JUN 1946

Use Inl mac: Y41 (679-1831) + Y24 (BϕC) into 50 ml colin C
Sh. 30°. 12M30.

12N. 7/1/46. Wash + plate into T(0). Save culture.

	P2	P12	
①	4		{ 7 S to T-1.
②	5		{ 2 R.
③ BCT.	27		
④ BϕP	14		

A. P12. Pick colonies from 3 and streak out. P13. Test rapid colonies.
T-1. = BCT [illegible] BC − BT. ≡ T(0) LR probably (T- P+ R.)].

	T-1.		
1	S	+	
2	S		
3	R		
4	R	P̄T̄R ✓ parental	BCϕPT+. See 272
5	R		
6	S		BCϕPT+ See 272.
7	R		
8	R		
9	R		
10	R		
11	R		
12	R		
13	R		
14	R		
15	R		
16.	R		

must purify on test / perhaps should streak out on OC agar.

B. P13. Streak out colonies of 1, 2.

	T-1
1	S
2	S
3	R
4	S
5	S
6	S
7	S
8	S
9.	R

A1U Test

C: streak out P13. Test P14.

	T-1	T(0)	BP —	Bϕ —	BϕP — BP	P
1	R	−	+	−	+	
→ 2	S R ✓	−	+	+	+	Prototrophs
3	R	−	+	−	+	
4	R	−	+	−	+	
5	R	−	+	−	+	
→ 6	S ✓	−	+	−	+	Prototrophs
7	R	−	+	−	+	
8	R	−	+	−	+	
9	R	+	+	+	+	
10	S	+	+	+	+	
11	R	−	+	−	+	
12	R	−	+	−	+	

Segregation of Biotinless: Etc.
$B^-d^-c^-$ × $B^+P^-T^-$

Check. neg.

	T(O) 10 ml.	T(B).
1. 254-144.	+	+
2. 254-146.	+	+
3. 254-52	+	+
4. 254-132	−	+
5. 254-42	+	+

Test other colonies from 254.

254-28.

1. BM − PT− BMPT+ BPT −? from BM/1 × PT

By auxan: BM 1, check. Parental.

Recotypes

12 JUL 1946

10P12. Establish Dracula. in 50ml coli ∞ 30°C.

1. Y9 (LM)
2. Y40
3 Y41
4 58-161
5 679-183
6 Y24 n.g.
7. ~~Y38~~ Y38 } for radiation.
8 Y39.
9 58-183X n.g.

~~Dra 50 ml coli~~ Store in refrigerator 7P13.

Dra 50 ml coli ∞:

① 161 + Y41
② 183 + Y40.

1. ① 6
2. ① B. 8
3 ② 9
4 ② B 19. See infra. Isolate and check for Bug, 11.
5 ~~See 262~~ Y40 + Y41 X-streak. 2 surface colonies. Will have to repeat procedure.
6. $5\ 10^{-2}$

Sex: cross-streaking

13 JUL 1946

7P13. Using loopful from 261, crossstreak on coli ∞.

A. 1, Streak 1 2.
7
5.
TLM 1. Y9 TLM 161 BM
2 183 TP
3 Y41 PTR
4. Y46 BMR
5

B. 11 Y40 BMR 183 PT
12 Y41 PTR

C 21 Y41 PTR 161 BM. Smeared.
22

D 31 Y38 Y39.

Seeage growth from B11 B12, suspend in H_2O and plate into T/O).

no colonies.

B/s: double mutants.

13 JUL 1946

Irradiate Y38 and Y39 cultures (see 260) [redacted]. and incor: colis.

11p13. Sh. 35°.

Y38 1 min
2 "
5 "

Test Y39 — dl leuc
dl isoleu.
l-leuc.

Y39 1 min
2 "
5 "

Detection plate Y38 5min. into T(arg.) at 10^{-7}. 4p14.
Y39 " into T(lc + isole.

Y38 - 2min
Y38 - 1 min 2 plates.

Y38 5min
1 — 600 cells. 4 colonies.
2
3 — 1
4 — 2
5 — 3
6 — 4.
7
8
9
10

1 min 11 — 350 cells. 1 colony.
" 12
2 min. 13 — 500 cells. 9 colonies.
14

Y39
21 — did not grow
22 — evidently [redacted] is not le or ise le strain.
23
24
25 — Test by auxan: e.g.g: histidine
26
27
28 — Specific for tests on Y38 —
29
30
31
32

Sex: triple cross

TLM × BφC

9P. 7/14/46. 1ml mix into coli∞+c Sh. 30°
Y9 × Y24

N15		Plate mito:	
1	0	0	
2	0	0	
3	0	0	
4	0	0	
11	B	0	
12	φ	0	
13	C	4	turbid
14	T	0	
15	L	4	
16	M	0	
21	BT	0	
22	BM	0	
23	φT	0	
24	φL	6. v.sm.	
25	φM	0	
26	CT		turbid
27	CL		turbid
28	CM		turbid
31	TLφ	ca. 16	
32	TMφ		
	BLT		turbid
	CTL		turbid

very disappointing. (c i.m. medium? or strains?)

Threw out plates.

679-680-Y9. 265

Y10.

July 16, 1946.

Check on requirements:

P16: Y9.

TLM	++
TL	—
TM	—
LM	—
TLM+Cyst	++
TL+Cyst	—

Growth is however not optimal at all: methionine; something else may be required. (Consider pab, homocysteine, choline, etc.

by TL + EAA, NEAA, YE, Vits.

TLM

TL∇E +++. others + or —.

Y10:

TB_1	—
LB_1	+.
TL	—
TLB_1	+++

probably some $T^+L^-B_1^-$ in the population. Re-isolate — streak out from TLB_1.

2/5 isolates tested came up on LB_1 as well as TLB_1. same as Y45. Other three - same 1. as Y10a. (or after 7/27 as Y10.

Killer E. coli.
Resistance

7/15/46.

P 15 Imoto & Hershey's "T" and "R".

A 16. Filter "T" and test for activity as R in plates.

1	T + R 1 ml ea.	+++
2	T 10^{-3} + R	+++
3	T + R·10^{-3}	++
4.	T 10^{-3} + R 10^{-3}	++.

no demonstrable killing.

17 JUL 1946

"Reacting" strains "3" and "14" received from Dienes AIT.
Transfer to subculture slants D3 D14.
streak plates 10A17. D3 swarmed only. Colonies in D14?

Nutritional Requirements: 11P17.
grows very rapidly compact colo

		9A18	9A19	
D3.	Past Mic Cyst	++	+++	
	PN	−	++	
	PC	+	+	to C. + slowly ++.
	NC	++	+++	
	Cyst – Vits.	+++	+++±	
D14	PNC	++	+++	
	PN	−	++	
	PC	±	+	to C + slowly ++.
	NC	++	+++	
	Cyst-Vits.	+++	+++±	

Repeat for asp. vit. req.

	Cyst + 10 B vits =	D3	~~D14~~	10P18. 35°
1		++		
2		++		
3		++		
4	+	+	f.	(mic)
5		++		
6		++		
7		++		
8		++		
9		++	+++	
10.		++	+++	

cystine is stimulatory; probably not adaptation.

Coincidental reversion

1 [illegible] 1956

Recd. from Ryan a "prototroph" obtained directly from 679-680. Subculture

1. A17 streak out on T(0). No colonies
2. Inoc loopful in T(0) No growth.

P19 - Inoc ca 10^7 cells into T(0). Use loopful to inoc T, L, TL:

0	+
T	—
L	—
TL	+++

Not prototroph!

M20. Inoc coli ~ 2% Use v. large inoculum. 30° C.

10P21. Plate out 1ml ea into:

1. T(lc) 10^3
2. T(thr) 0
3. T(0) 0
4. T(0) 0

240-5
Size variant (?)

P16. Inoc coli in 50 ml at 30° sh. c 240-5a ①
K-12. ②

P17. Dil 10^{-7} and plate in detection plates. T(0)

1. K-12 + A18.
2. 240-5 ++
3. both. +:++.

Phage: T-1 susceptible.

Pedigree:

236: 58-161 x 679-183 on minimal. Pick to H_2O + streak a minimal plate. Pick a colony to water + plate d. (240-1). 2% small colonies, but all prototrophic. Check now for mutability of colony size.

If anything 240-5 is the faster growing colony type
vs 240-5 large colonies at 24h
K-12 small v. distinguishable.

Repeat. Inoc 12M18.
Plate 6P19.

1M20 - Same result as above - K-12 colonies appear more slowly than 240-5 on T(0). They are indistinguishable on ∞! [Why was 240-5 first picked up as a small variant?].

TLM x BØC.

BØC x TLM

N17 Inl mor ea. into coli — ⩾ 30°

S30P18. Wash + plate Inl ÷.

		SP2a		
1	O	0		
2	B	0		BL
3	Ø	1	?	
4	C	57	T	[C]
5	T	0		
6	L			[Ø]
7	M	0		
8	BT	0		
9	BL	20	T	
10	BM	0		Compare — 264.
11	ØT	0		
12	ØL	0		
13	ØM	?2		
14	CT		T	
15	CL	52	T.	
16.	CM		T	

BCL?

Try Y18. BØC - TLB;

Sex: cross streak.

271

17 JUL 1946

N17. cross streak on colis 58·161 × 679-183.

1,2

3. Streak = mixed inoculum.

10P19. Plate nite T(0). ① @ ca. 10^8
② ● ca 10^9

no dup colonies. ∴ this is not a good lead.

259 – Recotypes? 272

July 18, 1946.
P17. P18', 10 P 18

	BΦCTP	BΦCT^{-P}	BΦCP^{-T}	BΦPT^{-C}	BCPT$^{-\Phi}$	ΦCPT^{-B}	BΦCPT^{-O}	
A4	++	–	–	+	+	+	+	$\bar{P}\bar{T}$/, parental.
A6	++	–	–	+	+	+	+	$\bar{B}\bar{T}$/, "

	BΦP	B	P	BP.	T–1	
C2	++	++	–	++	R✓	Recombination Types!
C6	++	–	++	++	S–	

Y38 - mutants.

See 263.

	Arg.	
1	++	
2	++	
3	Spreader (not coli	
4	—	
5	—	T (A)
6	—	T
7	++	
8	—	C - 9 - Methionine. check in lig. Y43 ✓
9	—	A only. → glutamic. Y49
10	—	T
11	—	T
12	—	T (e)

256 - 1	T(o)	+	acid: turbid; A.C.	(not coli)
2		+	— ACID	
3		+++		lost.
4		+.	turbid	

	T(Arg)	T(Meth)	T(A.M.)
Y43.	—	—	++

Y39 – mutants.

July 19, 1946.

10P19. Irradiate 24 hour culture Y39 in ∞ 2 mins. uv. in medium.

(1) plate 1ml in coli ∞ . p^s = 2 to 3.

(2) Inoc 1ml in 50ml coli ∞ sh 30°

Layer N22.

113 P20. Detection plates – T(histidine) 10^{-7} and 5×10^{-8}

ca 1200 colonies total. 10 small colonies. pick. 8P23.

to ∞ slants. T(H)

		T(H)		
1		++	probably not coli	
2	not coli	—		
3		—	T	
4		—	A	
5		—	B? —	
6		—	B —	B-3 Y44
7		—		
8	~~ag.~~ v poor on coli ∞.			+ on minimal (E2)
9		+		
10	n.g.			

auxanograph P25. – No vitamin sp. resp. in 6. Check in liquid + for yna. B-3 pab.

	12h.	24h.
H	—	—
H+ pab.	—	+++
" yna	+	+++
" M	±	±
" M+yna	+++	+++
-H +pab.	—	—

10γ.

Try 1. more pab
2. pab sterile filtered. (slow on pab)

yna replaces pab.

July 21, 1946.

Incc 1 drop each of Y24, Y41 in medium of 275a.

30° ~~shk~~ shk. 11P21 Plate into T(0). 3P. 22.

		Growth	G(1:10) of sesp.	plated Cells:	Colony count	×/cell.	
30°	1. Coli ∞	+3	86[2]	2×10^9	20	10^{-8}	+
	2. −glucose	+3	89	1.5×10^9	200	10^{-7}	
	3. −Yx	+2	1:5 88	7×10^8	2	10^{-8}	+
	4. pH variation: pH=8. →	a	78[2]	3×10^9	100	3×10^{-8}	+ − ++
		b	72[1]	4×10^9	40	10^{-8}	
		c	75[2]	3×10^9	30	10^{-8}	
		d	85[3]	2×10^9	10	10^{-8}	
	5 Beef x.	+3	79	3×10^9	5×10^3	10^{-6}	++++
	6 T(HCl)	+3	68	5×10^9	200	10^{-7}	++
	7 MaltEx	? + sesp. matter	1:2 81	6×10^8	0	0	−
	8 Coli hydrolys. T(0)	+3	79	3×10^9	10^4	10^{-6}	++++
	~~9~~						
	10 .2% peptone	±	1:2 93	2×10^8	0		−
	11. Coli ∞ vary salt. a 1%	+++	74	3×10^9	10	10^{-8}	+
	b 2%	++	82	3×10^9	10	10^{-8}	+
	c 5%	+++	80	3×10^9	0	0	−
	critical point? → d 10%	−					
	12 coli ∞ unsh.		93[2]	1×10^8	10	10^{-7}	++
	13 coli ∞ + cyst.		86[2]	1.5×10^8	1 − 10 ?	10^{-8}	+
	31. unsh. 25°		95	10^9	10	10^{-8}	+
	32. 38°		95[2]	10^9	0	0	−
	33 10°		93	10^9	1	10^{-9}	±
	41 u.v.		86 (73[2])	1.5×10^9	10	10^{-8}	+

[Salt inhibits recombination ??]

Δ.

25-30° opt.
unsh
−glucose.

inoculate 50 ml of the following media ẽ Y24 + Y41. wash.

30°

1. Coli ∞ (yx .3%; peptone .5% glucose .5%). See 276
2. yx .3% peptone .5% ~~#~~
3. Peptone .5%; glucose .5%
4. Peptone - yx - (glucose) in T(0) adjusted to various p H's. — 8 a, 7 b, 6 c, 5 d, 4 e. no growth
5. Buffer tract - yx. broth. .5%.
6. T(0) + N Z Case + ~~[redacted]~~ Vits.
7. Malt Extract 1%. (~~[redacted]~~).
8. T(0) + E coli hydrolysate .1%.
9. ~~Corn Meal agar stock.~~
10. .2% peptone + biotin

11. coli ∞ + 1% Nacl a
2% Nacl b
5% Nacl c
10% Nacl d. no growth

~~P~~ 31. Coli ∞ 25°

32. coli ∞ 38°

33. coli ∞ 10°.

41. Irradiate 1 min. ẽ u.v. then into coli ∞ 30° sh.

Conclusions:
Optimal:
pH 7-8 } buffered.
- glucose
- shaking
low salt
high nutrient N.
25-30°

Y10 = TLB,
Y41 = TPR

July 21, 1946.
Incoli-o, 1030 P21 1 drop each of:

- ✓ ① Y10 × Y41
- ✓ 2. Y10 × Y24
- ✓ 3. Y41 × Y24
- ✓ 4. Y43 × Y41
- 5. D3 × Y41
- 6. D14 × Y41 agglutination!
- 7. D3 × Y43
- 8. D14 × Y43. agglut!
- 9. " Y43
- 10 Y41.

~~[illegible]~~ T(0) T(B) T-1 (1st). etc. see infra.

PTR, × BφC

1.	B	③	36
2.	B		37
3.	B		64
4.	B		55
5.	0.		33

TLB, × BφC

11	0	②	-	1
12	B1		+	7 –
13	T		+	8
14	L			20
15	B		+	1
16	φ		+	4
17	C		T	5
18	B, B		++	17
19	B, φ		++	17 ✓✓
20	B, C		‡	7
21	T B		ǀ	9
22	T φ			9 many small
23	T C			1
24	L B			14 many v. small
25	L φ			14 "
26	L C		++	15

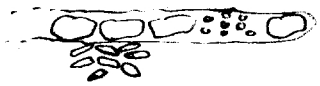
[illegible] (left margin beside 22–23: "...edually ...ish...")

See 276

TPR	31.	O	(4)		O
	32	A			4_3
AM	33	M		T	10^{-}
	34	T			15
	35	P			27
	36	AT			10^{1}
	37	AP			10^{2}
	38	MT		T	10^{3}
	39	MP			10^{3}
Y43	41.	O	(9)		O
	42	O			O
	43	A			50
	44	M	T. ca 10^{2}		100
Y41	51	O	(10)		O
	52	O		-	O
	53	T			100
	54	P			35
B3 Y41	61	O	(5)	T	
	62	O	(6)		
	63	O	(7)		
	64	O	(8)		

- star-like threads. —

microscopically long bundles of filaments & large cells of varying length + sometimes thicker up:



B. mycoides
see infra

Segregation of virus-resistance (T-1) 278

23 JUL 1946

Use plates 4C, 5, and 8 of the cross B♀C × PTr. Test surface colonies directly for resistance to T-1 See p. 275

4C. 5 R
13 S.

5 7 R
15 S

8 4 R
16 S

Total: 16 R /60 total. Ca 25% recombination of R ē either B+, T+ or P+

See 284. 5R/20.

Summary.

me	16	60
284	5	20
p.279	2	9
	23	89 = 26%
284	5	21
	28	110

Hand over symposium?

Aux conditions

According to 275, a buffered meat-extract or coli-hydrolysate enriched medium is best for producing new prototrophs. Check on this with other mutants.

Inocula: ① Y41 + Y24 as 275. Media: ~~T~~ = "Yeast Buf" broth = M×Y
② Y41 + 58-161 Ba = Bact. hydrol. 1 mg/ml
PMx = Nutrient Broth.
③ ~~Y9 + Y24. (which has yielded no prototrophs hitherto)~~. Y10 + Y43. (4-12 × B/A.)

Inoc. 1 drop each (stock cultures). Incubate 30° ⑤ shaking.
1245 A24. Plate 4P24.

	Medium.	Inoc.
1.	M×Y	①
2	M×Y	2
3	PMx	1
4	PMx	2
5	T(PMx)	1
6	T(PMx)	2
7	M×Y Ba	1
8	M×Y Ba	2
9.	M×Y	3.

Results are not encouraging.

How different from 275? — tense? shaking?

Try Y3 broth

See 279

For our conditions: Plate 4P24. (15h.)

11	1.	100	(100)
12	1 10^{-2}	1	
21		10	10
22	10^{-2}	0	
31 ~~23~~		100	(100)
32 ~~24~~	10^{-2}	1	
41 ~~25~~		100	100
42 ~~26~~	10^{-2}	0	
51 ~~27~~		200	(200)
52 ~~28~~	10^{-2}	2	
61 ~~29~~		50	50
62	10^{-2}	0	
71		10	
72	10^{-2}	0	
81		10	
82	10^{-2}	0	
91		0	
92	10^{-2}	0.	

For recombination types: 1030 P24

(2). 10^{-3}

1	O
2	B
3	M
4	P
5	T
6	MP
7	MT
8	BP
9	BT
10	O
11	O
12	O

too delute

n.g.

(1) 10^{-3}

21	O
22	O
23	O
24	B
25	B
26	B

K-12 x B/2.

11P24. 1 ml Y41 + Y43 in YB. Sh. 30°

N28. Grow into T(0). plates.

O

to 7P25, on desktop. Back on shaker.

1P27. Plate out. O

Y43 x Y44 O

Proteus + coli. 283

11P24. Inoc YB D14, Y41 Sh 30°

10A25. Inoc YB 1 ml each of above Sh 30°

& A27. Only typical bacilli.

[Repeat in yeast ext - peptone medium]

P27 Repeat in colloid.

a) D14 + Y41 — only bacilli

b) agar plate only atypical forms → practically exclusively the unusual organism (parasitic?) (actinomycete?) But true filaments of long cells like subtilis, staining well & saprogenic

Isolate + determine nutr. req., phage resistance, to exclude origin. from Proteus [How about Proteus × coli?] Study not from supernatant after large masses have settled.

Grows on plate like filamentous fungus. Refer to 283

B. mycoides according Tatum