. One is the yellow colony.

1/31/55

UV3: Col. picked, repl. to mini 1/29: 2 possible auxos (both rough, so possibly just poor replication). [UV3-3 & UV3-4)

WCG

Screen as follows: 1) min. 2) min + HC. 3) min + all AA groups together. 4) As 3, + VITS. 5) YNA.
Also include UV 3-1 + 3-2 in tests.

2/2/55

Rundowns of UV3 auxos: 2 days

	HC	AA	AAV	YNA	Ag 1	0
3-1	++	++	++	+	+	−
2	+	++	+++	+++	+++	−
3	±	+	−	−	+++	±
4	−	−	−	−	+++	−

2/3/55

UV3 rundowns, 3 days.

	0	HC	AA	AAV	YNA	Ag 1
3-1	±	++++	++++	++++	++	++++
2	±	+++	+++	+++	+++	++++
3	±	++	++++	−	−	++++
4	−	−	−	−	−	+++

These were kept for additional more days, no further growth in remained tubes, no change in other tubes

2/8/55

Inoc. AA rundowns of UV3-2 & 3-3. (Strepto, single additions)

2/9/55

Set up rundowns of UV3-4 as follows: HC, HC+V, HC+YNA, YNA, YNA+V, V, HC+YNA+V, YX. Grew only in YX

WCG

2/10/55

Rendrums, UV 3-2 & 3-3 (2 days)

	3-2	3-3		3-2	3-3	
O	–	–	A4	–	–	
A1	–	–	A5	–	–	
A2	–	–	~~VITS~~	+	not included	
A3	–	–	compl + vits	not included	++	To check for Tyrosine
			compl.	++	+++	

If these results check out after another 24 hrs, try 3-2 ⊂ individual vits + 3-3 ⊂ AA single omissions

2/12/55

Set up rendrums ⊂ individual vits ⊂ UV 3-2. (B-12 may be contaminated).

2/13/55

Run UV 3-2 vit rendrums.

2/14/55 UV 3-2

Vit rendrums: At about 28 hrs, fair growth in vits mix, trace of growth in biotin.

Would pay to do individual vit ⊂ all amps; behavioral has been constant ⊂ vit requirement, but mix may be toxic to some.

Also: 3-3 ⊂ ~~Leu~~ AA samples.

2/15/55 Vits, UV 3-2: Fair growth in biotin; other vits O

WCG

UV3-3 Incr. rendrous, AA groups.

2/17/55

UV 3-3 no growth in AA groups or in all AA's (2days)

2/24/55

UV 1-10 & 2-1 in vits: no growth in individual vits or mix. (Incr. 2/18)

2/22/55

UV 1-10 No growth in individual vits, but some growth in vit. mix (4 days) May have double requirement, or conc. of requ. vit in individual tube may be too high, since some are higher than in mix. Also may be reversion - see UV 2-1

UV 2-1 Growth in the following tubes (very slight)

Riboflavin	para	
B-12	vit mix	Reversions?
Pantothenate		Dirty tubes?

2/23/55 (5 days)

UV 1-10 Growth in: Biotin, choline, nic, para, vit mix (Dirty tubes)

UV 2-1 " " : Trace in those listed previously. Very scant even in vit mix.

2/24/55 Set up min, min + glycine (1 mg/10 ml), min + Vits, & min, vits, & glycine to see whether glycine will speed growth in vits 5 itself permitting growth (1-10 & 2-1)

WCG Jr. 2/25

3/1/55

UV 1-10 & 2-1 in glycine, vits:

At 3days, 1-10 ± in glycine, glycine + vits.

4days 0 —

glyc ±

vits +

vits+gl ++

2-1 at 4days. 0 —

glyc +

vits +

vits+glyc — (!?!)

3/4/55

UV 3-3	(2days)		3days		
0	—	—	A4	—	—
A1	++	++	A5	—	++
A2	—	—	all AA's	—	±
A3	—	+	compl	+++	+++

3/6/55 (UV3-3)

A1 rundowns since 3/4. Possibly trace of growth in lysine

3/7/55

A1 rundowns - 3days (at room temp; cold at night)

0	—	Meth	—
Lys	+	A1	—
Arg	—	compl	++
Cyst	±		

WCG

Try ē & s̄ lysine ē aeration: Usually lys - showed some rich clear-cut results because of more rapid growth.

3/8/55

Start culture of WCG 1 (S) for another UV exp't (#4) to try to get a readily identifiable auxotroph.

Ag 1, room temp, aeration

TRANSFER STABS

3/10/55

Excellent growth of WCG-1-S for irradiation. Irradiated 90 sec. (10^{-4} dil) Plated 0.2 ml/plate of 2×10^{-5} dilution (Ag 1 medium)

UV3-3 in min, lyp, A1 aerated: In 3 days, faint growth in all tubes.

3/14/55

UV4: 6 possible auxotrophs - 1 is a light yellow similar to UV3-1.

3/15/55

UV4-1 (yellow) is an auxotroph; all others grew in min.

3/18/55

UV5: 48 (approx) hr culture, Ag 1 broth, aerated. Irradiated 10^{-4} dilution for 90 sec. Plated .1 ml/plate of 10^{-5} dilution. Inc. 25°.

WCG

3/19/55

UV 4-1 & UV 3-1 (yellow): Streaked on complete. 3-1 rough, 4-1 smooth. Appearance ≅ rough & smooth WCG 1 except for color. Smell like Aerobacterium.

Try running down requirement(s)

Spread 4-1 broth (5 days old) on minimal; look for possible "reversions" to prototrophy which should appear as papillae - If any, what color? (i.e. is yellow color result of a metabolic block.)

Also - compare growth in complete broth $\bar{c}$ & $\bar{5}$ aeration $\bar{c}$ WCG-1.

3/21/55

UV 3-1 & 4-1 Both show scanty growth in complete broth $\bar{5}$ aeration, good growth aerated (but not so heavy as WCG-1)

4-1 spread on minimal - No papillae at 2 days.

Rundowns:

UV 3-1	++ in A4, + in A5, ± in others	} 2 days
UV 4-1	0 in all rundown tubes.	

3/22/55

UV 3-1 & 4-1 rundowns

4-1 no growth except in complete

3-1	A1	0	A5	+++	1) [illegible]
	A2	0	Vits	++	2) [illegible]
	A3	0	0	0	4) [illegible]
	A4	+++	compl.	+++	5) [illegible]

WCG

3/24/55

UV 5: Of 7 possible aspos picked, all show some growth in mini at 2 days. Two have only very faint turbidity; keep these, check especially for vits. [illegible] 4 days UV 5-1 UV 5-2

(3/19)

UV 4-1 Spread on Ag 2 plate: 1 papilla, which appeared in about 3 days. Appears to be slightly buff-colored. Pick, streak on complete, check on mini for color & prototrophy.

3/26/55

UV 3-1 Neurospora - 3 days (rm temp - cold at night)

B1	0	pyri	0	A4	++	A5	±
ribo	0	pant	0	hist	0	alan	0
nic	0	chol	++	Thre	0	glyc	±
paba	0	vits mix	0	glut	0	ser	0
bio	0			pro	0	OHP	+++
PGA	0			aspart	++		
Inos	++					compl	+++
K	0						

Streak out; recheck individual colonies.

3/28/55

Papilla from UV4-1 - picked from mini, streaked on complete - Colonies yellow. Pick several, check for auxotrophy.

NHC

3/31/55

UV3-1 : Rundowns mic. from 2 individual col. (2 days)

	(1)		(2)			(1)		(2)	(3 days)
0	0	0	0	±	A4	0	0	++	+++
vits	0	+	++	+++	hist	0	0	0	+
dist.	0	0	0	+	thre.	0	0	0	0
mos	0	0	0	0	glutn.	0	0	0	++
A5	+	+++	0	±	pro.	0	0	+	+++
alan	±	++	0	+	aspart	0	0	++	+++
glyc	0	0	0	+++	camp.	+++		+++	
Ser	0	0	0	0					
OHP	0	0	0	+					

Indicates erratic results prob due to reversions ?

5-1 & 5-2 rundowns: grew in min. However, there are apparently some inhibitions.

	5-1	5-2
0	+++	++
A1	+	±
A2	0	+
A3	±	0
A4	+++	++++
A5	0	0
Vits	+++	++++

4/2/55

Cols. from papilla from UV4-1; autotrophic: However, 1 streak - faint growth after 5 days, ~ 1 large colony, many small. On min, large col appears volute. Pick, streak in complete.

4/5/55

Final comparison of sundrums on two col. from UV 3-1 (ende. 3/29)

	(1)	(2)		(1)	(2)
O	±	+	A4	+	+++
rib	±	+++	hist	++	+
chol	±	++	threo	0	±
inos	±	+++	gluta.	++	++
A5	++++	+++	prol	+++	+++
alan	++++	++++	aspart	++	++
glyc	+++	++++			
ser	0	+++			
OHP	±	+++			

UV 4-1 Picked from large col. on mini: Streak mixed, yellow col as before, also a white, rough col. type. Latter extremely rough - probably a contaminant.

Might try plating UV 4-1 or 3-1 together & WCG 16. Main purpose would be to look for colonies & yellow & white sectors. First check nutrition of WCG 16; also make sure UV 4-1 (or 3-1) does not respond to biotin.

4/12/55

Spread water suspensions (from slants) of WCG 16 & UV 4-1 on plates of mini & ~~5~~ biotin: Also cross-brushed sa. & Sm.

4/14/55

WCG 16 - confluent growth on mini & bio; none ~~5~~ bio

UV 4-1 - no growth on either plate

WCG 16 S^S

UV 4-1 S^R

WCG

4/22/55

Results of attempted cross of WC616 & UV-4-1 (4 days 25°)

Controls: WC616 can grow ē SM at 200 µg/ml

" " no growth s̄ biotin.

UV 4-1 Some background s̄ SM, none ē SM, though culture appeared to be S^R

With mixtures, confluent growth on all media. Growth is white, i.e. WC616

/ min
min + SM
min + SM + biotin

3-3 AA groups

~~Plated crosses of KK 33-48 on~~ For loop dilutions to be plated in min agar: 0.1 → 1.0, 1.0 → 10.0 loop, 10 → plate

1-10 HC − ; in AA single group omissions, grew well in all tubes except min control. AA gr. sing additions all − ; late growth (4 days) in some vits

2-1 HC+ ; poor growth in all AA single omission tubes. Late growth (4 days) in some vits.

3-1 (yellow) HC + ; AA's together + ; poor in YNA

3-2 HC fairly +, AA's ~~together +~~ better in AAV ; good YNA
BIOTIN

3-3 HC +, AA's together strong +, AAV − ; YNA −

3-4 Grows only in complete or min + YX
(Have tried HC, HCV, HCYNA, YNA, YNAV, V, HCYNAV

Possible schemes [illegible] using WCG16 + UV 4-1
See whether both [illegible] less S^S.

If could obtain:

yellow S^R x^- B^+
wh S^S x^+ B^-

Plate on	What could grow?	Unselected:
1) min	all x+ B+	color, S
2) min + [illegible]	only x+ B+ S^R	color
3) min + biotin + [illegible]	all x+ S^R	color, B

Lys Arg Meth Cyst