

5/8/46.

Blend 368-7 (43-3 mycelium in exc. leuc.) ~~blend~~, sterile, 11P9
 Transfer remainder of blend to sterile flask + store cold.

1. 5ml unblended medium - 43-3

2. " " " " - 10A.

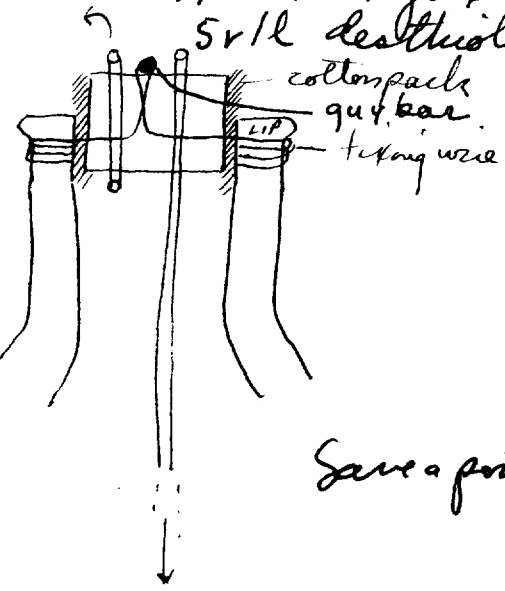
Add 5ml extract + 5ml F(0) + auid. 1mg dl-leucine

	no.	P11.	P13	P22
3	ext. + F(0)	—	±	±
4	" "	10A	+++	+++
5	" "	43-3	±	±
6.	" " + leuc	—	++	++
7	" " " "	10A	+++	+++
8.	" " " "	43-3.	++	++

No evidence of inhibition.

Production of Neurospora. 5/14/46.

in 20 l. Pyrex reactor, 10 l. of a Fries \bar{c} 2% glucose,
5 v/l desthiobiotin. Obs \bar{c} 847



Inoculate 6/8/46. 12 M.
Harvest A12

Yield: ca 150-200g. dry

Send to Graf in 95% alcohol (before drying)

Save a portion and dry for hydrolysis.

Mutants by selection.

N-381

5/14/46.

349-AS x 25a.

5/14-20 isol. Isolate ascospores, 1/peithum. 7 germ. / 20.
Color Morph. F(0).

1	n.g.		
2	"		
3	"		
4	"		
5	"		
6	+	+	++
7	n.g.		
8	n.g.		
9	n.g.		
10	n.g.		
11	n.g.		
12	-	+	++
13	+	+	++
14	+ -	-	
15	n.g.		
16	-	+	++
17	+	+	++
18	n.g.		
19	+	+	++
20	n.g.		

Isogenic N-storles
Isolations.

N 382

5/28/46 SY7 x 360-6. (S series.) 4/30 phototroph

- A. 1 n9
- 2 "
- 3
- 4
- a ✓ 5 ✓
- 6 n9
- 7
- 8 "
- 9
- 10 "
- 11.

G(0)

SY7 x 1633a (70-26) 4/30 pal-
p

- 21 -
- 22 +
- 23 +
- ✓ 24 -
- 25 -
- 26 +
- 27 n.9
- 28 ~~n.9~~ +
- 29 n.9 +
- 30 n.9.

SY7 x 378-3 5/10 mastel

- a 31 -
- a 32 - store
- A ✓ 33 +
- 34 -
- 35 +
- 36 +
- 37 +
- 38 n9
- 39 n9
- 40. n9

37401a x SY7



378-3 x SY7



382-52

nic a x put A
4540 5331.

dated 4/28. Isolate:

5/14: v
infectible.

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11

o nic put nic-put

~~none germinated.~~
Reheat.

4/27-5/14.

21 isolates 2/pur. unassociated virus.

366-3 x 25a. (Lc-4637+~~15300~~-15300).
Only 6 germs!

#41 is	Color	F(0)
giants-pore 21	+	++
n.g. 22	-(pf)	"
23	+	"
24	+	"
25	+	"
26	± (dil)	"

note
so far!

multiple mutants

N-383

4540a x 370-12 (4540-5531 A7. dil)

5/28/46 - G (nic) ♀ (pnt) ♂ (pnt-nic) Color Sex.

fltyg.	n.g.	-	+	±	Sex
1					
2 ✓	-	-	+	±	A
3 ✓	-	-	+	+	a
4 ✓	-	-	-	-	
5 ✓	-	-	+	-	A
6 ✓	-	-	+	+	a
7 n.g.					
8 "					
9 "					
10 "					
11 "					
12 "					
13 "					
14 "					

most spores are not colored.
 sp. A. select for deep pigment.
 sp. B. " " light "

B

21
22
23
24
25 n.g.
26 n.g.
27
28
29

5/28/46. pnt ^{lc - pnt-lc.} Sex.
 5531 A x 33757 a.

✓

Random

31
32
33
34 ✓
35
36
37

border.

??

41	+	-
42	+	-
43	+	-
44	+	+
45	+	+
46	+	+
47	+	+
48		

border

51 n.g.		
52 n.g.		
53	+	-
54 n.g.		
55	+	+
56	+	+
57	-	+

1633a x SY7A.

N-383a

58-6 x SY7.

4/20-5/14.

5/14. -
all spores
colorless.

~~366-3 x 33757a.~~

G(0).

58-6 x SY7.

find more isolates.

5/14:
several pairs.
3 sporoph-
saw sp. P1.

12 pairs
4 sporophytes

	1	
	2	N.G.
	3	
	4	N.G.
	5	
	6	
	7	49
P3	8	+
	9	+
	11	+
P4	12	
	13	
	14	
P5	15	+
	16	
	17	
P6	18	
	19	
	24	+

PAB - adaptation

384

358-6 x 847A

5/28/46

6 (6).

Random -
from
2700
pint.

- | | |
|----|---|
| 1 | + |
| 2 | + |
| 3 | + |
| 4 | + |
| 5 | + |
| 6 | + |
| 7 | + |
| 8 | + |
| 9 | + |
| 10 | + |
| 11 | + |
| 13 | + |

~~generalist.~~

29 vol.

184.9. 1st
heating

all morph.
OK. compare
successive
ratios.

Isolate protogameticia as far as possible of extraneous spores. However some had discharged. 5/18/46

Test on F(0) A31.

	Color.	F(0)	F(prot)
371-11. 1	+	-	+
P: 2	+	-	-
3	+	-	-
SS31-15300+ 4	+	-	-
37401 5	+	+	-
X 6	+	-	-
SS31-37401 7	+	-	-
8	+	-	-
9	+	-	-
10	-	-	-
11	+	-	-
12	+	-	-
13	+ +	-	-
14	+	-	-
15	+ -	-	-
16	+	-	-
17	+	+	-
18	+	-	-
19	+	-	-
20	+	-	-
21	+	-	-
22	ns	3+	-
23	ns	18-	-
371-12 31	+	-	-
32	+	+	-
do. 33	+	-	-
34	+	+	-
35	+	-	-
36	±	+	-
37	±	-	-
38	+	-	-
39	+	-	-
40	+	-	-
41	+	-	-
42	+	-	-

	Color	F(0)	F(prot)
371-1551	+	+	-
52	+	+	-
P: SS31- 53	±	+	-
37401 54	+	+	-
X 55	±	+	-
SS31-15300 56	+	+	-
+ 57	+	-	-
37401 58	±	+	-
heteroc. 59	+	+	-
60	+	+	-
61	+	+	-
62	+	+	±

→ slow ++. ??

371-16 71	+	-	+
72	+	-	+
SS31-37401 73	+	-	+
X 74	+	-	+
SS31-15300 75	+	-	+
und 76	+	-	+
37401 77	+	-	+
unip. 78	+	-	+
condig 79	+	-	+
at fertil 80	+	-	+
81	+	-	+
82	-	-	+
83	+	-	+

Why should protogameticia be more effective?

Nuclear origin, etc.

N 386.

6/1/46

Proc. F(0) plates \bar{c} 4545a + 37401a. for heterocayototaxia
P1.

P3. No growth. (hard starter?)

P4. Repeat \bar{c} fresh cultures.

P6 - no heterocayototaxia growth!!! see 389. Compare

L_1 and L_2 ; $L_1^- + L_2^+$.
 Leucine
 inhibition

6/1/46. 25° broz 4P.

Use 43-3 as L_2^- 368-22 as L_2^+ . 10A as L_1^+ $L_1^- = L_2-4637A$.
 SPI.

broz N plates in the following in pairs, \bar{z} control plate singly. all OK.

1. $L_2^- + L_1^+$
 prec. with.

2. $L_2^- + L_1^-$

3. $L_2^+ + L_1^+$

4. $L_2^+ + L_1^-$

5. $L_2^{\check{+}} + L_2^{\check{-}}$

3PM 6/2/46

Isolate 387 hyphal tips to mid. medium. \odot is solid; \circ is hyf.

Incubate at 30°. p4

	1 to \odot Color	G(o) - color.		5 to \odot Color.	G(o) - color.
L1+ 71		+		71	± +
72		+		72	- +
L2 73		+		73	± +
74		+		74	± +
75		+		75	± +
76		+		76	± +
	1 to \circ		L2+ L2	5 to \circ	
11	++	-		81	++ -
12	"	-		82	-
13	"	-		83	++ -
14	"	-		84	" +
15	+	-		85	" ++
16	++	-		86	" ++
	2 to \odot				
L2- 71		-		77	+ +
72		-			
L2+ 73		-			
74		-			
75		-			
76		-			
	3 to \odot				
L1+ 31		-			
2		-			
L2+ 3		-			
4		-			
5		-			
6		-			
	5 to \circ				
No pins 41	+	-			
2	+	-			
3	+	-			
4	+	-			
	4 to \circ				
L1- 51		+			
2		+			
L2+ 3		+			
4		+			
5		+			
6		+			
	4 to \circ				
61	++	-			
62	"	-			
63	"	-			
64	"	±			
65	"	±			
66	"	-			

The color here is quite hyp. Unless a further mutation of 15300 is considered, this stock is highly suspicious. again should be rejected.

No inhibition demonstrated.

see:

Segregation in hetero conyocis.

389

6/4/46. Broc F(0) plate \bar{c} 94-4 + 37401 a. Use edges of plate for controls.

P6 - no growth!

Repeat P10.

- initial hyphae, but no extended growth!

In vitro activity: synthesis of pantothenic acid by *Neurospora*.

N 391

6/5/46.

50 ml Fries +

broc 5471030A5.

1. —

2. 1 mg β -alanine + 1 mg pantoic acid.

Harvest: 11 P.M. (6 1/2 days).

A. Medium

B. Mycelium in 10 cc H_2O . Then ~~still~~ boil, and remove mycelium for digestion.

Use medium 50% in assay; extract 0.1 ml / 50 Fries.

broc 5531. 12 N 12.

1. F(0) 50 ml.

2. 1A.

3. 1B.

4. 2A.

5. 2B.

no demonstrable
response in
2 days.

9 JUN 1946

In previous experiments, color markers were used; here another biochemical mutant type is employed: 33757-4540 + other single mutants.

361-6 is 33757-4540 A. P10:
 Au F(10) plates + 1 mg d.l. conc. / 10 ml:

~~P10~~ P10.

Isolate 4P11.

α 361-6 + 5531 A.

β 361-6 + 16117 A.

γ. 361-6 + 1633-15300 A.

To F(1c) small leg. hyphae
 1230A13 1130A13

To F(10).

	1230A13	1130A13
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
11	-	-
12	-	-
13	-	-
14	-	-
15	+	++
16	-	-
21	-	-
22	-	-
23	-	-
24	-	-
25	-	-
26	-	-

	1230A13	
51	-	
52	-	
53	-	
54	-	✓
55	-	
56	-	
61	-	-
62	-	-
63	-	±
64	-	-
65	-	-
66	-	±
71	-	-
72	-	-
73	-	-
74	-	-
75	-	-
76	-	-

To Noo slants.

15, 62, 66 to Noo large slants.

α 81 +++
 82 +++
 83 +++

β 91 +
 92 +
 93 +

γ 41 +++
 42 +++
 43 +++

A24. teston: F(10) F(1c) F(1cnic) F(F1C)

15
 62
 66

Selection vs. Dominance

N-393.

Test 392 on le, vic, etc.

There may have been deficient leucine in the σ medium.

clads.

	le	vic	le-vic
81	+ (±?)	+	+
82	+ (±?)	+	+
83.	-	+	+
91	+	+	+
92	+	+	+
93	+	+	+
41.		+	+
42		+	+

hirsuticulus hetero argus.

N-394.

6/13/46. 13 JUN 1946

12N.
See N-83. N. stophila 299A + N. cessa 1633-15300A (70-27). on F(0) plate

Isolate hyphal tips to minimal liquid. 4P14.

1 —
2 —
3 —
4 —
5 —

Take a block of agar + mycelium + inoculate F(0) plate:

11. a few hyphae grew out and covered plate. Conidia spottily white + colored in various areas.

Syntrichium? or unstable *hirsuticulus* colony?

Heterocaryon transformation
in vitro.

N-395

14 JUN 1940

1. Inoc F(0) \bar{c} 5531A + 4540A. 1130P14.

3P15. Isolate hyphae to F(0) ~~in~~ 1 ml tubes.


1
2
3
4
5.

1A16. Inoc prot-cornmeal \bar{c} 383-3

Chlamydomonas newportii.

A-1

Araucarion Prospect. St. was found to bloom after every rain.
Collected 5/18/46, and found Stichococcus, Bacillariae and a variety of
Chlamydomonads. Purify by centrifuging + phototaxis, and more.

 into Mores' 15.

P22 - some green growth noted - a few filaments & some sediment

A24 - micro exam. showed numerous flagellates. Plant struck
out when culture is heavier.

P1 - streaked on Mores' 15 agar. + micro. liq. \bar{c} culture

Endomyces - Killing & Tentants

36. hour culture in F(0). Shaken at 30°.

radiate in quartz tube 2 mins., etc. inoc. coli CM plates

1. Control. dil. 1:25,000. 1:125,000 - (29) - 36,000,000.
 1:10 dens. = $\sigma = 89$ $d = 0.51$ $d_{avg} = 50\%$ $G_{0.31} =$

7P27: small colonies & hyphal radiations.

2. Inoc. 2 min. 1 ml

- 1:50
- 1:2500
- 1:125000
- 1:625000

(11) 275,000 S = .0076

pSurvival = 2.1

3. 1 ml to coli CM. 6P26. coli CM plates. $d = 1340 = 95 \times 10^6$ 2P28. Dilute to 10^{-6} approx. 100/ml and spread over etc.

sample {
 1. Spread 1cc over surface.
 2. Embed in 5cc surface agar.
 3. Embed in 5cc surface agar.
 4. ...
 5. as coli procedure.

T50

Wavy

Fairly uniform. Liquid on surface w. spreading growth. and across plate.

F(0).
 6. Spread 1cc over surface
 7. Embed in surface agar
 8. Embed in surface agar
 9. Embed & cover heavily.
 10. Embed & cover heavily.
 11. Embed & cover heavily.

" Yedonies. P1. 2A30.
 " not up yet.
 to 38°. 7 colonies!

Layne's complete 1P2
 Nothing came up

ps = -log survival This should be a dose.
 = log (1/1 - killing)

Schizosaccharomyces octosporus (20). 6/1

1. Vitamin Requirements.

1. T(0) + Biotin, Thiamin, Riboflavin, nicin, put, inositol.

- 1.
2. - biotin
3. - thiamin
4. - riboflavin
5. - nic m.g.
6. - put
7. - inos.
8. - pab
9. - folii
10. - B₆

6/5.	1 coli	6/6
F)	2 HC+V	±
	3 HC	±
	4. V	±.

unknown factors?

Anyhow, octosporus is genetically not satisfactory for mutant production as it diploidizes very readily. Use *S. pombe* which requires biotin, nic, put + inositol. Compare *S. castellii* which does not req. put

1/7/46.

1. plating density. susp. in H₂O. G = 93. = 31.5 d.u.

hemocytometer count. 136, 138 = 137 / 10⁻⁴ ml.
= 1.4 x 10⁶ / ml.

∴ 1 density unit = 4.3 x 10⁴ / ml.

Vitamin Requirements - stated as biotin, pnt, nic, moa.

		Pg.
1. Frest + vits.	+++	+++
2. " + 4 vits.	+	+++
3 - pnt	-	++
4 - nic	-	-
5 - moa.	-	-
6. T - + vits.	+	+++

1230A7. *Proz coli* 37° sh. - growth only fair worse than
use 10 vits. omit 1 & 11.

1A9 *Proz.*
1 - B₁ (~~Protococcus~~)

- 2 - B₂
- 3
- 4
- 5
- 6
- 7 etc.
- 8
- 9
- 10

see A5.

50 ml 11. F (vits.) 37° shaker.
12. " 37° shaking.

Vitamin Requirements S. pombe.

A-5

19 JUN 1946

19 JUN A-1

Compass A-1.

37°

Fries + vitamin supplement: 10 B vits. - 14d. F con'ts. des'this brot.

	IP 9	10 A10	(put)	put
1 - B ₁	+	++		
2 - B ₂	+	++	nic	nic
3 - pat	+	++	inos	inos
4 - nic	-	-	nic	nic
5 - pat	+	++		OK.
6 - B ₆	-	±		
7 - pat	-	+		
8 - chol	+	++		
9 - inos.	-	-		
10 - Biotin	+	++		

Compass shaking 5 unshaken cultures. 37°

11 F-vits	sh.	+
12. "	unsh.	-
13 colise	sh.	+
14. "	unsh.	-

Growth is poorer than in tubes.
Relatively anaerobic conditions

~~Vitamin Req.~~

A10.

Fries:
21 put / inos, nic, folie (biotin)
22 - put
23 - inos
24 - folie

In colony type, plate A4-2 into F(P) = Fries + pomb vits

$$6 \times 10^{-10} = 78^2$$

$$d = 1051$$

$$(45000,000)$$

$$\text{dil. } 10^6$$

P13 colonies finally noted. Resemble bacterial colonies.

Inoculum?? too old.

PAB - uspm securities.

E 2

8/20/46.

Sp. 16
1946-1947

I PAB		Transmission 36 hrs							
s/p from T(0)									
	off	0	.001	.003	.01	.03	.1	.3	1
Blank		96.0							
71	T L	100		99.1		99	99.0	98.1	97.4
744	H	100	99.2	99.2	99.2	99.1	99.1		
L 13-334	O	99		99.1	99	99.9	99.5		
L 15-162	O	99.2	99.2	99.3	99	99	99		
L 16-162	O		99	99	99.2	99	99		

II		744 (#17)							
Blank		s/p from 04 16							
	0	100	99.2	99	99	99	99	99	99
744									

III		#22							

IV		#11 750							
Blank									
	0	.001	.003	.01	.03	.1			
75	98	91.3	92.4		93	90			
	100	87.5	88.1	93.2	91	93			
	Blank	#9							

(82)