

Exp. List of Experiments

7/15/62 - 8/14/63

- ✓ 101 Competence medium for SB 202 (shk^{-} - ind^{-} - his^{-} - typ^{-})
- ✓ 102 Effect of different medium on competence (SB202) during 30°C incubation
- ✓ 103 Colony selection for higher competence
- ✓ 104 Assay precision for transformants.
- ✓ 105 Competence medium for SB 5 (ind^{-} , his^{-} , ura^{-}) - freezing cultures.
- ✓ 106 Competence after storage in ice-bath (SB5)
- ✓ 107 Competence after storage in KCN (SB5)
- ✓ 108 Partial denaturation of DNA SB503 (FU^R) by heating & testing for high biological activity of the fraction heated to 93.5°C with various auxotrophs
- ✓ 109 Same as exp. 108 except DNA used is SB 19 ⑤
- ✓ 110 High T_m of cys^{+} in SB3 & SB424, their "complementation" & lack of linkage to his^{-}
- ✓ 111 Treating DNA with 1-cyclohexyl-3(- α) morpholinyl-(4)-ethyl carbodiimide metho-p-toluene sulfonate [= compound I]
- ✓ 112 Induce mutation in DNA by Nitrous acid treatment (also see exp 122)

Expt.

- 113 Denature DNA in formaldehyde solution (1%)
- ✓ 114 Cysteine mutants - variation in competence or transformability.
- 115 Effect of heating DNA on linkage relationship (SB 202)
- 116 Denature DNA in formaldehyde solutions - less than 1% conc. of HCHO
- 117 Renaturation of DNA heated in HCHO - S.S.C. (also 121)
- 118 Inhibition of *cys⁺* transformants by large number of recipient cells on *do + trypt + his* plates.
- 119 Centrifugation of DNA heated in SSC-HCHO to determine whether denatured DNA is single stranded or double-stranded.
- 120 HCHO as mutagen.
- 121 Renaturation of DNA heated in HCHO - ssc (also 117)
- 122 Growth curves of the transformed culture vs. increase in total no. of transformants
- 123 Induce mutation in DNA by nitrous acid treatment during renaturation
- 124 Induce mutation in DNA by nitrous acid treatment during renaturation
- 125 SB 22 - check out markers for use as reversion indicator strain
- 126 Competence procedure for SB 22 = *ind⁻ his⁻ arg⁻ ade⁻*
- 127 Induce mutation in DNA SB 22 by nitrous acid treatment during renaturation
- 128 Induce mutation in DNA SB 543 "
- 39 Osmotic sensitivity of transformants

8/20/63 MF

Summary

Competence medium for strains with multiple requirements.
(exp. 101, 102, 105, 126.)

1. Good competent cultures can be obtained by using Spiz + AA (10x)* + tryp (20 µg/ml) as first conditioning medium with 37°C incubation with shaking for 4½ hours (or till Klett Reading reaches ~ 200). This medium works well with all strains tried having amino acid requirements e.g. ind⁻, his⁻, cys⁻, arg⁻, leu⁻, tyro⁻, shk⁻ in strains SB 202, SB 505, SB 543,
2. For second conditioning ~~step~~ period simply dilute 1:5 or 1:10 into CHT-1 & incubate at 30°C for 90 min.
3. For nucleic acid requirements, add 50 µg/ml to 100 µg/ml to the Spiz + AA + tryp medium for 37°C incubation & then dilute 1:5 or 1:10 into CHT-1 for 30°C incubation as usual. Strains tried out satisfactorily are SB 5 (ind⁻ his⁻ ura⁻) use 100 µg/ml uracil & SB 22 (ind⁻ his⁻ arg⁻ ade⁻) use 50 µg/ml ~~100 µg/ml~~ adenine.
4. As alternative to dry-ice alcohol freezing & storage of competent cells, ice-bath & KCN were tried. Overnight storage of competent cultures in ice bath resulted in excessive lysis (98-99%) & 48 hr. storage resulted in total loss of competent cells (exp. 106). When KCN 10^{-2} - 10^{-4} M was present in culture during ice-bath storage excessive lysis also occurred.

* (exp. 107)

* AA (10x) = Amino acids 10-times recommended amount in: Methods in Medical Research vol. 3: 5-22 by J. Lederberg, 1950.

Summary - experiments in search for new linkage groups
with high melting temperatures (T_m)
(exp. 108, 109, 110, 111, 112, 113, 114, 120)

I Screening for high T_m among markers in existing strains.

1. DNA was heated to 93.5°C for 15 min. in Ethylene glycol bath. This heated DNA fraction, still has high his. activity, is used in spot test ~~of~~ with 46 selected strains from stock collection having either a single requirement or one requirement combined with ind^- to see if any of these markers has high melting temperature like his. (For marker & strain used see exp. 108 b). It is interesting to note among ~~these~~ markers tested there were 6 other histidine strains all showed low T_m . The 7 different arginine strains chosen & had such poor competence it was not possible to make any comparison to classify them as high T_m or low T_m . Only 1 out of the 7 arginine strains gave good competence comparable to the other markers (Arg⁺: SB 9). The results of the spot tests showed (exp. 109 b) that 3 cys⁻ strains (SB 62, SB 424, SB 37) & 1 glycine (SB 87) strain could have high T_m . Further test (exp. 109 c) showed SB 424 as having high T_m & also very competent.
2. ~~cysteine~~ cys⁻ (SB 424) is not linked to his⁺ (exp. 109 d)
3. Another cys⁻ strain SB 3 (ind^- his⁺ cys⁻) also showed high T_m for ~~cys⁻~~ marker & non-linkage to either his⁺ or ind^- . However cys⁻ in SB 3 transforms poorly (10-20 times less than his⁺) & this cys⁻ does not complement SB 424 cys⁻ (exp. 110 c). The more competent cys⁻ marker from SB 424 was incorporated into SB 5

to give rise to a better strain ind-his; $cys^+ = SB505$ replacing
 $SB3$ (exp. 114)

4. Melting curve for cys^+ ($SB3$) was determined & found to be higher than that of his, i.e. his: $T_m = 93.5^\circ C$ & $cys^+: T_m = 95^\circ C$ (exp. 110 d, e). Also cys^+ gave very little residual activity upon further heating beyond $96^\circ C$ while his, always retained more residual activity.

II. Induce mutation in DNA linked with either his, or cys

1. p -toluene sulfonate of Cyclocarbodimide was used to induce mutation in DNA $SB19$ (heat denatured). After ~~centrifugation~~ dialysis The treated DNA was rendered & used to transform a his- strain ($SO1$). The his $^+$ transformants (selected on AA-his plates) were tooth picked & tested for any possible newly induced amino acid requirement. None was found among 5600 his $^+$ transformant colonies tested (exp. 114 d)
2. Nitrous acid was used as mutagen for DNA treatment. Attempts were made to determine any amino acid requiring auxotroph linked with either his $^+$ or cys $^+$ transformants. 12,353 colonies were tested & no new mutant was found among either his $^+$ or cys $^+$ transformants. 2 mutants were picked up on NA plates after penicillin treatment however, these can be spontaneous mutations ^{in the recipient population} having no relationship with Nitrous acid treatment of DNA. (exp. 112)

3. HCHO as mutagen for DNA - 9,550 his $^+$ or cys $^+$ transformants tested

No new linked mutants detected. (~~exp. 112~~ + 120)

Effect of HCHO on Denaturation & Renaturation of DNA.

(exp. 113, 116, 117, 119, 121)

- When 1% HCHO was present in S.S.C. melting temperature T_m shifted down approximately 20°C compared with melting curves done in S.S.C. (exp. 113 d)

	<u>S.S.C.</u>	<u>S.S.C.+1% HCHO</u>
ind : T_m	91.5°C	71.3°C
his : T_m	93.0°C	72.2°C

- When .1% HCHO is present in S.S.C. T_m of ind, his, & cys all shifted down less - to $83^\circ - 84^\circ\text{C}$ range. With .01% HCHO T_m falls in range $86^\circ - 88^\circ\text{C}$. Residual activity of all markers after heating to 100°C were reduced when HCHO is present (exp. 116 d, d' & e')
- Because of the high residual activity of his, it is difficult to separate markers his, & cys by the difference in T_m .
- Presence of .03% HCHO during denaturation gave slight amount of renaturation (exp. 117 d) 0.065% recovery of native activity vs. 89% recovery in the absence of HCHO (Comparison unfair because the 89% recovery started from denatured sample with 55% residual activity while HCHO sample started from denatured sample with .001% activity) Much better renaturation were obtained when HCHO denatured DNA was first dialyzed (exp. 119)
- HCHO-SSC denatured DNA & S.S.C. heat-denatured DNA were bands of in CsCl ultracentrifugation. The calculated densities are
 S.S.C. heat denatured DNA $\rho = 1.720$
 HCHO-SSC DNA $\rho = 1.723$ (while Native DNA $\rho = 1.703$)

Therefore the Hctto.ssc denatured DNA is banding very close to ~~heat~~ standard heat denatured material, & probably single-stranded (exp. 119)

Nitrous acid treated DNA - to induce reverse mutations.
(exp. 123, 124, 125, 127, 128)

In order to improve the efficiency of assay for induced mutation, reversions of selected ~~of~~ autotrophic markers were chosen. DNA's from multiply marked strains e.g. SB 202 (*ind⁻*, *his⁻*, *trp⁻*, *shk⁻*), SB 543 (*ind⁻*, *his⁻*, *cys⁻*, *leu⁻*) & SB 22 (*ind⁻*, *his⁻*, *arg⁻*, *ade⁻*) were made & treated with NaNO₂ in acetate buffer during renaturation at 66°-67°C. The treated DNA's were then used in transformations using the original strain from which the DNA was made as recipient. An increase in ~~reversion~~^{mutation} was looked for if rate of any of the markers used would indicate mutagenic effect of nitrous acid on DNA. So far, no increase of reversions has been detected.

During the course of these experiments it was learned several points were learned & should be useful in future experiments:

1. In order to obtain good renaturation it is important to have high DNA concentration during renaturation e.g. with 20 µg/ml. DNA one can recover 30% - 90% of native ~~activity~~ biological activity. If 5 µg/ml DNA was used the results are very poor - perhaps only 1-5% recovery.
2. In order to use high conc. of DNA, the denatured samples are necessarily high in residual activity 1-5%.
3. 2 hour heating of denatured DNA (without fast cool) at 66°-67°C

is adequate for renaturation, no further slow ~~the~~ cool good from 66°C to room temperature is necessary (exp. 123e). This fact ~~gives~~ allows for immediate assay of treated DNA instead of waiting overnight.

4. The pH used for nitrous acid treatment is 4.2. A 10 min. exposure of this low pH & relatively high temperature 66°C does not destroy DNA excessively. One can recover about 50% as much biological activity from these acid treatment as compared with the standard renaturation procedure.
5. Optimal conditions for treatment in order to produce DNA with approximately $\frac{1}{3}$ the activity of standard renaturation were: 10 min. treatment using NaNO_2 concentrations of .1M to .2M. Reaction is terminated by addition of Na_2HPO_4 to change the pH to 7.