1/11/55

Irradiation appd to WCG -1 S. Used overnight aerated culture of gown in Ag I broth, raw temp.
Irradiated 10^{-4} dilution; ef'd, washed, resuspended in saline, then diluted.
90 sec irradiation to big lamp.
Diluted further to give 10^{-7} and 5 \times 10^{-7} dilutions of original culture on plates.
Inc. 25°C incubator

1/13/55

9:00 AM: Irradiated culture plated 1/11: Dilutions used too high: 5 \times 10^{-7} \rightarrow \text{about} 20 / plate. Colonies still very small; i.e., another day before replicating.

Started culture for second irradiation: 0.1 ml Ag I broth culture (not aerated) \rightarrow Ag I broth aerated, raw temp.

1/14/55

Replicated 5 plates of 5 \times 10^{-7} dilution to measure. 6th plate contaminated, and morula, pit, spot to complete for later replication. Also pick the few colonies from 10^{-7} dilution to complete.

1/15/55

Examined plates ref'd to mini from 1/11 irradiation: 9 possible auxotrophs from 245 colonies. Positive spotted on complete.
Some colonies very rough; use caution in identity. If possible, irradiation on target colonies.
Second UV run, WCG-1-5;
Culture: Aerated at room temp overnight, stored
at aerated at room temp another 24 hrs
(24h), pellet washed w. saline; diluted to 10ml in
saline. 10⁻⁴ dilution spread on 90 sec.
Plating: 0.1 ml and 0.2 ml 5x10⁻⁶ dilution.

17/55
Second UV run: Very few colonies visible at 48 hrs!
Continued incubation at 25°. By 2:30 p.m. colonies on
plates began to develop.

"Cure" from first run: Of the 9 spotted on complete,
three are gummy; look like typical Agrobacterium
growth. Culture was rough & retain battery. Duplicate
all to minimal.
Of colony picked from 1st run plates were spotted, then repl to
mini, 1 of 66 appears to be an auxotroph. Test
along with other 9.

Made up 2 minimal tubes. Include medium, one (drop)
water suspension from WCG-1-5 stab into 2) mini + N₂5 added.

Used 1 drop 1% sterile N₂5/tube (10 ml) 3) " " not added.

1/18/55
"Cure" from first UV run. 4 of 10 grew well on
mini. in 24 hrs. These included all the 3 "typical"
gummy growths. 2 of the rough type show poor
growth on replica but to run to complete at 24 hrs. Remaining 2 replicates show good growth on complete, good growth on mini. The 10th "cemo", brushed on plate to which others were replicated, shows four growth on complete at 24 hrs, no growth on mini. Continue incubation of these plates until early pm.

From 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 cemolatent.

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

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

Check on Ag 2 remained: No growth overnight on mini tubes & a 5 cm area. Inoculated Ag 1 tube (complete) slightly turbid. Room temp probably quite low during night.
1/19/55

Check of minimal liquid (see 1/17): At 2 days, very good growth of WC-17-1.5 in mini (5% Na₂S) aerated. About the same turbidity in un aerated minimal as in aerated complete.

Why Na₂S and aerated needed for best growth? Seem contradictory. Effect of aerations may be largely respiration rather than O₂ availability, but at pH slightly below 7, should think sulfide would be rapidly lost.

Set up:

- mini + Na₂S 3 aerated
- mini 5% Na₂S
- mini + Na₂S 3 un aerated
- mini 5% Na₂S

1/20/55

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

*10 picked from first run appears to be amphotroph. Start prepare new UV tubes. Growth is rather weak even in complete. How be sure of identity?*

Second UV run: Of 53 col. picked, 10 are poorly-growing rough type. If this is a contaminant, I certainly appear
1/21/55

Effect of aeration on 0.5% Na₂S: (48 hrs)

- Not aerated: Little difference in 0.5% Na₂S. Poor growth.
- Aerated, no Na₂S: Better growth than 5% aeration.
- Aerated, 0% Na₂S: Very good growth; end 10⁹/ml

1/22/55

Since A82 suspensions on UV1-10, UV2-1, and WCG1/5 from water suspensions from A82.

UV2-1 grows very poorly when streaked on complete.

"Rough" strains streaked on complete + glucose + mannitol look the same as A82 strain.

1/24/55

<table>
<thead>
<tr>
<th>Strains</th>
<th>WCG1/5</th>
<th>UV1-10</th>
<th>UV2-1</th>
<th>UV1-10</th>
<th>UV2-1</th>
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<td>++</td>
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<td>-</td>
<td></td>
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</tr>
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<tr>
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<tr>
<td>A4</td>
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<tr>
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<td>++</td>
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1/25/55

Graudins (3 days)

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<tr>
<th></th>
<th>WCGIS</th>
<th>1-10</th>
<th>2-1</th>
<th></th>
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<th>1-10</th>
<th>2-1</th>
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<td>-</td>
<td></td>
<td>AAG</td>
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<tr>
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<td>-</td>
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<td>HC</td>
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<td>YnA</td>
<td>+</td>
<td>-</td>
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<tr>
<td>3</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td></td>
<td>VITS</td>
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<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
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<td>-</td>
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<td>empf</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

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

UV units (43)

24 hr aerated culture (Ag1, room temp)

90 sec, Pl. 0.1 & 0.2 and 10^-5 dil. (4 replicates 10^-4)

1/28/55

UV 3 : Replicated to minimal from plates > 100 col. Picked from plate < 10 col. 8 spotted on comple.

One yellow (or cream) colony. Picked to stab, labelled UV 3-1. Colony was same size, shape, height, & texture as others on plate. Check motility, growth > 3 an 3 weeks, 0 4 5 aeration.
1/28/55

Rundown, single omission of 5 or genes (2 days)

<table>
<thead>
<tr>
<th></th>
<th>UV1-10</th>
<th>2-1</th>
<th>1-10</th>
<th>2-1</th>
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<tbody>
<tr>
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<td>0</td>
<td>0</td>
<td>-5</td>
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</tr>
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<td>-1</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>-2</td>
<td>+</td>
<td>+</td>
<td>all</td>
<td>+</td>
</tr>
<tr>
<td>-3</td>
<td>+</td>
<td>+</td>
<td>HC</td>
<td>+</td>
</tr>
<tr>
<td>-4</td>
<td>+</td>
<td>+</td>
<td>empl</td>
<td>+++</td>
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</table>

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

1/29/55

Rundown, single omission, 3 days

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<th>2-1</th>
<th>1-10</th>
<th>2-1</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-5</td>
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</tr>
<tr>
<td>-1</td>
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<tr>
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<td>+</td>
<td>all</td>
<td>+++</td>
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<tr>
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<td>+</td>
<td>HC</td>
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<tr>
<td>-4</td>
<td>+</td>
<td>+</td>
<td>empl</td>
<td>+++</td>
</tr>
</tbody>
</table>

3rd UV run: Replicated to mini. col. picked to complete 1/28.

Direct replication: 2 possible axp set of ca 50 col. One is the yellow colony.

1/31/55

UV3: Col. picked, repl. to mini 1/29: 2 possible axp (both rough, so possibly post replication). (UV3-3 & UV3-4)
Screen as follows: 1) min.  2) min + HC.  3) min + all AA groups together.  4) As 3, + V175.  5) YNA.
Also include UV3-1 + 3-2 in tests.

2/2/55

Random of UV3 asper:  2 days

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>AA</th>
<th>AAV</th>
<th>YNA</th>
<th>Ag 1</th>
<th>O</th>
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<tr>
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<td>+++</td>
<td>+++</td>
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<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

2/3/55

UV3 rundown, 3 days.

<table>
<thead>
<tr>
<th></th>
<th>O</th>
<th>HC</th>
<th>AA</th>
<th>AAV</th>
<th>YNA</th>
<th>Ag 1</th>
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<tbody>
<tr>
<td>3-1</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
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<td>+</td>
<td>+++</td>
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<tr>
<td>4</td>
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<td>+++</td>
</tr>
</tbody>
</table>

2/8/55

Inst. AA rundown of UV3-2 + 3-3. (Shimpo, single addition)

2/9/55

Set up rundown of UV3-4 as follows: HC, HC+V, HC+YNA, YNA, YNA+V, V, HC+YNA+V, YX. There only on YX.
3/10/55

Rendoms, UV3-2 + 3-3 (2 days)

\[
\begin{array}{cccccc}
 & 3-2 & 3-3 & 3-2 & 3-3 \\
0 & - & - & A4 & - & - \\
A1 & - & - & A5 & - & - \\
A2 & - & - & VTS & + & net+inhibited \\
A3 & - & - & completive, inhibit & ++ & to check for
\end{array}
\]

If these results check out after another 24 hrs, try 3-2 = individual
vito + 3-3 = 0+ single omission

2/12/55

Set up rendoms = individual vito in UV3-2. (B-12
may be contaminated).

2/13/55

See UV3-2 vid rendoms.

2/14/55

UV3-2

Vito rendoms: At about 28 hrs, fair growth in vito mix,
trace of growth in broth.

Would pay to do individual vito in all arrays; further aid has been
consistent in vid requirement, but may vary to topic 6 same.
Also: 3-3 = don't AA groups.

2/15/55

Vito, UV3-2: fair growth in broth; other vito 0

WCA
UV3-3 Inst. rambums, A1 groups.

2/17/55
UV3-3 no growth in A1 groups or in all A3's (2 days)

2/18/55
UV1-10 6 2-1 in vito: no growth in individual vits or mix. (here 2/18)

2/22/55
UV1-10 No growth in individual vits, but some growth in vial mix (4 days) May have double requirement, or case of rogue vial, individual tube may be too high, since some are higher than in mix. Also may be reversals - see UV2-1
UV2-1 Growth in the following tubes (very slight)
Riboflavin + paba
B-12 + vid mix + reversals?
Pantothenate + paba Dirty tubes?

2/23/55 (5 days)
UV1-10 Growth in : Biotin, choline, nic, paba, vid mix
UV2-1 """" Trace in those listed previously. Very scant even in vid mix

2/24/55
Set-up mini, mini + glycine (1 mg/10 mc), mini + Vita, & mini, into & glycine to see whether glycine will speed growth in vito & itself permitting growth (1-10 & 2-1)

w.g. Jan 2/25
3/1/55

UV 1-10 & 21 in glycine, vto:

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

4 days: 0 -

glyc ±

vto +

vto + gl ±

2-1 at 4 days: 0 -

glyc +

vto +

vto + gl ± (!??)

3/4/55

UV 3-3 (2 days) 3 days

0 - - A4 - -

A1 + + + A5 - +

A2 - - all AA5 - ±

A3 - + comple + + + +

3/6/55

(UV 3-3)

A1 rundown on 3/4. Possibly time of growth in glycine

3/7/55

A1 rundown - 3 days (at nat temp, cold at night)

0 -

LAP +

LAP - comple + +

Try to 5 % glycine 2% aeration. Usually LAP should give

WCG

much clearer cut result. Because of more rapid growth.
3/8/55
Start culture of WCG 1 for another UV expl (1/4) tot 3 expl.

Ag 1, room temp, aerated.

TRANSFER STABS

3/10/55
Excellent growth of WCG 1-5 for irradiation. Plated 0.2 ml/plate of 2x10^{-5} dilution (Ag 1 medium).

UV 3-3 in mini, log, Ag aerated: in 3 days, forward growth in all tubes.

3/14/55
UV 4: 6 possible amputophores - 1 is a light yellow similar to UV 3-1.

3/15/55
UV 4-1 (yellow) is an amputophore, all others grew in mini.

3/18/55
UV 5: 48 (4psi) in culture, Ag 1 broth, aerated. Irradiated 10^{-4} dilution for 90 sec. Plated 0.1 ml/plate of 10^{-5} dilution. Inc. 25°.
3/17/55

UV4-1 & UV3-1 (yellow): Streaked on complete. 3-1 rough, 4-1 smooth. Appearance == rough & smooth WCG-1 stuff for color. Smell like Agrobacterium.

Try running down requirements:
Spread 4-1 broth (5 days old) on minimal; look for possible "reversion" 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 of 3 & 5 aerated vs WCG-1.

3/21/55

UV 3-1 & 4-1. Both show scanty growth in broth & aerated, good growth aerated (but not as heavy as WCG-1)
4-1 spread on minimal — No papillae at 2 days.

Rundowns:
UV 3-1 ++ in A4, + in A5, + in A3,
UV 4-1 ++ in all rundown tubes.

3/22/55

UV 3-1 & 4-1 rundown

4-1 no growth except in complete.

3-1 A1 ++ A5 ++
A2 ++ A7 ++
A3 ++
A4 ++ complement ++

WCG
3/24/55

UV 5: Of 7 possible superspikes, all show some growth in mini at 2 days. Check have only very faint turbidity, keep these, check especially for vito. UV 4 may be omitted from UV 5. V 5-1452

(3/12)

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

3/26/55

UV 3-1 Sandrous - 3 days (xen temp - cold at night)

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<tbody>
<tr>
<td>B1</td>
<td>o</td>
</tr>
<tr>
<td>sib</td>
<td>o</td>
</tr>
<tr>
<td>nvi</td>
<td>o</td>
</tr>
<tr>
<td>paln</td>
<td>o</td>
</tr>
<tr>
<td>bsc</td>
<td>o</td>
</tr>
<tr>
<td>PGA</td>
<td>o</td>
</tr>
<tr>
<td>K</td>
<td>o</td>
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</tbody>
</table>

Struck out, exclude individual colonies.

3/28/55

Papilla from UV 4-1 - picked from media, streaked on complete. Colonies yellow. Pick several, check for prototrophy.
3/31/55

UV3-1: Aundems ini. from 2 individual col. (2 days)

<table>
<thead>
<tr>
<th></th>
<th>(1)</th>
<th>(2)</th>
<th>(1)</th>
<th>(2)</th>
<th>(3 days)</th>
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<tr>
<td></td>
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<td>A4</td>
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<td></td>
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0 + P 0 0 0 +

[Indicates erratic results put due to recursion?]

5-1 & 5-2 Aundems grew in mini. However, there are apparently some variations:

<table>
<thead>
<tr>
<th>Col.</th>
<th>5-1</th>
<th>5-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

4/1/55

Col. from papilla from UV4-1, autotrophs. However, 1 strain did not grow after 5 days, 1 large colony many small. On min, large col appears solute. Pet, streak in complete.
4/5/55

Final comparison of mutants on two cultures from UV 3-1.

<table>
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<tr>
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<th>(1)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
<td>vit</td>
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<td>diph</td>
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<td>++</td>
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<td>riss</td>
<td>±</td>
<td>+++</td>
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<tr>
<td>A-5</td>
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<td>+++</td>
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<tr>
<td>alan</td>
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</tr>
<tr>
<td>OH P</td>
<td>±</td>
<td>+++</td>
</tr>
</tbody>
</table>

4/12/55

UV 4-1 picked from large col. in mini. Struck mildly, yellow.
Col as before, also a white, rough col. type. Latter extremely
rough - probably a contaminant.

4/14/55

Spread water suspensions (from slants) of WCG 16 & UV 4-1 on
plates of mini E 4.5 butdim: Also cross-brushed one w/ SKI.

WCG 16 - growth on mini E 4.5; none 5 E 4.
UV 4-1 - no growth on mini plate.

WCG 16 5
UV 4-1 5R
Results of attempted cross of WC616 & UV-4-1 (4 days 25°C)

Culture: WC616 can grow in SM at 200 mg/ml
      no growth in SM

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

With mixtures, confluent growth on all media: Growth
      is white, w.t. = WC616
3-3 AA groups

1-10 HC- in AA single suspension, grow well in all tubes except mini initial. AA single addition all - late growth (4 day) in none.

2-1 HC+ prominent growth in all AA single suspension tubes. Late growth (4 day) in some tubes.

3-1 (yellow) HC+ AAs together + poor in YNA

3-2 HC poor + AAs together + better in AAV, good YNA

3-3 HC+, AAs together strong +, AAV-, YNA-

3-4 Shows only one complete or mini + YX
(Have trial HC, HCV, HCYNA, YNA, YNAV, V, HC YNAV)

Possible combinations are missing HCA, 1b, UV 4-1
See whether both grow in less 5%.

If would define?

Yellow SR x + B+
White SS x + B-

Plat am
1) mini
2) mini + A
3) mini + block + C (Y)

Meth cell grown?
all + B+
Only x + B + SR
all x + SR

Tuberized:
color, S
color, B

Lee and Niwa, 1991