

Auxanography: pre-nicub. time series,
use ϕ al.

184.

Probably 2 zones/plate.

Inoc T(B) plates mainly \approx 58-278. ~~to~~ 230 P21. Incubate
to $t \approx 28^\circ$. Then ~~suppl.~~ \approx drop of dl selan. 6:50 to 38° incub.

A22

1A 3:30
B. 6P > A.

2C 8P \gg A. Best: 6 hr. precultivation.
~~D 8P: H-12.~~ Inhibited around streak; then zone of growth as supra.

3.

4.

8P: ABCD.

Auxanograms var. unidentified mutants. As above.

58-5880 A,C,(B) C9,(6+7?) Methionine? Rechecks.

175-3 - inh. HC

175-7 A.

175-8 D.

175-9 C?

175-11 inh. A,C.

175-10 inh. hydro. casein. Seems protot.

- 175-12 A D1 inh. D7 Proline with tyrosine

175-14 -

- 172 28 A,D. D1 inh. D7,8 Proline

" + cyst

- 172 30 A,D. D1 " " Proline

" + cyst

not a u.g. series.

Need better washing.

Check densitometer calibration

4/22/46

Use culture of 278 which has given plate counts of ca. 3×10^9 .

$1:3 = \text{ca. } 10^9$. Green filter 540.
 A. (73) Absorb = 0. Stand = 0.
~~42'~~

St. 91²

Red filter 660.

A	74.
St. 20mg/ml	53
10mg/ml	25
	48 ²

Green filter Abs = 0.

74'

	Dilv.	Dens.	Dens/dil	
A.	43	367	367	$367 = 3^{2/3} = 11/3 \approx 10^9 \text{ cells}$
A/2	65.	187.	374	$1d = \text{ca. } 3 \times 10^6$
A/3	75'	124	372	$\text{ca. } 10^6$
A/6.	87	060.5	363	12.3×10^6
A/9	91	041.0	369.	11.1×10^6

mean = 369.
 m.d = 5
 $= 1.4\%$.

Ultra-violet mutations: type

186.

4/22/46.

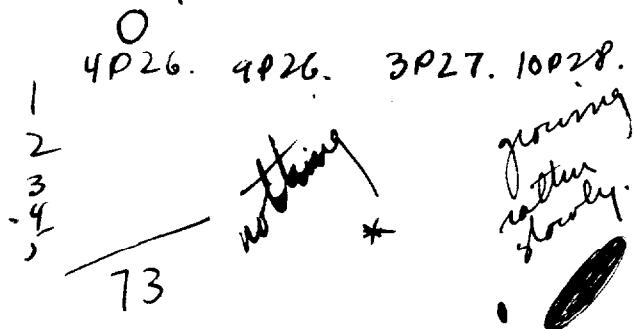
24 hours shaken tube culture 58-278. Irradiate at 11 cm. for 2 min. Mix 1 ml into another 10 ml and shake 24 hours. ().
9P22.

$\frac{60}{125} \times 10^0$ dil 1:500 - ca. 7500
 $1:75,000 - \frac{125}{50}$ These figures check.
These figures check.

∴ survival is ca. $500 \times 7500 = 3750,000$ out of 3×10^9
= 1% = 2 min.

N25. Dil $1:2.5 \times 10^6$ and plate into $\tilde{\tau}(\phi, B)$. 38°. 5 plates.

1230P26 Layer coli complete.



* There are 12 prototroph colonies on this plate. In addition, ca 50-60 new colonies have appeared, first noted all this time. The other plates are similar. Made prototrophies & continue incubation. In addition, there is a single colony of intermediate type. 7P27 - 12 min. 11 cm. (See also 183)

See 194.

4/23/46.

No good

Syntegenism
FPA. 187.

I 58-278 (δ) + 58-161 (methionine). As. 181. heat. 38°.

boc. 1230A2H. T(B) medium. .01r. 10r=1cc. Add 10cc.

	ϕ	M.	2Y6. 386. M24 2P25	II P25	G d. II P25. II P26
1	M	0	0	-	100
2	"	0	1	+	98
3	"	0	3	+	98
4	"	5	5	++	98
5	"	0	10	+	95
6	"	10	10	++	98
7	"	0	0	++	95
8	"	0	0	-	0.02
9	"	3	0	+	0.01
10	"	5	5	-	0.02
11	"	10	0	-	0.03
12	ϕ	10.	0	+	+++
13	ϕ	250	10	++	-
21	ϕ	0	0	-	-
22	"	5	5	++	+++
23	"	5	5	++	-
24.	"	5	5	+	+

every thing still clear

II

	ϕ	FPA		
31	phi	0		
32	0			
33	100r	100r		
34.	200r	100r		
	200r	200r.		
	200r	0.	See 13.	
	0	0	See 7.	
	0	+	See 181.	

77.

2. q. Old culture medium?
See 193.

4/26/46. Dose incomplete. U.V. radiation 11cm etc.
 $\overline{k_{RAD}}$

26. 0. 7×10^8 1:12,500,000.

56.

A. 1 2 MIN. 250,000.

1 ca. 10^5 . Killing. 10^{-3} surv.

2 2,500,000.

0 $p^S = 3/2 m.$

B. 1 5 MIN. 1:1
 2 1:100 4,500 1:
 3 1:5000 1:
 4 1:250,000 1:250,000 1:
 5 1:2,500,000 1:2,500,000 0

7P27

dose flasks of 50 ml complete coli \pm 1 ml of each dosage above.

D	1 6.	.1	probably anti.
	2 7.	.1	
	3 8.	.1	
dil c	4 9.	#1	
5 10.	#1	2	
6 11.	#1	1	
7 12.	#1	0	
8 13.	#1	1	
9 14.	#1	0	
10 15.	#1	1	
11 16.	#1	0	
12 17.	#1	1	
13 18.	#1	0	
14 19.	#1	1	
15 20.	#1	0	
16 21.	#1	1	
17 22.	#1	0	
18 23.	#1	1	
19 24.	#1	0	
20 25.	#1	1	
21 26.	#1	0	
22 27.	#1	1	
23 28.	#1	0	
24 29.	#1	1	
25 30.	#1	0	
26 31.	#1	1	
27 32.	#1	0	
28 33.	#1	1	
29 34.	#1	0	

This strain is certainly less resistant than 58-278, and should hardly be designated 58/r. 19P A. 1:12,500,000 1030 P 27. into T(B, 0) bottom + cover.

11	12.	13.	14.	15.	16. complete.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50							
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Y23-Aquane 0 56 250.

2 55 } 250.

3P290 4A30X 10P30 ⊕ 1030 P 1 □

sm coln. Total
250

Richter, 197

Triple mutants.

192

4/26/46

24 hour culture shaken at 30° 58-278. ~~1 ml into 5 ml~~

4P26. Phage units: culture 1:12,500,000. into T(B, φ). 3P26. ^{0.01 ml into CM} 192 A.

1	<u>150A28</u>	<u>1230P28</u>	<u>10P28</u>	<u>10A29</u>	<u>10P30</u>	<u>$\frac{(35)}{1}$ P1</u>	26 37 29 38 31 <u>161</u>
2	=	=	-	-	-	<i>N. crassa</i> contam.	
3	=	=	-	-	-		
4	=	=	-	-	-	2 36,37.	
5	=	=	-	-	-	2 38,39.	

Quadruplicate 2 virus. \rightarrow 5 total

10. 1:25,000. in coli complete. centrifuge + resuspend. 750 ca. $\frac{1}{2}$ % survival.

1:25,000 in T(Bφ) etc.

Layer	complete	11°	•	•	-	•	4	172
		12	=	=	-	•	6	253
		13	=	=	-	•	0	227
		14	=	①	-	,	1	242
		15	=	-	-	-	0	198
		<u>10P27</u>		1 ml into 50 ml coli. 1. 192 B. 1:12,500,000. Bottom				
		3D29	O	4A30 X	10P30	Δ	— SP3	<u>17(4B)</u>
		18h.		36h.				
		21	0	3				32
		22	0					
		23	1	1				
	<u>$\times 25000$</u>	24	0	0				
	<u>750</u>	25	2					
	<u>$38 \times 12,500,000$</u>							
	<u>50</u>	2						

240 tested

N - contamination n.g. top pick.

20.

35
31

31 complete.

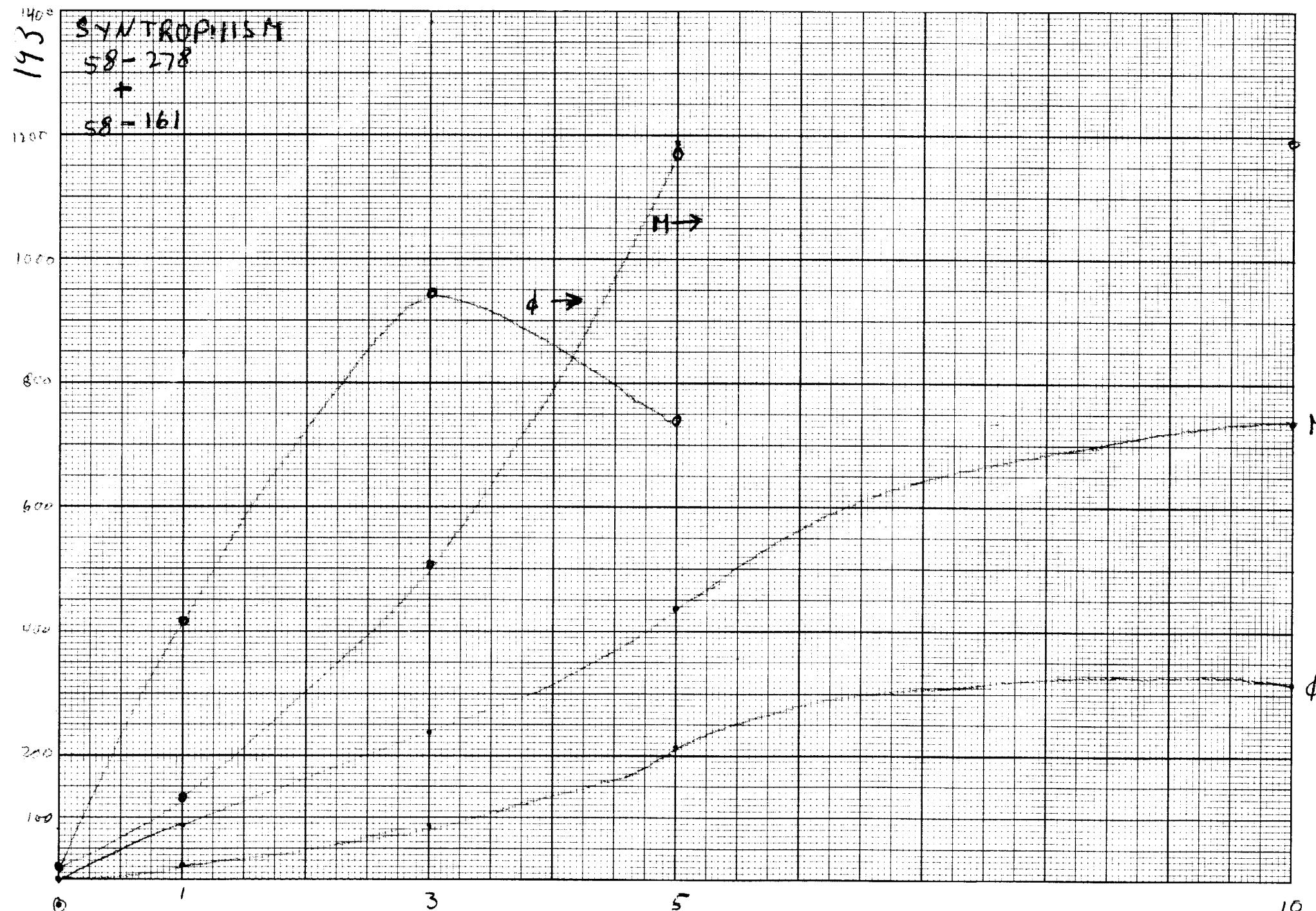
Cover & saline - glucose - agar.
Lawn & complete 10A 29.

Prototroph unusually large here!

Incubate 1-15 at 31° after layering. do. 21-31.

Picks 1947

MS, ϕ



Syntrophus

193

Meth. - O₂ Al.

Proline.

Use fresher cultures for inoculum.

4/27. inoc 1230 428. 3r°.

58-278 (♂) and 58-161 (M.)

^{34h.}

Inoc. Suppl. M. 10P28 11A29

(77° G)

D

As before 10 ml T(B) +.
48h. 60h. 72h.

12M29. 12N30. 11P30 % M.

(77) 72° 77

1	φ M	5	0	-	100		100	100	100
2	" "	5	1	±	98	88	98 ³	98 ³	98
3	" "	5	3	+	94 ³	235	94 ²	94	94 ³
4	" "	5	5	+	90 ²	434	90	91 ²	90
5	1610 "	5	10	+±	84 ²	731	84 ¹	85	86 ²
6	" "	5	100	++	63 ¹	1973	66	67 ²	66
11	φ	0	5	-	99 ²	22	100	100	100
12		1	5	-	99 ²	22	98 ³	98 ³	98 ³
13		3	5	±	98	88	97 ²	96	92 ³
14		5	5	±	95 ¹	212	93 ³	88 ¹	77 ² * No peptone.
15		10	5	+	93	315	90	79 ³	73 ² * no plating!
16		200	5	+++	61 ¹	2129	60 ²	61 ¹	61
21	M+φ	0	5	+	90 ¹	446 -M5	81	72 ³	54
22		1	5	+	82 ¹	848 414	74 ³	77 ³	44
23		3	5	+	72 ³	1582 948	72 ¹	72 ³	
24		5	5	+	76 ¹	1117 743	70	73 ²	73
25		10	5						
31		5	0	+	96	177 -65	62 ¹	63	
32		5	1	+	43 ¹	321 139	67	69 ³	41
33		5	3	+	84 ³	718 506	71 ¹	73	
34		5	5	+±	72 ³	1352 1170	72 ¹	72	50
35		5	10.	++	72 ¹	1412 200	71	73 ²	74

679-183 (P) + 679-662 (G).

in T (the. 1/2 mg).

N2

41	G	Proline 0	-	100		100	100	100	-
42		3	-	100		100	100	100	+++
43		10	-	100		100	99 ²	97 ¹	+++
44		100	-	92 ²		84	83 ²	83	+++
51	P.	0	-	100		100	100	100	-
52		3	±	97 ¹		97 ³	98 ²	96 ²	+
53		10	±	96 ¹		95 ¹	96 ²	93 ²	+
54		100	+±	69 ¹		68 ¹	68 ³	68 ²	+++
61	P+G	0	-	100		100	100	100	-
62		3	±	98 ¹		96	81	79 ¹	+
63		10	±	96 ³		81 ³	74 ¹	79 ¹	+++
64		100	+±	66 ³		68	68	66 ¹	+++

Note - change to EST. 2A28.

* Adaptation.

193 b.

1 AM. 4/30/46.

Remove 1 ml of culture ~~medium~~ from tubes 22 + 32
and plate into $\Gamma(x)$ at a dil. of ca 1,250,000. Bottom & cover.

- 22a. $x = \text{biotin} + \text{methionine}$.
 b. $x = " + \text{phenylalanine}$.
 c. $x = "$.

After counting 1 mutant, cover
the experiment & count the
other. Dil. only ~~500~~ 500
for 1.5% plates.

3P2

22a. 52. (v. small colonies) ~~3~~

b. 114. 10P30. (\rightarrow 125) ~~3~~

22a. 67. (later 114 \rightarrow) ~~3~~

b. 14A ~~3~~

32c ^(1:500.) 3 colonies seen 10P30.

L	S. Total	M %.	later:
117	125 (251)	<u>46.5</u>	42.5
w.t. 0	25 ^{94?}	<u>43.0</u>	
(!)			
114.	149 d = 1308.	<u>43.2</u>	39.3
w.t.	<u>00.1%</u>		
145			do.

22c. 0. ✓

Divide by 2500 for ratio of mutants
(later, at 4P1, d = 1739.).

Notes Bro. 1A30.

Layer & hetero: 4P1 (i.e., 22a \neq phenylalanine).

At 3P2, examine & recount. Small colonies of the heterologous mutant have appeared.

Early counts are erroneous. Methionineless are much slower to develop visibly or by layering. At 3P2, they are however quite distinct but too small to be counted readily.

930P2: $\frac{1}{2}$ smaller colonies intergrading with large but still distinguishable.

$\frac{1}{2}$ small colonies still minute, but enumerable. Count A3

N3 - No colonies still v. small. Not properly enumerable

193c.

Analyze syntrophic cultures.

4P1.

Culture	M	R	♂	G	D	Colony count as:	T(B)	T(M)	T(♂)	F(♀) + ♂	T(♂) + M
	% M.				Deletion						
F _{two} groups line.	193-21	5	0	54		$10^3 > 10^4 - 5 \times 10^6$	194	$\frac{1}{10} \times 170$	170	369	380
	-22	5	1	44		0	106	144	144	230	247
	31	0	5	—		$> 10^5$	(N) — (N) —				
	32	1	5	41		$\text{ca } 3000$	(N) 133	" 202	" 202	357.	
	34	5	5	50		" 7	(N) 158	$\frac{1}{10} \times 161$	161	330.	
but 58-278 adapted.	14	5	5	,		$10^6 - 0$! / / / /	$\frac{1}{10} \times 246$	246		

What is sign. of
adaptation test.

62 10v

(N) T(G)(T), T(T),
 $\frac{1}{10} \times 392$ " 0 " "T(0+✓) (part of plate)
 $\frac{1}{10} \times 380$.

1st counts 930P2

∴ assume that another
 $\frac{1}{10}$ = protinless.Layer is complete 12 M₂. These
marked ✓

Type differential is maintained indefinitely.

Thus, culture 31 was adapted (no detection of 14 or 0 cells)

21 is already adapted, and contains, without tally, 3 cell
types.

% M.

% M.

21 M	52.5
21 ♂	55.3.
R	53.2
22 M	46.0
22 ♂	43.7
R	42.5

34 M	49.7
34 ♂	49.5
R	49.5

31 M	
31 ♂	*
R	*
32 M	
32 ♂	43.5
R	39.3
w.t.	= .9

Spontaneous mutants.
Small colony variants.

194.

58-218		A3a Picks colonies on 183 plates to 1 ml coli complete liquid.					
		Test on to CH Test on ml Test					
		T(B, Ø). slants P7. BØ 5/11 BØ 5/18 - A20					
18332	1	+	-	-	+	+	+
	2	+	-	-	-	-	-
	3	-	-	-	-	-	-
18329	4	+	-	-	-	-	✓
	5	-	-	-	-	-	-
	6	"	-	-	-	-	-
	7	-	-	-	-	-	-
183-31	8	-	v. sparse	more heavily	-	-	✓
	9	"	-	n.g.	-	-	-
183-30	10	-	-	do.	±	±	✓
58-218	11	-	nq P22	-	±	±	-
spont ↑	12	"	nq P22	-	±	±	-
	13	-	nq P22	-	±	±	-
	14	-	nq P22	-	±	±	-

P1-7.							
186-15.	21	+	-	-	-	-	-
>70%	22	"	-	nq	-	att	-
on 58-218	23	"	-	nq	-	-	-
+uv	24	"	-	-	-	-	-
	25	"	-	-	-	-	-
	26	"	-	-	-	-	-
	27	"	-	-	-	-	✓
	28	"	-	-	-	-	-
	29	-	-	-	-	-	-
	30	-*	-	-	-	-	This had a granular

21a. to 1 ml complete; 10% grow on shaker at 31°.

Came up slowly & to a low level.

* suspicious consistency.

medium later found n.g.

58-218

++ ++

Every O was a prototroph.

These are the 5 hour colonies.

must examine plates daily

for 3-4 days.

Syntrophus

58-278 + 58-4899.

4/30/46. 530PM

T(B) round +

10P1

N2

1. 5r ♂	58-278	++	++
2. "	58-4899	++	+++
3.	58-278 + 58-4899.	++	+++

4.9. - too much fat

8/29/46.

syntrophism - plate proc.

1945a

Pour plates of T(B) or T(r) & various organisms as indicated
 230P29. Incubate at r.t. to 230A 30. Proc. surface =
 homologous & heterologous E. coli. & *Nemopspora* 5801 & 21863.

By plate: Proc: 1 2 3

58-161 HC -278 homologous N.21863
 + sl. growth streak " " " "
 considerable P3. SP1.

58-278 HC -161 " N.21863
 + - - -

679-183 HC 662 " N.21863
 ++ sl. growth streak. - - Colanic incomplete ε stain zone,

679-662.
 (hairy -
 canary). HC 183 " N.21863
 + - - -

-662.
 no precipit. + N.5801
 growth;
 do colistin. N.21863

O



196.

Response of ~~S~~ 679 - 662
to glut & proline.

4/30/46. Is response to peot, which is delayed, an adaptation?

5:30 PM.

base T(T) + suppl. ē 679-662

1. proline 200 μ
2. glut.ac. 200 μ

abandons temporarily.

And 1-2 transfusions each soln.

different colonial appearance. Stains & acid + urease:

cystococcii: #

58-278

Cystococcii

1) Spontaneous:	5 / 161.	.15	X	.031
2) U.V. - (t=0)	10 / 1099	.014	197-61	.0099
3) U.V. (t=24 h)	(24 / 240.)	.001		.10

χ^2 tests:

	f_0	f		χ^2_{unc}	
1) - 2)	2	5	161	$= \frac{(2-5)^2}{2} + \frac{(10-13)^2}{13}$	
	13.	10	1099	= 5.2.	$p = .023$
	15		<u>1260</u>	$201 = \frac{(3-5)^2}{3} + \frac{9}{13}$	
				2.0.	
				$p = .15.$	

	f_0	f		χ^2	
1) - 3).	12	5	161	$= \frac{(5-12)^2}{12} + \frac{(24-17)^2}{24}$	
	17	24	240	= 4.1 + 2.0	
	29		<u>401</u>		

	f_0	f		χ^2	
2) - 3	28	10	1099	$= \frac{(10-28)^2}{28} + \frac{(24-6)^2}{6}$	
	6	24	240	= 11.6 + 54	
	34		<u>1339</u>		

$$p = \ll .001$$

Picks instant "colonies
to coli complete

197

See 194

583

All colonies exc. O were of des't. size at pickling.

Plate.	Design.	#	Complete test. ^{B6 (agricol)}	B6 test ^{sample 5/11}	B6 ^{From plate A12}	B6	-	Arc. B6C	Agar
91-12	X	31	+	A1			-	-	
	58X	32	+	2			+	-	
91-13	X	33	+	3			+	-	
91-14	X	34	++!	4	+ 111		+	-	
92-1	Δ	35	+	5			-	-	
92-4	Θ	36	"	6			+	-	
92-5	Θ	37	"	7			+	-	
	Θ	38	"	8			+	-	
	Θ	39	"	9			+	-	
		40		10					
92-11	Θ	41	"	11	x				+
	Θ	42		12	x				
	Θ	43		13	?				
	Θ	44	"	14			-		
	Θ	45		15			-		
	Θ	46	"	16			-		
	Θ	47	"	17	x		-		
	Θ	48	"	18			-		
	Θ	49	"	19			-		
	Θ	51		20			-		
	Θ	52		21			-		
	Θ	54	"	22			-		
	Θ	55		23			-		
	Θ	56		24			-		
	Θ	57		25			-		
	Θ	58	"	B1			-		
	Θ	59	"	2			-		
	Θ	60	"	3			-		
	Θ	61		4			-		
92-14	Θ	62	"	5			-		
	Θ	63		6			-		
92-15	Θ	64	++	8			+		
	Θ	65	+	9			++		
	Θ	66	+	10			±		
92-21	-O	67	-	11			+		
	Θ	68	+	12			++		
	Θ	69	-	13			+		
	Θ	70	+	14			+		
92-23	Δ	71	+	15			+		
92-24	-	72		16			+		
	X	73		17			+		
	Θ	74		18	1		++		
	Θ	75		19			+		
92-25	Δ	76		20			+		
	Θ	77		21			++		
92-27	Θ	78		22			+		
	Θ	79		23			++		
	Θ	80		24			+		
				25					

Seems to grow unusually rapidly. *

Non-growth, but no diff 583-278 by P12.

+

							BPC
192-19	-	81	+	c1			
-30	0	82	+	2		±	✓ +
17-22	-	83	+	3		-	✓ +
58-278				4			++
58				5			

An exceptionally high proportion of mutants is indicated.
These have to be auxanographed now.

~~with~~ In series 191-35 to 82, 58-278 treated in v.v.

5 - grow on minimal

39 . grow on biotin + dal + cyst.

1 (#61) - ?

Auxanography: 1947 mutants.

1972

Plates not sterile.

Pour plates 10 P 18. Inc 30°.
1230A - A) & 10X B) etc. Inc 35°.

5/18/46.

SA 19.9A
AD
Y11
Y13
Y14
Y18
Y19
Y20
Y2

Sp
DSA

1 Proline-

A 24
 $\bar{T}(\text{Bc}\phi)$ Final identity

-

C SA
B SA
D SA
Turbid
— I. (Thiamin)
Turbid

4
6
Y21 A A?
25 A B?
turbid.

Y12 D6 AD
5580 A
29 A —
31 A C —
32 A —
33 A AD —
34 A D? A —

C SA —
D II A. —
I ??

(23)

35 A —
36 A —
37 A —
39 A —
43 A C —
44 A C —
48 A —
49 A —
51 A —
52 A —
54 A —
55 A —
56 A —
57 A —

C II A —
C II A —

Growth +
 $T(\text{cyst})$ +
liquid. +

58	AD	D II A	6	$B\phi C$		24
59	AD	D II A	6	"		25
60	A			"	+	
61	turbid A				-	
62	AD	D II A	6	$B\phi C$		26
63	AD	D II A	6	"		27
65	A	D II A	6	"		28
66	AD	DSA	-6? (Cyst)	"		29
67						
68	A D AD	D II A	6	$B\phi C$		30
69	- A	D II A	6	"		
70	A AD	D II A	6	"	+	31
72	A				+	
74	A D	D II A	6	"		
75	AD	D II A	6	"		32
78	AD	D II A	6	"		33
79	AD	D II A	6	"		34
80	AD	DSA	6?	Cyst		35
81	A D	D SA	6?	Cyst		36
83	AD	DSA	6	"		37
21	A	-				

12 N
put palat
C, B, D.
Pilose
det.
Error!

where?

Method A: Bact. hydrolysate C 198.

bac. 200 ml in 500 ml Erlenmeyer \in K-12, at shaker 31°. SP 5/3

1. *Escherichia coli* complete

2. T(0).

(weak superficially)

① Harvest SP 6 into 20 ml 6 N HCl. reflux 1A7 - 1B7 (calc 6×10^{10} cells)
distill off HCl + water to volume of ca 5 ml.

Centrifuge humic down, dilute to 20 ml + store as hydrolysate 198.
(light goldenbrown color.) for future filtrations + assay.

The T(0) has not been growing well.

5/8/48

Mix 10ml col & B/r from constant. 1130A8.
1130P9 d = 1.4900

Irradiate as above for ~~20 sec.~~ t. (ps. calc. as 4.9/min.).
constant killing curve points.

	Rad. t.	Dil.	Count.	ps.	ps/t
1.	0	$1:12\frac{1}{2} \cdot 10^6$	86.	1.2×10^9	0
1.	"		83.		
2.	20 sec.	10^2	>>		
3.		10^4	$\text{ca } 10^4$		
4.	.	10^6	558 $\text{ca } 900$	$.9 \times 10^9$.12
5.	60 sec.	10^2	>>		
6.		10^4	$\text{ca } 10^3$		
7.		10^6	558	$.56 \times 10^9$.33
8.		.1	>>		

Mix 1 ml of a and b into 10ml col x.

Use b. only. Apply mutant method. 1130P90.

$$\text{dil. } 2 \cdot \frac{\text{dopt}}{1.6120} = 12.5 \times 10^6$$

$$10^4 \cdot 0 \quad 28h. \Delta \quad 60h.$$

$$12M12 \quad 6P13 \quad 3A15$$

1, 2, 3, 4

marked
colonies all
still dist.
small

no growth
seen.

650.

10. not layered. 6. 2

1130P11 (24 hours) - colonies in T/0 visible but very small. Hold ~~at~~ ^{HF 6500} layer.

145P12 38 hours. - good colony spread; size sl. small but uniform.

Layer \approx 2P12.

1130P12 colonies sl. larger than 10.

Picks to complete lig. 1ml:

Picks to complete. A21.

G(∞) G(0).

- | | | |
|----|----|---|
| 1. | O | + |
| 2. | O | + |
| 3. | O | + |
| 4. | O | |
| 5. | O | |
| 6. | O | |
| 7. | Δ | |
| 8. | Δ. | |

h.g.

3/2 ++

Decanograph 5/23.

3 - C8 - Arginine
 4 - C1-2 - Leucine or isoleucine!

Sact. Hydrolysate D.

201.

5/4/46.

Broz K-12 into 200 ml T(0) at 98. Use fresh culture.

A. - on shaker qd.

B - unshaken

medium n.g. Repeat AII.

see 200, 202 ff.

CO 2

Precursors + bacterial mass
activity.

Remove alcohol from bacterial suspensions 2 and 7 by centrifuging & decanting, and hydrolysing by refluxing in 6N HCl 1 hour.

Calculate product in "densityunits/ml" by dividing original optical density by the ~~the~~ ultimate concentration. e.g. #16 hydrolysate if final volume is 20 ml and 100% recovery is assumed would be:

$$848 \times \frac{250}{20} = 1060 \text{ d/ml.}$$

This may be useful in calculating recoveries of various substrates.

Precursors and filtrate activities

202

5/11/46.

				31° (on shaker -)	Fuel growth
		Amnt.		Growth.	
1. <i>Escherichia coli</i> K-12	the following. 10P11	250 ml	2P12	1130 P	61:10. d
Coli Minimal +		500 ml	250 ml wash + 250 shake	2+	96 177
1. + O				3+	82 848
✓ 2. glutamic acid 5 mg.	50 ml		+++	+++	67 1707
✓ 3. anthranilic acid 5 mg. Hawest 10A16	"		-	±	86 655
✓ 4. citrulline 5 mg.	"		++	4+	71 ³ 1442
5. pantothenyl lactone anthranilic 1 mg.	"		++	4+	76 ² lost. F163
✓ 6. phenylalanine 5 mg.	"		+++	4+	not done.
7. O	"		++	4+	71 ¹ (74) 1472

The most efficient growth is evidently in small shake flasks.
Hawest N14. Same filtrates & bacterial mass.

(Pool bacteria of # 1, ~~2, 3~~ for hydrolysis. Preserve others in alcohol.
Collect in 0.25 ml 6 NHCl + reflux 4P14. — 3A15 on 11 hours lost
P141.

E. coli K-12 50 ml.

8. pantothenyl lactone 1 mg
 β -alanine 1 mg

84
lost on centrifuging
(73+)

N. ceca ~~#~~ SY7 in Fuel + 50 ml.

9. pantothenyl lactone 1 mg
 β -alanine 1 mg.

Hawest. 11A17.

Sample 5 ml (st.) from 9 add to 5 ml F10
centrifuge & monodate \approx 5531.

202

BA15. 30°

(79)

	droz.	1	2	3	4	5	6
1	58-3214.	0	202-7	1ml	1ml 202-7	1ml 202-7	
	"	-	±	±	202-2	Impool	b1 drogl
2	R572-228	100	—	100	100	99	
	"	-	"	1mg citr	202-4	1mg arg.	1mg cit
3	58-5030	100	—	100	—	—	
	"	-	±	±	202-6	1mg tyrosine	1mg phenol
4	K-12	100	—	100	—	—	
	"	+	+++	++	+++	++	
5	"	+++	++	"			
6	"	"	"				
7	"	"	"				

all + are ca 79, indicating
traces of substrates. Larger
quantities of filtrates should
be tested, until these are def.
by 1:10.

1st reading "P15.

2d reading A16.

3d reading 19P16

Interaction of nutritional requirements

203

5/10/46.

679 - 183.

Dose 1130 P 10. (10 hour culture !)

	T. (r)	P. (r)	830 P 11. 21.4.
1	500	500	100
2	"	10	93
3	"	30	81 (proline limiting value.)
4	"	100	62
5	"	300	63
6	1	500	100
7	10	"	97
8	30	"	93'
9	100	"	
10	300	"	83 ³ (threonine limiting value.)
11	1000	"	71
			59
12	100	10	94
13		30	84 ← <u>not depressed below T lim.</u>
14		100	±
15		300	±
			±.
16	1	30	100
17	10	30	97 ³
18	30	30	95
19	100	30	83 ← Response at higher proline.
20	300	30	81'
21.	1000	30.	82.

(18)

The requirements seem to be independent, with a step cut-off when the limited growth is reached. Set up another, clearer exp. to establish this, using levels of

Proline = 40 r

Threonine = 100 r.

~~204~~
d = 6.79 - 18.5

b = 5.8 - 16.1.

204

5/13/46.

Spec 3A vs. 30°

(tG M17)

8 A II 7

505

281

796

257

D. ED calc.

	Broz	Biotin	nr Meth.	Tan. + Polar	UP 15	10P16	8 A II 7	
1	-	-	-	50	10	±	+	89 ³
2	A	-	-	500	-	±	+	18 ³
3	-	10	50	-	-	+	+	185 ¹
4	3	10	3	-	-	±	+	94 ¹
11	x + B	10	3	500	10	last.	had glucose	77 ¹
12	"	10	3	50	100	+	++	1121
13	"	.3	100	500	100	+	++	538
14	"	.3	100	20	100	+	++	76 ¹
15	"	.3	100	20	100	+	++	1177
16	"	10	50	-	100	+	++	1077
21	B	.3	3	-	-	+	+	73 ²
22	A	-	-	50	10	+	+	1337
23	A + B	.3	3	50	10	++	++	1301
31	A + B	10	-	500	-	-	-	70
2	"	.01	-	"	-	-	-	74 ¹
3	"	.03	-	"	-	-	-	+
4	"	.1	-	"	-	-	-	+
5	"	.3	-	"	-	±	++	+
6	"	.1	-	"	-	±	++	+
7	"	3	-	"	-	+	++	+
41	B	"	-	"	-	-	-	+
2	"	.01	-	"	-	-	-	100
3	"	.03	-	"	-	-	-	100
4	"	.1	-	"	-	-	-	100
5	"	.3	-	"	-	±	++	99 ²
6	"	.1	-	"	-	±	++	97 ²
7	"	3	-	"	-	+	++	93 ²
51	B	"	3	"	-	+	+	+
52	"	"	3	"	-	++	+	++
53	"	"	3	"	-	++	+	+
54	"	"	3	"	-	++	+	+
61	58-278	"	3	balance.	-	±	+	++
62	"	"	3	-	-	±	+	++
63	"	"	3	-	-	±	+	++
64	"	"	3	-	-	±	+	++
65	d +	coli		-	-	-	-	-
66	B	100		-	-	++ 77 ²	? adapt?	-

10P16 - spec 51 and 52 = 58-3214. (d)

77³

Sep 207

Syntrophism - Ser.

2049

679-183 x 58-161.

1/2 values - 81 ca. angular.

This experiment is designed for:

- critical conditions of syntrophism.
- substrates in culture medium
- recombinations.

679	200 r	T
183	30 r	P
58	.7 m r	B
161.	10 r.	M

1st. Need data on interaction of requirements. — independent.

- a. 4 1:1 interactions: use excess and 1 optimal
- BT
 - BP
 - MT
 - MP.
- + PM
TM
BP
BT.

Analyse for recombination types.

2:2. 1 optimal for each.

e
f
g
h.

3:1. 1 opt for

BM
TP

Critical conditions:

- delayed inoculum
- excess BT. provide M in range 0-5 r.
- 278 adept. series at 5 r.

Dyntrophism.

~~205~~
205

10% proline

All 58-cultures & .01% Protein
679- ε.5 ug threonine

1130P10. 30°

5/10/46.

noz A	noz B. Supp.	BT.	1130P11.	1130P12	1130A13	Ex. (78)	Syn. !!
✓ 1. 58-3214.	—	✓ BT.	+	+	+	85	
✓ 2. —	679-183	BT.	+	+	+	89 ³	
✓ 3. —	679-662	BT.	—	±	±	98	
✓ 4. 58-3214	679-183	BT.	++	+	+	88	
✓ 5. 58-3214	679-662	BT.	+	+	++	74 ²	
— 6. 58-3214	—	B	+	+	+	90 ¹	
— 7. ✓	Y1	"	+	+	+	93 ³	
8. ✓	Y1	"	+	+	+	93 ³	
proline	9	Y13	"	—	—		
arg?	10 ✓	Y13	"	+	+	89	
— 11	209-301R	"	++	+++	(autol?)	77	
12 ✓	209-301R	"	++	+++		74 ¹	
13 ✓	58-2651	"	+	+	+	90 ¹	
14. ✓		"	+	+	+	90	
15 ✓	58-3232	"	+	+	+	90 ¹	
16 ✓		"	+	+	+	89	
17 ✓	58-52-55	"	+	+	+	87 ²	
18 ✓		"	+	+	+	84 ¹	
— 19 ✓	5450	"	—	+	+	91	
20 ✓		"	+	+	+	91	
— 21 ✓	6049	"	—	+	+	91 ¹	
22 ✓		"	+	+	+	92	
23 ✓	6177	"	+	+	+	89 ²	
24 ✓		"	+	+	+	91	

All available proliners are identical
exc 679-662 which uses glutamic acid.

Note 1 cell contains $\approx 10^{-12}$ g H_2O . ~~ca 10⁻¹² g~~

$$\text{at pH 7. the } [H^+] = 10^{-7} \times 6.02 \times 10^{-3} \text{ g.} \\ = 6 \times 10^{-3} \text{ molecules/g.}$$

This is $\approx 60 H^+$ / cell. at pH 7

1% of the time, there will be only 60

A replacement of 30 r / sec or is quite reasonable
for protein 10-40% of the dry weight.

$$\text{dry wt./cell} \quad d = 3 \times 10^6 \text{ cells.} \approx 1r$$

$$\therefore 1 \text{ cell} \approx .3 \text{ rr dry} = 3 \times 10^{-3} \text{ dry.}$$

ca. 1 rr wet.

This is less than previous estimates: (8×10^{-3})

Bacteria production.

T(0) in 4 liter lots in 4L Pyrex bottles.

1.

2.

3. Without asparagine.

Aerate by aspirator suction; cotton - in glass air filter;

Mon 14-12 2A15. 5/15/46. Room temperature.

11A16 - 1, 2 ++ 3 ± growing slowly
 12 M16 - 1, 2 are nearly opaque; 3 ++ A17. 3+++
 930 P17 - cell indistinguishable. Harvest - pool in large container
 + put in ice box.
 l. 1.
 2.
 3.

Both are 90° at 10:1

Remove 250 ml samples of 2+3 for centrifugation.

Allow to settle in cold room at 0°C. for two weeks.

P2 - separate by syringes into ⁽¹⁾ 4L, ⁽²⁾ 4L, ⁽³⁾ 3L, 1L fractions, ca.
 and reject fraction 2. Allow others to settle further; centrifuge
 small samples from 3+4 and collect & accumulate the harvest.

P.S. Deyaggregated harvest after collecting in saline - note sticky nature
 Cells largely intact - and wash in H₂O. Dry 1 over 3m Cl₂ (2) at
 100° 2-3 hours. Extract in 100cc Et₂O. overnight.
 Dry weight was 3.406 g. Assume ca 75% yield of the bacteria, approx.
 After extraction 3.3180 ca 3%

There were $12000 \times 10 \times 43 = 5,200,000$ 5.2×10^6 density units $\stackrel{\circ}{=}$
 3.406 gm. or 1 density unit $\stackrel{\circ}{=}$ 1 r dry wt. bacteria. and
 average culture volume 10ml $\stackrel{\circ}{=}$ G = 60 has about 2 mg dry
 bacteria in it.

Assays of Y-hocn hydrolysate
Nutrosopha.

frac 1A6. 30°.

A. Biotin. SY7A. 9A8.

- | | |
|------------------------------------|-----|
| 1. 25cc "Biotin"-free Fries. | ± |
| SY7. | |
| 2. do. + 1 ml 206A. | +++ |
| 1 ml 206A. | |
| 3. do. + 1 ml 206A + .05 r biotin. | +++ |

B. Inositol. 34701 a.

- | | |
|--------------------------------|---|
| 1. 10cc Fries. | - |
| 2. + 1 ml 206A. | - |
| 3. + 5 ml 206A. | - |
| 4. + 5 ml 206A + 5 r inositol. | + |
| 5. 5 r inositol. | + |

C PAB. 1633A

- | | |
|-----------------------------|-----|
| 1. 25cc Fries. | - |
| 2. " + 1 ml 206A. | +++ |
| 3. " + .1 ml 206A | + |
| 4. " + 1 r pab. | +++ |
| 5. " + 1 ml 206A + 1 r pab. | +++ |

While "appreciable" biotin and pab are available, there is no detectable inositol in this fraction of *E. coli*.

6/6/46.

Take a 1 gm sample (1.014 g.) + reflux in 18% (6N) HCl
at 100-110°.

at 4 hours digestion, 20.5 cc was present. Remove
5 ml sample + continue digestion. — Make up to 20cc.
lost.

4

Remove another 5 ml. = $\frac{1}{4} \times \frac{3}{4} \times 1.014\text{g.}$

neutralize \ominus NaOH. Store as $\stackrel{\ominus}{=} 19.0\text{ mg}$ Concentrate +
assay 4 hr. hydrolysate 206a.

Continue hydrolysis of remainder to 24 hours. Concentrate,
neutralize and dilute to a conc. of 20 mg /cc. Store in cold

See 234 for proline, arginine assay of hydrolysate

Use 204 - synkaryotic cultures:

Mixtures of BM. + TP. P. 5/17/46.

#	Strain excess:		P19	Lysin:	% BM.
1.	.. Cost				
2.	12 BT.	Plate 1:1000 in T(0).	0		
3.	"	1:1000 in T(BT) 3	1-3		
4.	"	10 ⁻⁶ BT	0		
5.	"	10 ⁻⁶ BM.	∞		
6.	"	10 ⁻⁶ BM.	PT		
11.	13 MT	10 ⁻³	0.	0	
12.		10 ⁻³	BP	0	
13.		10 ⁻⁶	BP	0	
14.		10 ⁻⁶	BM	∞	
15.		10 ⁻⁶	BM.	PT	
21.	14. MP	10 ⁻³	0	0	
22.		10 ⁻³	MT	0	
23.		10 ⁻⁶	MP	0	
24.		10 ⁻⁶	BM	∞	
25.		10 ⁻⁶	BM.	PT	
31.	Wash:	10 ⁻⁷	∞		
32.		10 ⁻³	0	0	
33.		10 ⁻³	BT	4	4-7
34.		10 ⁻³	BP	0	
35.		10 ⁻³	MT	0	
36.		10 ⁻³	MP	cont?	8
37.		10 ⁻⁷	BM.	∞	
38.		10 ⁻⁷	BM.	PT.	

Where possible recombinations:
 colonies represent pick to
 a complete hybrid (A, A)1.
 (BT). A1.

See 212.

P21. to slants + test on:
 latest on variois = #5.

T(0) T(B) T(T) T(BT)
 1 P22 P22

1	-	±	✓	±	✓	++	✓
2	-	-	±	✓	+	-	✓
3	-	✓	±	✓	±	-	✓
4	-	✓	±	✓	✓	-	✓
5	±	✓	±	✓	✓	-	✓
6	±	±	++	±	✓	++	✓
7	Streak out 1 on a complete plate.						

A BT strain? Call it 58-~~xx~~⁶¹⁹.

Why no growth? trace reg.

Six

679-183 x 58-161

v. 204.

d

3

5/21/46.

Inc. ^{mr} Biotin Methionine Threonine Proline

30° 2A 23.
12 h. P2

	d	10 ✓	3	500 ✓	10	+
1	d	10 ✓	3	500 ✓	10	++
2	β	10 ✓	3	500 ✓	10	++
3	d + β	10 ✓	3	500 ✓	10	++
4	d + β	10 ✓	3	500 ✓	10	++
5	d + β	10 ✓	3	500 ✓	10	++
6	d	10 ✓	3	500 ✓	100 ✓	+++
7	d	10 ✓	3	500 ✓	100 ✓	+++
8	β	10 ✓	100 ✓	500 ✓	10	++
9	β	10 ✓	100 ✓	500 ✓	10	++
10	d	10 ✓	100 ✓	500 ✓	100 ✓	++
11.	β	10 ✓	100 ✓	500 ✓	100 ✓	++
12	d + β	10 ✓	100 ✓	500 ✓	100 ✓	++

BT.

MT.

BP.

MP

21.	d	.3	100 ✓	500 ✓	10	+
	β	.3	100 ✓	500 ✓	10	++
	d + β	.3	100 ✓	500 ✓	10	++

? autolysis or
phage??

31.	d	10 ✓	3	30	100 ✓	+
	β	10 ✓	3	30	100 ✓	+
	d + β	10 ✓	3	30	100 ✓	+

41.	d	10 .3	100 ✓	30	100 ✓	+
	β	10 .3	100 ✓	30	100 ✓	+
	d + β	10 .3	100 ✓	300	100 ✓	+

False off shaker
11A28

51.	coli	d	, 50 ml flask, shaker	d	+Y	
52	"	β		β	+Y	
53	"	d + β		d + β	+Y	