Exp. List of Experiments 7/15/62 – 8/14/63

101 Competence medium for SB 202 (shk^- ind^- – his_2^- – 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^-_1, ura^-) – freezing cultures.

106 Competence after storage in ice-bath (SB5)

107 Competence after storage in KCN (SB5)

108 Partial denaturation of DNA SB303 (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 Tm of cys^+ in SB3 & SB424, their non-complementation & lack of linkage to his,

111 Treating DNA with 1- cyclohexyl - 3 (-2) morpholinyl - (4) - ethyl carbodiimide metho-p- toluene sulfonate [= compound I]

112. Induce mutation in DNA by Nitrous acid treatment (also see exp 123

Exp.

113 Denature DNA in formaldehyde solution (1%)

✓ 114 Cysteine mutants – variation in competence or transformability.

115. Effect of heating DNA on linkage relationship (SB202)

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+trp+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 SB22 – check out markers for use as reversion indicator strain

126 Competence procedure for SB22 = ind$^-$ his$^-$ arg$^-$ ade$^-$

127 Induce mutation in DNA SB22 by nitrous acid treatment during renaturation

128 Induce mutation in DNA SB543 " " " " " "

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 (20μg/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⁻, typ⁻, shk⁻ in strains SB202, SB505, SB543.

2. For second conditioning ~~period~~ period simply dilute 1:5 or 1:10 into CHF-1 & incubate at 30°c for 90 min.

3. For nucleic acid requirements, add 50μg/ml to 100μg/ml to the Spiz + AA + tryp medium for 37°c incubation & then dilute 1:5 or 1:10 into CHF-1 for 30°c incubation as usual. Strains tried out satisfactorily are SB5 (ind⁻ his⁻ ura⁻) use 100μg/ml uracil & SB22 (ind⁻ his⁻ arg⁻ ade⁻) use 50μg/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.

8/20/63
MJ

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

I Screening for high Tm 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 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 of strain used see exp. 108h). It is interesting to note among the markers tested there were 6 other histidine strains all showed low Tm. The 7 different arginine strains chosen & had such poor competence it was not possible to make any comparison to classify them as high Tm or low Tm. Only 1 out of the 7 arginine strains gave good competence comparable to the other markers (Arg4 : SB9
The results of the spot tests showed (exp. 109b) that 3 cystine strains (SB62, SB424, SB37) & 1 glycine (SB87) strain could have high Tm. Further test (exp. 109c) showed SB 424 as having high Tm & also very competent.
2. cys1⁻ (SB424) is not linked to his1 (exp. 109d
3. Another cys⁻ strain SB 3 (ind⁻ his⁻ cys⁻) & also showed high Tm for cys marker & non-linkage to either his1 or ind. However cys⁻ in SB3 transforms poorly (10-20 times less than his1) & this cys⁻ does not complement SB424 cys⁻ & (exp. 110c). The more competent cys1 marker from SB424 was incorporated into SB5

to give rise to a better strain ind⁻ his₁⁻ cya₁⁻ = SB505 replacing SB3 (exp. 114)

4. Melting curve for cya⁺ (SB3) was determined & found to be higher than that of his₁ i.e. his₁ : T_m = 93.5°C & cya⁺ : T_m = 95.5°C (exp. 110 d,e). Also cya⁺ gave very little residual activity upon further heating beyond 96°C while his₁ always retained more residual activity

II. Induce mutation in DNA linked with either his₁ or cya

1. p-toluene sulfonate & Cyclocarbodiimide was used to induce mutation in DNA SB19 (heat denatured). After ~~conductivity~~ dialysis The treated DNA was renatured & used to transform a his₁⁻ strain (S01). 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. 111 d)

2. Nitrous acid was used as mutagen for DNA treatment. Attempts were made to determine any amino acid requiring auxotroph linked with either his₁⁺ or cya⁺ transformants. 12,353 colonies were tested & no new mutant was found among either his₁⁺ or cya⁺ transformants. ~~#~~ 2 mutants were picked up on NA plates after penicillin treatment in the recipient population however, these can be spontaneous mutations having no relationship with Nitrous acid treatment of DNA. (exp. 112)

3. HCHO as mutagen for DNA – 9,550 his₁⁺ or cya₁⁺ Transformants tested

no new linked mutants detected. (exp. 112 & 120)

Effect of HCHO on Denaturation & Renaturation of DNA.
(exp. 113, 116, 117, 119, 121)

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

	S.S.C.	S.S.C. + 1% HCHO
ind : Tm	91.5°C	71.3°C
his$_1$: Tm	93.0°C	72.2°C

2. When .1% HCHO is present in S.S.C. Tm of ind, his, & cya all shifted down less — to 83°-84°C range. With .01% HCHO Tm fell in range 86°-88°C. Residual activity of all markers after heating to 100°C were reduced when HCHO is present (exp. 116 d, d' & e)

3. Because of the high residual activity of his, it is difficult to separate markers his, & cya by the difference in Tm.

4. 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 5.5% 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. a)

5. HCHO-SSC denatured DNA & SSC heat-denatured DNA were banded in CsCl ultracentrifugation. The calculated densities are

SSC heat denatured DNA $\rho = 1.720$

HCHO-SSC " " DNA $\rho = 1.723$ (while Native DNA $\rho = 1.703$

Therefore the HCHO·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 auxotrophic markers were chosen. DNA's from multiply marked strains e.g. SB 202 (ind⁻, his₂⁻, typ⁻, shk⁻), SB 573 (ind⁻, his₁⁻, cys⁻ leu⁻) & SB 22 (ind⁻, his₁⁻ arg⁻, ade⁻) were made & treated with $NaNO_2$ 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 ~~rate was looked for~~ 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 20μg/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 good renaturation, no further slow ~~cool~~ cool from 66°C to room temperature is necessary (exp. 123e). This fact ~~~~ 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 1/3 the activity of standard renaturation were: 10 min. treatment using $NaNO_2$ concentration of .1M to .2M. Reaction is terminated by addition of Na_2HPO_4 to change the pH to 7.