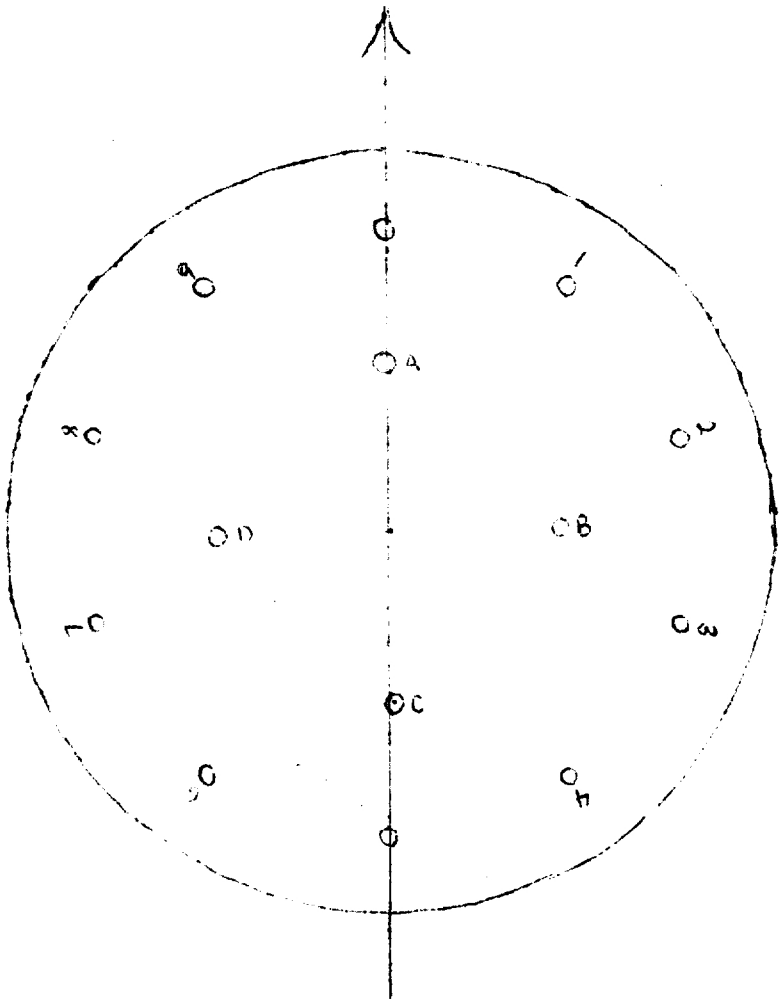
YALE UNIVERSITY
OSBORN BOTANICAL LABORATORY
NEW HAVEN, CONNECTICUT

EXPERIMENTS IN THE GENETICS OF BACTERIA

1946- 1947.

Joshua Lederberg.



AUXANOGRAM STENCIL -

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Abbreviations:

F(x) = Fries supplemented ē x. (Neurospora minimal).
CM = complete medium (essentially aminoac + vits.)

Ca = HC = Hydrolyzed Casein.
Vit = Vitamins B + hydrolyzed YNA
YNA = yeast nucleic acid
EAA = EA "essential" amino acids =
NEAA = NA non - EAA.
⊘ = minimal
⊘ = complete
T(x) = coll ⊘ = Tatum + Gray's + min. suppl.

Amino acids.

1 leucine
2 isoleu
3 valine
4 phen.
5 trypt.
~~6 tyrosine~~
7 lysine
8 arginine

9. methionine
10 histidine
11 ~~threonine~~
12 aspart
13 glutam
14 proline
15 HO- "
16 glycine

17 alanine
18 cysteine
6 tyrosine

Ind = Indole
Anth = Anthranilic.

Vits. V 1 Thiamin
2 Riboflavin
3 PAB
4 NIC
5 Foliac
6 Pyridoxin
7 Pant

8 choline
9 inositol
10 Biotin

Purines + Pyr.

3/23/46. – 3/24 Y 160.

Test 156 – Strains. Dil. surface growth in H_2O + test as indic.

Incr 9P23. 33°
1st Reading: 12 h.
2nd 24h. 9A24.

#	O		C-M		HC		Vct		Eaa	Naa	
1	–	–	++	+++	++	+++	–	–	+	+	– –
3	–	–	++	+++	++	+++	–	–			
4	–	–	++	+++	++	+++	–	–			
7	+	++	++	+++	++	+++	+	++			
9	±	++	++	+++	++	+++	–	+ –			
10.	+	++	++	+++	++	+++	+	+++			

check!

(This agrees ε the previous interpretation that 7–10 may readily back-mutate. 1, 3, 4 should be tested further. of (1).
Note poor growth in ε 9. 9. Evidently strong inhibition is present. Wh. is inhibiting?!)

Incr: dil. from HC
9P24. 36h.

1: T( )
trypt –
thr –
lys –
arg –
dal –
meth –
v-i + +++
leuc – +
hist –
HC +++
O –

usol. – ++

Repeat 9: Incr dil from CM. 24h. 1st. 36h 2

T (Vct) – +
Vct – +
Vct + ++

T (O) ++ +++
O ++ +++
O ++ ++

T (HC) +++

Test 160 [156-1] for adaptation. 161.

9P26. 37°.

162

	Incr. into 0.	12h.	24h	Therefore these responses are key way of growth, rather than adaptation!
1	~~HC~~	0	—	
2	V-iso.	0	—	
3	lc	0	—	
4	cool.	0	—	

1130A28 V 3:30 — 1130A28 O 3:30

156-9.5	from HC				+	+
6	" V2T	onto CM slants for testing		=	*	-
7	" O.			-	-	∠

dilute + test 2P27.

		5	±	+	++
		6	±	-	+
		7	-	-	+

Test 160 for growth vs. adaptation - 9P26. into T(0)

	12h	24h.	48h	930A29	10A30	testing O	A29	10P29	10A30
1. from HC	—	—	—	+++ ✓				±	+++
2 " V-i	—	—	—	2P27 -					
3 lc	—	—	—	—	—				
4 cool	—	—	—	—	±				

Test 156-9 substrains.

a. to CM slants 5 from HC 6 from vit 7 from O

			1130A28	330P28	8P28	A29 Test	O	V
to vit + 2P27 HC	5 - vit	—	±	++			±	+++ — —
min.	O	+	++	+++			+	+++ + ++
V	6 - vit	—	±	++				
	O	—	+	+++				
O	7 - vit	—	⊕	±				
	O	—	+	+++				

This behavior is remarkable. What is inhibitory? What is the type of genetic modification?

See 164.

Mutant identification.
"Auxanography"

Jan 7 1948

	histidine	+ C. Hydrolyzed Casein →
E-8		
161-6		

ca. $10^{4.5}$ colonies per plate. They are visible for a radius of 1 cm in both cases around the HC, then thin out somewhat.

161-6 – not scattered large colonies, but quite numerous small. ∴ not "adaptation". ∴ vitamin effect is directly on growth, not adaptation. Ap. of g.c.a. not clear. Contains grows faster on e-a-a. than on s.c.a.a. but this may not be a specific response. Need L-15 for control. Inhib. by biotin??

P 37 ca 10·12 "colonies" full size are seen in the e-a-plate, presumably adaptants

Try heavier inoculum.

See Auxanography – p. 168.

3/28 Mutant identification, patching. 163a

Plate histidineless (E-8) and 161-6 heavily onto T7 agar. When solidified, add a loopful of

(a) HC
(b) histidine .1% to each.

7P28

Tart "washed agar #1" for biotin. Agar was had by 10 transfers through dist. H_2O, + 2× ± 95% alcohol, dried in a dessicator ± $ZnCl_2$.

Plate "58" (biotinless E coli) in 2% agar unwashed
" " washed
" " "washed + 1× biotin"

P31 — no colonies!

Broc. on surface (streak) 8P31.

A2 — Well developed colonies only where biotin was added. None elsewhere!!

Auto-answer

Test various T-L. mutants.
Retest 156-1 142-17.
Test 156-2, 3.

4 1130P28 inoc.
1 930A29 ·1·
2 12 N
3 9P29

	0	TL	TL(HC)	[illegible]+eaa	TL+neaa			
142-17	− − −	− − −	+ ++ +++	− + +	− − −		= Y10	
-36	− − =	− − 10A30 − +++	++ +++	+ ++ ++	− − +++		(adapted??)	
-54	− − −	− − −	− + +	− ± ±	− − −		= (Y9)	
-57	− − −	− +++	++ +++	+ ++	− + +++	n.g.		
	0	HC	neaa	leuc (1)	isoleuc (2)	[illegible]	val+isol. (23)	val+leuc. (13)
156-1	− − =	++ +++	− − =	± ++++	− ± ± (check!)		− + +++	± ++ +++
	0	HC	neaa	eaa				
156-3	− − =	++ +++	− − =	++ +++				
156-4.	− − =	++ +++	− − =	++ +++				
E-1	0 10A30	HC	neaa	eaa 10A30				
	− − −	++ +++	+ ++ +++	− − −				
Test 156-9 on various vitamins.	eaa	0	HC	Vits.	V1	V2	V3	V4
	+ ++ +++	= = +++	++ +++	= = = ++ ± +++ 10A30	− + +++	− + +++	− + +++	− + +++
	neaa	[illegible]	V6	V7	V8	V9	V10.	V11-ynah.
	− + +++	[illegible]	− ± +++	− + ++	− + ++	++ +++	− − +	− + +++
E-6	0 →	− − −	−					
E-1 + E-6 (3x).	0 →	− − −	− 10A30	✓ P3				

inoc. 530P9. suppl A B C D 1130P Retest 142-17- auxanogram.

9A10 − D +++ A +. suppl. c̄ vitamins.
Many adapted colonies! 12M10. No resp. response. − Entire plate somewhat turbid
to new plate 4P10. Incubate 4h. before suppl c̄ vits. etc.
12M10 − Thiamin.

3/29 — Identify mutants on hand

1. 12N30
2. 4P30

12M30 Inoc.

12M31 Inoc. 1) 4P31 2) 10A1. 3/2P1

			val	?	try	lys	arg	meth	biot
		TL	TL+3	TL-4	TL-5	TL-7	TL-8	TL-9	TL-10
1	142-17						- ±	± ± +++	
2	142-36 N.G.	- +++	+++	- +++	± +++	- +++	- +++	- +++	- +++
3	142-54	- - -	- - -	- - -	- - -	- - -	- - -	- +++	- - -

~~7~~ ~~8~~ ~~9~~ — Postpone inoc.

Check 4/2/46 TL-9 +++ no others.
✓ 4/3/ ∴ probably both methionine.

0 cult.

		11	10	1	2	3	4	5	7	8	9
4	156-1				± ±* ✓ + +++	• – –					
5	156-3	–	– – ✓	+ +++	± ± +++	– ✓	– ✓	– – ✓	– ✓	– ✓	– ✓
6.	156-4.	± ±	– –	± +++	± ± +	–	–	– –	–	–	–

No further growth by 10A1.

(1) 10A30.

Check: 4/2 — OK.

* anyone's prep. other is Tatum's. may not be enough used.

~~1. Try 8.~~

See infra for recheck on 142-17 + 54.

5P4. — Plate 142-17 + 142-54 into T(TL) agar. 1 ml inoc. Incubate to 9P4, then do auxanograms on Essential a.a. on 54, only "9" on 17 using double depth agar.

	HL	B	1	2	3	4	5	6	7	8	9	10		C	D	!	Check!
17	++	~~++~~	–	–	–	–	–	–	–	–	–	–		–	++	!	8P5
54			–	–	–	–	–	–	–	–	+	–	–	–	–		8P5

Check 54 on hybrid 10P5. — 12N7(1)

∴ 54 is TL Meth.

→	M	MT	ML	MTL	TL
	–	–	–	++	–

Hydrolysate C. 166a

200 (2×100) T(O) 72 hour culture. Centrifuge cells down, put in 10 ml 6 N HCl, seal tube + keep in boiling H_2O for 24 h.

Lost during hydrolysis.

Try again.

3/31 - 4/

167a.

Mutants by ultra-violet irradiation.

10A 31. Incr 50ml/125 ml flask coli C-M ē 58 (Tatum's biotinless coli) and grow on shaker, slowest speed, at room temperature.

① 9A1 – 1ml sample to 50ml coli CM.

②. Irradiate in quartz tube, 11 cm from tube, 15 ~~sec~~ min. Incr. 1ml into 50ml coli CM. Grow 1, 2 on shaker.

No appreciable growth in 24 hours. Dosage too high. Try 5 mins. (1 colony at 1:50 dil.)
P2 finally came up.

A2. Use 167A1 + irradiate as above, 5 min. Do in tupl.
11A2.

Estimate (a) before irradiation.
Dil $\frac{1}{500 \cdot 500 \cdot 50}$ + plate into YBG. 1a.

b. after irradiation.

1:1	50^0	710.
1:50	50^1	9
1:2500	50^2	
1:125 000	50^3	
1:6250 000	50^4	

c. In (a) prepare last dilution in saline also. Irradiate + compare sal. + H_2O after 48h. Do in tupl. 1c.

~~d. Test colonies from b (1:1) and use for 58/2 studies~~:

2a+2c 10P2. O O
3a+c 9[30]P3.

U.V. irradiation;

~~#.~~ 15 minute irradiation A1.

Before irradiation, plate counts not made
after 1:50 — 1.
[illegible] 1 ml. 2P ~~#~~3 dilute 1:25 x 10^6 + use method II,
plates 1-5 for mutants (T(o) + dr-botin)
Colonies first apparent 9P4. 10A5 layer YBG. (Agar too soft).

1
2P4. 2 ~~Plates~~ so soft as to be ~~almost~~ useless.
3
~~#5~~ 4
T(o) / YBG 5. Pick colonies before [illegible]. to >8/radiation-resistant.
Test on T(o) - all grow —

5 minute irradiation: A2 see 172
i ii iii

Control plate counts: *1a — (last dil in T(o))
1:12,500,000. *1c — last dil in saline).

+ send for viability study

After irradiation, 1b : 1 ml 710 } not very different. Incubate + shake alternately.
1:50 9

c) Viability of control in sal, H_2O. — Apparently very low in this dilution

			i	ii
Incubate last dilution	10P2	2a	0	0
flasks of 1a, 1c + sample.	10P2	2c	0	0
Plate in YBG	P3	3a	0	0
	P3	3c	0	0

Auto immunology

I 10A1. halo 2 cm diam 2 1 cm fringe around H.C.

Nothing over 10^{-3} dilutions By 8P, there was a very faint response to H
At 8P, >3 cm diameter.

II Supplement 10A1. By 2P., a distinct turbidity was visible over HC & a faint one over histidine. By 8P this was very distinct & H. less impressive over histidine. Both ca 2.5 cm diameter..

B). Supplement other portions of both plates, as above, 8 3/4 P. (after prolonged incubation.

10^{30}P — 1B-dist turbidity under HC.
— 1 colony.

12 hour incubation probably optimal
maximum size is above or less optimal + maybe reduced for frequently occurring types.

3) Add HC qp 4. — No response desired

Incubation, hence at ca 35° unless stated.

~~Auxo~~-auxanography

8P31 - Plate heavily (ca 10^6) into 7(0) 2% agar ± 8 (histidine)

I Add, as cooling drops of HC + histidine (10%, .1% resp.) + dil. 1:1000 resp.

II Incubate 14h. fast.

III Cover + incubate to P4. add HC. Then ~~[illegible]~~ to determine survival.

P2 - # 48 adapted colonies. ←
Try more conc. agar.
P4 ←

IV 4/2 - 3. Try as above ± 3, 4, 6% agar.

No diffusion to speak of.
∴ 2% is best

V 4/3 - Use of indicator - plate ± 8 as above ± 10r/cc
Methyl Red (also 20r/ 50r/). Medium is alkaline to the indicator.

156 – 9.

See 161.

4/2/46.

I Plate 161 – Theavily in 20/o 7(0) agar plate. Add suppl. HC & Biotin to surface 10P

10A – Turbidity increased over HC.
~~Noticeably~~ clearer ~~or~~ under biotin. (0.05ml 1:1/ml)

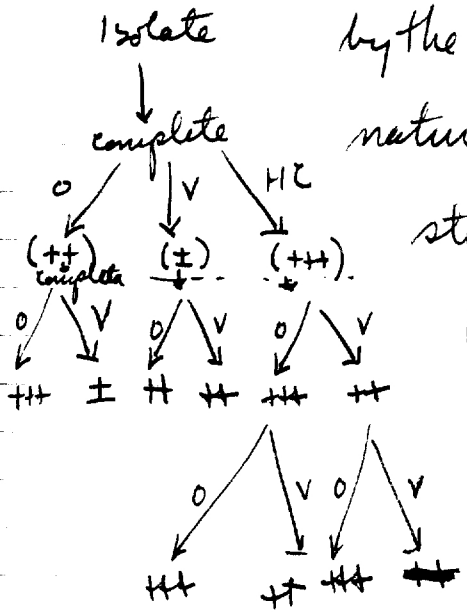
5P. do. The biotin area is definitely less turbid than the rest of the plate. The plate is ~~fairly~~ fairly dense but somewhat darker under HC.

9P4. Differentials essentially disappeared. ∴ 156-9 is not an adapter, but perhaps a slow grower & perhaps inhibited by biotin. Check E 115! (At best adaptation is not genetic or "mutational")

See 173.

Study of 156-9

1. Isolated by Method C. from γ-irrad L15.
2. Responds rapidly to HC, but grows on min.
3. Is slower on vitamins, or on biotin, but eventually grows up.
4. No "adapted" colonies seen on auxanography on 1st attempt (163.)
5. Exp. 161. Grown on HC, Vits and O. (Exp. 160). Then transfund to complete slants, growth tested on O + V. To all appearances, that grown on minimal was most sensitive to biotin. Apparently an exposure to HC or to V limits response to V, but not enough to complete. Also, growth on minimal reinforces susceptibility. This is not supported by the trial results on the HC line!!! The fundamental nature of the phenomenon is not clear. It should be more striking on plates than otherwise. Also, all these results have to be checked.



I

Inoculum ca 10^6

Data

	Medium	Date	Requirement					
1. 58.	T(0)	4/3	Biotin	P/4. 0	4/5 0			
2. E-6	T(0).	4/3	Methionine	P4. 0				
3 58-5198	T(0)	4/5	indole	0				
4 58-5417			uracil	9				
5 58-5636			indole	>10 (plate microcolly turbid) compare 5198				
6 679-680	T		[illegible]	7				
7 "	L		[illegible]	1				
8 "	0		[illegible]	0				
9 679	0		[illegible]	2				
10 58-5631	0		[illegible]	1				
11 58-161	0		[illegible]	0				
12 58-2621	0		[illegible]	0				
13 58-5173	0		[illegible]	0				
14 58-118	0		[illegible]	0				
15 58-336	0		[illegible]	0				
16 679 662	0		[illegible]	0				
17 679-[illegible]	0		[illegible]	1				
18 58-4[illegible]	0		[illegible]	0				
19 58-3214	0		[illegible]	[illegible]				
20 58-3[illegible]	0		[illegible]	0.				

Mutant Reversions

Pour ca. 1 ml of inoculum (ca. 10^9) into T/O, as was indicated and incubate 48 hours; look for adapted colonies. Data on 170a.

Mustard.
E-3-Histidine sep 168

58 (Bertani).
E-6 Methionine. } Probably excess carryover: plates streaked.

Mustard induced — consequently each mutate also.

$(-N\text{-}et)_n + H_2O \rightarrow -NH + EtOH$

Check for ratio improvement: 58-63a

Dey.

4/3/46.

5P3 – Cross-streak on minimal plate:

E-6 (methionine), 58 (biotin) + TL.

P5 no growth.

9:30 P5 – On minimal liquid, the following:

	Exp.				
1	679-680	T-L			
2	"	"			
3	"	"			
4	58-278	B-Ø	No growth in any by N9.		
5	"				
6	Both	—			
7					
8			10P10	(1st drop x 10^8/cc)	

N9. Repeat above & heavier, fresher inocula. in T(0).

1		TL	±	±	
2		TL	±	±	
11		TL + Bø$_1$	+	++	
12		TL + Bø$_2$	+	++	
13		TL + Bø$_1$	+	++	
14		Bø$_2$	±	++	
21		Bø$_1$	±	++	
22		Bø$_1$	±	++	
31.	On complete	TL + Bø$_1$	–	+	
32		TL + Bø$_2$	–	+	
33	On ~~complete~~ test in T(0)	Bø$_1$ + Bø$_2$	+	++	+ +

T(0) 1030 P9 – P10 930 P10 — P11.

2P9 Cross streak on T(0) again

41.	Bø$_1$ × TL	nothing by P14.	
42.	Bø$_2$ × TL.		

Note

Streak of minimal plates

See 176.

+	+++	Try plating out from C-M.
++	+++	
+	+++	

T(0) 930 P10 — P11.

. . something happened in the complete cultures between the 1st + 2nd tests in min.

19+27 [illegible]

[illegible] of 6 [illegible] + 9 [illegible] [illegible]

	Plate #	Total.	Mutants	Mutants	#s.Y.		[illegible]	Rate.
			1230 A7	1130 A.7			[illegible]	
	1	218	3 [1-3]	1 [4]			1 [5]	
	2	213	4 [6-9]	5 [10-14]			2 [15 16]	
	3	220	2 [17 18]	3 [19 21]			1 [22]	
	4	194	5 [23-7]	3 [28-30]			2 [31-2]	
lost	~~5~~	~~209~~	~~2 [33-4]~~	~~2 [35-6]~~			~~0~~	
	6	160	1 [31]	0			0	
37/		1214	17	14.			6	
		Retest						
Y(58-...)		T (b)	HC	Vits.	Auxanogram.			
1		+ ✓						
2		+ ✓						
3		+ ✓						
→ 4	58-Y11	— ✓			HC; D?; ?;		...	
5		+						
6		+						
7		+						
8		+						
9		+						
Proline 10	Y12	+ ±			A · B C?			
11	~~[illegible]~~	− X+			hybrid plate sl. inh. HC			
→ 12	Y13	+ −						
13	~~58-Y13~~	− X+			hybrid plate			
→ 14	~~58-Y13~~ Y14	+ −			B?			
15		+						
16		+ ✓						
17		+ ✓						
18		+ ✓						
20		+ ✓						
21		↓						
22								
23								
24								
25								
26								

Ultra-Violet Irradiation: See 167A.

Irradiate 58 coli. 5 mins at 11cm in quartz tube on shaker.

Inoculum before irradiation / ml. ~~(4 x 10^8)~~. (8×10^{-8}).

i	1/q. (455)	$= 5.7 \times 10^9$	1/c (249) $= 3.12 \times 10^9$
ii	1/q 97.	$= 1.23 \times 10^9$	1/c (129) $= 1.64 \times 10^9$
iii	1/q. 300	$= 3.75 \times 10^9$	1/c. (252) $= 3.1 \times 10^9$
		10 7	7.8
		3	/3
		$= 3.6 \times 10^9$	2.6

3×10^9 = mean.

Survivors = 710 = $\frac{7 \times 10^2}{3 \times 10^9}$

$= 2 \times 10^{-7}$

.00002 % survival. in 5 min.

[Ironic there were ca 50/ml after 15 mins, there must be heterogeneity in u-v susceptibility.]

Shake 48 hours + dil: 12.5×10^6. (9p4). Plate 4 in T(B).

2:30 P6. Jagger.
12:30 A7. Pick mutants (ca. 1%.) see data.

[See 183]

All mutants this time were picked 21 hours after layering.

Score in this group is 5/12.

3 probable.

5 mutants in 1200 cells.

comp. ε spont.

5/6000.

	found	Exp.
sp	5	1.7
uv.	5.	8

$\frac{10}{7200}$ — Exp. are too small.

$\frac{(8-5)^2}{5} = 1.8 = \chi^2$ too small for sig.

		T (Bod).	HC	Vits.	Auxano.	✓
Proline ~~27~~	Y15					
28	~~58-Y14~~	— ✓			HCl 1z; T;	
also test: Proline - 29	Y16	+ ✓				
30	~~58-Y15~~	— ✓			HC large zone; D; A	
31		+ ✓				
32		+ ✓				
33		+				
34		+				
35		+				
36		+				
37		+ ✓				
Tatum's 58	carteof.	+ ✓				
58-278	"	— ✓				
Total: 5 stable						
1214 colonies.						
5580.					HC; AAA????	

4/5/46 156-9 vs. L15.

Biotin

Incr. 3P 9 asmf. (1) 9A10. (2) 9P10

	0	0	V.+	Biot.	valine			
L15	+++ ✓	+++ ✓	− ++	+ +++	+++ ✓			
156-9.	+++ ✓	++ ✓	− ±	++ +++				
K12	+++ ✓	+ ++	− +	− +++				

Biotin is then not the only factor + fluoric some inhibition by a vitamin of 156-9.

Add 1 drop 1N HCl / 10cc
for preservation?

Also consider:

Eff. pH indep (for pump. preservation).
benzene

Concentration dep. of substrate.
(Res might be sharper after pre-incubation).

4/9/46. **Auxanography—**

Optimal inoculum size. Plate into T(B) varying samples of an inoculum culty. ca 10^8/cc. of 58-278 (B-φ)

Incubate 12 hours & supplement c̄ HC + c̄ φ. [B4 = Phenylalan.]

Inoc: 530P9 Suppl. 1630 9A10.

		Diam. φ	Diam HC.
1 ml	1	1.8 ++	2.3 +++
.1 ml	2	2.5 ++	3cm ++
.02 ml	3	2cm ++	3cm +
.002 ml	4	? ±	2cm +
1:250000 .000004 ml	5	2 +	4cm +

↑ distinctness

The method can be used at any inoculum size, but is most neat c̄ heavy inocula. For very adaptable strains, it may be important.

OPTIMAL AGAR DEPTH. 4/10. Inoc. 1ml undil culture into varying agar depth. Inoc ~~[illegible]~~ ~~Incubate~~ to 11 30 P9.

Supplement

A - φ - at t = 0. B: φ at 1230P11.

		A (11A.)	730P	B. 230P
1	5ml	nothing!	practically nothing	fair response
2	10ml			
3	15ml			
4	30ml			

It makes very little difference what depth agar is used. 10–15 ml is quite OK.

TIME OF INCUBATION. Inoc. 58-278 into T(B) agar 9P11

Suppl.	t	9A12	7P12
①	10P	0	±
②	12M	+	+
③	~~[illegible]~~		
④	10A12	~~[illegible]~~	−
⑤			
⑥			
⑦			

← optimal < 12h.

quality of zone.

sample is 6000.

eg. 5 mutants.

compare 175.

hydrolysate A 1:10.

* Contam. ? Neurospora + thiamine!

4/9 - 10 .. 1/6.

Spontaneous mutants in 58.

530P9 broz t.t. ē coli CM ē 58. Shake at RT.

9P10 – dil 1: 12,500,000 + plate out by method II in T (biotin).
Incubate at 35° to 11P11 Plates recorded!
Count 1130 AT 6P13.

*1			
*2			
*3			
4	1000	1	
5	"		2-5
6	"	6	
7	"	7	8-9
8	"	10	
9	"		11,12
*10			
		124	30 h.
Add .1ml of A/10 in lieu of biotin.			
11.			

Prick to CM 830P14 n.v. satisfactorily
None of the 1st series would have qualified except by flexibility criterion. 1 (B). V. sm. inoculum from colonies required.

1	+
2	+
3	–
4	+
5	+
6	+
7	–
8	–
9	–
10	vegetative in CM
11	–
12	v.g. int CM.

Same 1/4

Inf.

4/12/46

Broc. c-M c̄: + shake A12 — P13

1. TL
2. Bφ1
3. TL+Bφ1.

12 M13.		T(0)	P16.	P17	P17: add	A18	P19.
Test 1 } loopful inoc	4	–	–	–	1 loopful of	–	–
2	5	–	–	–	complete	–	–
3	6	±	+++		coli medium		
1+2	7	±	+++		to tubes		
				–	4, 5 and 9		
3 } v.sm. inoc	8	–	–	–	13, 15+17		–
1+2 }	9	–	–	‡ –		–	– killed?

See 171. Plate 31. into T(0) + cover. M13. (TL×Bφ1 in C-M).

1:25000 ~~1:50~~	10	} No colonies P16	Cover c̄ φAl+B.
1:12,500,000	11	}	Nothing came up.

			P17	A18	P18
	12	3 into CM, c̄ shaking at 37° 1030 A13			
small inoc.	13	Test: (6).	–	– ‡	+
large inoc.	14		++	+++	
P16.					
Test 6 sm	15		–	+++ ‡	
6 large	16		–	+++	
7 sm	17		–	– ‡	–
7 large	18		–	–	–

∴ the carryover is as might as in medium count of cells! There has been no recombination.

Oceanography.

Preservatives.

I Plate K-12 in T(10). Add drops of HCl - diluted.

Center	Conc HCl (10N)	4 cm inhibition	
2	1:10	1 cm inh.	
4	1:100	No inhibition	try 1 drop HCl/ 10 cc bacteria, etc.
6	1:1000	No inh	
8	$1:10^4$	No inh.	

I. Benzene.

1.	Center -	pure Benzene	Clear zone
2.	10 -	Benzene - water.	OK
3.	5 -	Alcohol 95%.	OK
4.		Chloroform	OK.

Pour plates ~ ca 10^6 organisms aerial + hyph. when hard, streak II. on surface.

1. B Φ
2. B Φ
3.

4/17

	I.	hyph	II.
1.	B Φ	Φ	K-12
2.	B Φ	Φ	K-12
3.	B Φ	Φ	K-12

See N-363 for expt. on symbiosis with N. B3757.
E 181.

A19. About each streak is a clear zone, ringed ~ a range of greater density, fading off to a small turbidity.

Ans complete P19 — No diffusible zone found. K-12 somewhat inhibited.

Salt resistance mutants.

after Stevens + Tatum.

4/17/46. S8/

1. Plate [redacted], ca 10^3, into complete plates +

1. $CuSO_4$. [redacted] 10 mg
2. $HgCl_2$ .1 mg

A19. Nothing came up. use less.

A Penicillium sp. contaminant did grow on D!

Syntrophism

58-161 + 58-273
14 ϕ,

10 ml T(b) in dens. tubes. Biotin .01γ. Suppl. + inoculate as indicated (in γ/10 ml): 1030 P19. Incubate at ~~28~~ 28°

Dens. readings (galv., uncor. tubes).

#	Inoc	Sup: ϕ	M	930P20.	1P21	330P22	9P22	9P23	Tube #
1	M	10	10	–	+	80	80	+1.5	
2	ϕ	10	10	–	–	~~93~~	95	–	!
3	M	0	0	–	–	93²	93²	–	
4	ϕ	0	0	–	–	94²	93²	–	
5	K-12	0	0	++	+++	58²	57²	+++	
11	ϕ+M.	1	0		–	94	93²	–	
12	"		1		±	90	83	+++	
(13)			3		±	68	61²	+++	
(14)			10		–	83	75²	+++	
21	"	3	0		–	94'	94²	–	
22			1		–	93	93	±	
23			3		–	75'	63	+++	
(24)			10		+	66	67	+++	
31	"	10	0		±	88	84	+++	
32			1		–	93	92'	±	
33			3		+	84	13³	+++	
34			10	–	±	81³	80²	+1.5	
41	"	0	0		–	92'	92'	–	
42			1		–	92	91'	±	
43			3		–	93	93	–	
44			10.	–	–	95'	do.	–	
		75% = ½ max.				73²	73'		

45. Plate ϕ+M into plates ± 5γ methionine; 100γ phenylalanine/plate to see whether this is a good separation
ϕ el. inadequate

~~M~~ 22 to 38°. This does sum to squid thing up.

#13

Dil 1:50,50,500 = ~~1:1,250,000~~ = 1:2,500,000. + inoc. into

A. T(B)	0	
B. T(B+M)	40	21.5%
C. T(B+ϕ)	146	

186

FPA.

4/14/46
26 Cal!

Asbefore:

		Supplements.		
5	broc	∅	Tyr.	FPA.
51	K-12	0	0	10r
52		0	0	100r
53		10r	0	100r
54		100r	0	100r
55		0	10r	100r
56		0	100r	100r
57		10r	10r	100r
58	58-278	1r	0	0
59		10r	0	0
60		100r	0	0
61		10r	100r	0
62		10r	0	10r
63		10r	0	50r
64		10r	0	100r
65		0	0	100r
66		100r	0	10r
67		100r	0	50r
68		100r	0	100r
69		10r	10r	100r
70		0	0	50r
				100r
71	58-4899	0	0	100r
72	58-5030	0	0	100r
73		0	10	0
74.		100	10	0

Many aspects of this experiment are coextensive with the investigation of the aromatic a.a. mutants + are to be postponed until this is carried through by ELT, et.al.

4/19. Utilization of FPA:

			430P20	3^{30}P22	9P22	P23
81	58-278	10	-	96		-
82		100	-	97		-
83	58-4899	10	-	96^{2}		-
84		100	-	97		-
85	58-5030	10	±?	95	75^{3} ∓	+++ (green tint)
86		100	-	67	65^{1} ∓	+++

∓ test on minimal: they grow.

Mutant detection:

Viability

Pour (with shaken) 58-278, dil. to 1:12,500,000 into T(6) plates + cover as in mutant detection. 830 P19. Count complete at time t_1. Colony diameter recorded at t_n. Incubate at 38°.

	t_1	hours	h^1 430P20	h^2 830P	10A21	4P21	8P21	12M21	Count.
1	9P19	1	19 5		+++ (excludable variation.				
2	"	1	4			← do			362
3	1130	3	17		+++				352
4	"	3	6			← do			
5	930A20	12	7	< #1.	+++				379
6	"	12	3			← do.			407 (41?)
7	830P20	23		—	++		+++		363
8	"	23		—					5
9	10A21	36			—	±+	+	++	ca. 380. ← too many bottom colonies
10	8P21	48.						++ (9A22)	349
~~11~~	~~12M21~~	~~48~~							353
12	1mg 6	6	6	∴ 7 hours is badly too long for period II. Past runs 4.9.					

Bottom colonies troublesome!

Repeat 9P20: clearer agar. as above. Pour bottom layer. Inoculum from 48 hour shaken culture is complete. Incubate 30°.

21	9P20	9	+	++	++	+++		0	131	−9
22	9P20.	9	+	++	++	+++		0	139	−1
23	1P21			±	+	++		16	138	−2
24	1P21.			±	+	++		16	151	+11
25	8P2[illegible]	23			−	+	+++	23	154.	+14
26	8P2[illegible]	26			−	+	+++	23	133	−7
27	930A22			Uneven pour: lumpy.			++	36	139	−1
28	"			" " "			++	36	123	−17
29	3P23	7:15P.		(5) colonies quite distinct micr. indicates (3-5 h)				64	156.	+16
** 30	1130A24.	26°		130P: — (7) sm. colonies					145	+5
31	"	30°		colonies micr. dia. avg. (2) sm colonies					135	−5
32	" (2×)	38°		!!! (8) " "				3½ da. 86.	340. (2×)	
33	CM.-P.C.	+		+++				0	146	+16
34	Plate cent	+		+++				0	143	+13
35	"	+		+++				0	138	+8
				11A21.	8P21	12M21	10A22	P23		Δ.

Count. $\Sigma\Delta^2$ =

m = 140. 1097.

σ = ~~9.~~ 8.85

$\sigma_{calc.}^{140}$ = 14.

Viability of 58-278 at 38° is excellent for ± 48 hours.

* 7 small colonies picked 10P22. punctum.

Rick colonies 11A30. see 194.

* 15 units = 1 mm.