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# STUDIES ON THE ORGANIZATION OF GENES CONTROLLING LYSINE BIOSYNTHESIS IN *NEUROSPORA CRASSA*

## V. Genetic fine structure of locus lysine-5.

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### Abstract

Gentical studies on lys-5 mutants have revealed that they are highly sterile as well as produce mostly inviable mutant acospore progency when crossed to other strains. In addition to the other known methods, back crossing of lys-5 mutants, markers and double mutants repeatedly to the parental wild type Emerson strain has proved to be effective in improving their fertility.

Mapping of 38 lys-5 mutants with the help of triple point crosses gave the length of the locus lys-5 as about 1.315 contimorgan. When the position of mutants belonging to different complementation groups is examined on the genetic map, it is found that they are dispersed along the locus and occupy discontinuous regions of the map.

## Introduction

The study of the organization of genes is of prime importance. As a number of U.V. induced lys-5 mutants had been obtained (Ahmad et al 1979). It was decided to investigate the genetic fine structure of this locus.

## Materials and Methods

*Strains:* Lysine-5 mutants induced by Ahmad and his coworkers: A203, A213, A223, A224, A226, A227, A240, A243, A245, A247, A249, A252, A257, A259, A263, A277, A284, A287, A289, A290, A916, A923, A924, A934, A935, A945, A946, A947, A950, A973, A975, A979, A1032, A1043, A1048, A1075. Parental wild type strains: Emerson A (EmA) (5296): Emerson a (Ema) (5297).

Lysine-5 mutants induced by other workers:

Asco a(37402) Good (1951): STL-7A

The above two mutants were kindly provided by Dr. D.R. Stadler. Mutants of linkage group VI tested and used have been tabulated in Table 1.

Stocks were subcultured on Vogel's minimal medium (V.M.) (Vogel,1956) supplemented with 20mg lysine, adenine or cysteine. For making crosses, Westergaard's medium (Westergaard & Mitchell, 1947) supplemented normally with 20mg of the required amino-acid and Suyama's medium (Suyama, Woodward, & Sarachek, 1958) with 50mg lysine. and 20mg adenine were usually used.

Improvement in fertility of crosses was effected by giving 4 to 6 rapid subcultures to the two strains before crossing, increasing phosphate to 0.6 or 0.8%, use of mycelial extract from a highly fertile cross (Ahmad et al, 1967) or mammalian-hormones (Ahmad & Rahman, 1969). Back crossing of mutants, markers and double mutants, for a number of generations was undertaken to achieve fertility of triple point crosses used for mapping the mutants. A few crosses proved to be fertile when one parent was inoculated first and conidia from the second parent were put in after 6 days of inoculation of the first parent. Mutants which became leaky, during the investigation, were back crossed with EmA and non-leaky isolates of the respective mutants were obtained from amongst the progeny.

## Experiments and Results

### *Choice of markers for determining the order of lys-5 mutants.*

### *Cysteine-1 and Cysteine-2.*

When cys-1 (84605) and cys-2 (38401) were tested on V.M., cys-2 was found to be much more leaky than cys-2, so of the two cysteine markers, cys-1 was chosen. However, cys-1, being to some extent leaky was used only in a few crosses (Table 3).

### *Ylo (Y30539y)*

When ylo was utilized as a marker in triple and quadruple point crosses, it was found that very few growing spores had ylo phenotype. That ylo was not being freely expressed in the progeny became still more obvious when data from a reciprocal cross of

Table 1. **Showing mutants of linkage group VI tested and used for genetic fine structure studies.**

| Mutant | Mutant Symbol | Isolation number | Phenotype | Person who kindly sent it |
|---|---|---|---|---|
| Adenin-8 | ad-8 | | Requires adenine | Dr. D.R. Stadler |
| Cysteine-2 | cys-2 | 38401 | Requires cysteine (or methionine) | –do– |
| Cysteine-1 | cys-1 | 84605 | –do– | Dr. M.B.Mitchell |
| Yellow | ylo | Y30539y | Yellow conidia | –do– |

**Table 2.** Expression of ylo phenotype of the ylo mutant under different light conditions (72 hours after treatment).

| Designation of mutants | Expression of formation and colour of conidia under diffuse sunlight | | electric light | | direct sunlight through water | | blue light | | yellow light | | red light | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | formation | colour | formation | colour | formation | colour | formation | colour | formation | colour | formation | colour |
| ylo Ext-la | Best | Yellow | Good | Yellow | Good | Yellow | Moderate | Pale Yellow | Moderate | Pale Yellow | Moderate | Yellow |
| ylo Ext-2A | ,, | ,, | ,, | ,, | ,, | ,, | Good | ,, | ,, | ,, | ,, | ,, |
| ylo Ext-3A | Moderate | ,, | Moderate | Pale Yellow | Moderate | Pale Yellow | Moderate | Yellow | Moderate | White | ,, | ,, |
| ylo Ext-3a | Best | Best Yellow | Good | Yellow | Good | Yellow | ,, | ,, | ,, | Pale Yellow | Good | ,, |
| ylo Ext-4A | ,, | Yellow | Moderate | Pale Yellow | ,, | ,, | ,, | Pale Yellow | ,, | ,, | ,, | ,, |
| ylo Ext-6A | ,, | ,, | Good | Yellow | Moderate | Pale Yellow | ,, | ,, | ,, | ,, | Moderate | ,, |
| ylo Ext-7A | ,, | Best Yellow | Moderate | ,, | ,, | Yellow | Good | ,, | Good | ,, | ,, | ,, |
| ylo Ext-8A | ,, | ,, | Good | ,, | Good | ,, | ,, | Yellow | ,, | Yellow | Good | ,, |
| ylo Ext-9A | ,, | Yellow | Very Poor | ,, | ,, | ,, | Moderate | Pale Yellow | Good | Pale Yellow | ,, | ,, |
| ylo Ext-11A | ,, | ,, | Good | ,, | ,, | ,, | Good | ,, | Moderate | ,, | ,, | ,, |
| ylo Ext-12A | ,, | ,, | ,, | ,, | ,, | ,, | ,, | ,, | ,, | ,, | ,, | ,, |

A245 and asco, utilizing ylo as a marker were obtained. No Ylo strains were recovered amongst the progeny of either of the two reciprocal crosses as shown below:

A245, Ylo Ext 5A x asco (7th) 11a: 14 growing spores, all wild.

Asco (7th) Ylo Ext 1A x A245a : 24 growing spores, all wild.

Therefore, Ylo was back crossed to the parental wild type, Em, to obtain an isolate which would readily express the Ylo phenotype. Furthermore, since sunlight is known to play a role in the development of pigments, it was decided to study the effect of different types as well as of different wavelengths of light.

Effects of diffused sun light, i.e. sun light in shade, direct sun light which was passed through pale blue coloured water to eliminate the heat effect, electric light, blue, yellow and red coloured lights were studied on the production of conidia by 11 isolates of ylo obtained after back-crossing it with Em (Table 2). Not only the expression of Ylo was better in diffused sun light but also the formation of conidia was best under the influence of this type of light.

Of the 11 Ylo isolates studied, two isolates, Ylo Ext-3a and ylo-Ext-7A showed best development of yellow pigment in the conidia. Ylo Ext-7A was crossed with Ema. Fifty good growing spores were isolated. Forty single spore cultures survived. When they were classified, 20 proved to be ylo and 29 wild type. This was close to 1:1 segregation of ylo and wild type.

In subsequent work ylo Ext 7A was used and lysine recombinant prototrophs were allowed to develop in diffused sun light. But even then ylo kept lagging behind in expression. For example, in the reciprocal crosses of A263 with asco, no ylo progeny was found amongst the recombinants (Table 3). This lead to our abandoning this marker and fall back upon ad-8 for ordering the various lysine-5 mutants along the locus.

### *ad-8*

This marker is located distal to asco, while cys-1 and ylo are located proximal to it (Fincham & Day, 1971). While this marker was not leaky and could also be depended upon for expression but it promoted sterility in crosses, inviability of spores and reduction in the number of spores shed. Back crossing it upto 6th generation with Em, improved its performance and as the back cross generations progressed the handicaps were gradually reduced.

### *Improvement of asco and asco ad-8 double mutants through back crossing*

Asco suffered from sterility in its crosses with markers like ad-8, cys-1 and Ylo as

Table 3. Order and Distances of LYS-5 Mutants

mk = Marker used in triple point crosses. Specific marker used, is mentioned in each cross.
mk1 = Marker used with the parent mentioned first in the quadruple point cross.
mk2 = Marker used with the parent mentioned next in the quadruple point cross.
e.g. In cross 4: A224 ylo-Ext-2A x asco (8th) ad-8 (6th) Ext-2. mk1 = ylo; mk2 = ad-8.
$d_1$ = deduced number of inviable spores where ad-8 has been used as a marker. Deduction based on average of inviable spores in quadruple point crosses involving ad-8, asco and A213, A224 and A284 = 496% of viable spores.
$d_2$ = deduced number of inviable spores where ylo has been used as a marker = 76%.
* pseudowilds and true wilds were distinguished by crossing the wild type growing spore with Em and estimating mutant spores in the progeny.
† Absence of ylo recombinants from reciprocal crosses showing lack of expression of ylo.

| | Inviable spores | | Number of asco spores | | | | | | | | | | Based on viable spores | Based on total of viable and inviable spores | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lys-5 | | Triple point crosses | | | Quadruple point crosses | | | | | | | |
| Cross | Number | percent of viable spores | auxo-trophs | proto-trophs | Lys-5+ mk+ | lys- mk- | pseudo-wild | lys-5+ mk-1+ mk-2+ | lys-5+ mk-1- mk-2+ | lys-5+ mk-1+ mk-2- | lys-5+ mk-1- mk-2- | order and map distance | order and map distance | mean map distance centi-morgan |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| A203 x asco (8th) ad-8 (6th) 5th Ext-1A | $d_1$(13349) | – | 2629 | 63 | 2 | 5 | 56 | | | | | ad-8 A203 Asco<br>0.5201 | A203 Asco<br>.087 | |
| A213 ylo Ext-2A x Asco (7th) lla | 1220 | 32% | 3850 | 6 | 3 | – | 3 | | | | | Asco A213 ylo<br>0.156 | Asco A213<br>.118 | |
| A213 ylo Ext-9A x Asco (7th) lla | 2555 | 52% | 4865 | 9 | 3 | – | 6 | | | | | Asco A213 ylo<br>0.123 | Asco A213<br>.081 | Asco A213<br>0.139 |
| A213 ylo (mk1) Ext-9A x Asco (8th) ad-8 (mk2) 5790 Ext-2a | 5790 | 124% | 4656 | 14 | – | – | 4 | 10 | 0 | 0 | 0 | ad-8 asco A213<br>0.428 | Asco A213<br>.191 | |
| A213 cys-1 Ext-1A xA223 ad-8 (3rd) Ext-3a | $d_1$(54879) | – | 11063 | 1 | 1 | – | – | – | – | – | – | A223 A213 cys-1<br>.018 | A223 A213<br>.003 | |
| A223 ylo Ext-2a x Asco (5th) Ext-1a | $d_2$(4735) | | 6229 | 1 | | 1 | | | | | | A223 asco ylo<br>.032 | A223 asco<br>.018 | A223 asco<br>.05995 or .060 |
| A223 Ext-4A x Asco (8th) ad-8 (6th) Ext-1a | $d_1$(13084) | | 2625 | 13 | 2 | 5 | 6 | | | | | ad-8 A223 asco<br>0.531 | A223 asco<br>.089 | |

Table Continued

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A224 ylo (mk1) Ext-2A x Asco (8th) ad-8 (mk2) (6th) Ext-2a | 2070 | 639% | 316 | 8 | | | 2 | 2 | 0 | 2 | 2 | ad-8 A224asco ylo<br>3.704 | A224 asco<br>0.500 | |
| A227 Ext-1A x asco (8th) ad-8 (6th) Ext-1a | $d_1$(12231) | | 2453 | 13 | 2 | 6 | 5 | | | | | ad-8 A227 asco<br>.649 | A227 asco<br>.108 | |
| A227 ad-8 (6th) Ext-1A x A259a | $d_1$(10679) | | 2119 | 17 | 10 | 4 | 3 | | | | | ad-8 A227 A259<br>1.302 | A227 A259<br>.218 | |
| A226 ad-8 (6th) Ext-2a x A287 Ext-1A | $d_1$(2842) | | 561 | 12 | 9 | 3 | 0 | | | | | ad-8 A226 A287<br>4.189 | A226 A287<br>.703 | |
| A240 ylo Ext-1A x asco (7th) Ext-11a | 5175 | 63% | 8128 | 41 | 15 | 6 | 20 | | | | | ad-8 asco A240<br>.514 | Asco A240<br>.315 | Asco A240<br>.272 |
| A240 ylo Ext-2A x asco (7th) Ext-11a | 3655 | 54% | 6537 | 19 | 9 | 2 | 8 | | | | | ad-8 asco A240<br>.336 | Asco A240<br>.215 | |
| A243a x asco (8th) ad-8 (6th) 3rd 30A | $d_1$(12474) | | 2467 | 48 | 13 | 8 | 27 | | | | | ad-8 asco A243<br>1.670 | Asco A243<br>.280 | |
| A245a x asco (8th) ad-8 (6th) Ext-5A | $d_1$(11180) | | 2232 | 22 | 2 | 9 | 11 | | | | | ad-8 A245 asco<br>0.976 | A245 asco<br>.160 | |
| A245a x asco (7th) ylo Ext-1A | 7153 | 104% | 6846 | 24 | 16 | 0 | 8 | | | | | A245 asco ylo<br>0.466 | A245 asco<br>.342 | A245 asco<br>.455 |
| A245 + ylo (2nd) Ext-5A x asco (7th) Ext-11a | 2162 | 170% | 1260 | 14 | 9 | 0 | 5 | | | | | asco A245 ylo<br>1.413 | asco A245<br>.815 | |
| A247 ad-8 (6th) Ext-5a x asco (8th) 1A | $d_1$(19519) | | 3216 | 59 | 11 | 22 | 26 | | | | | ad-8 asco A247<br>2.015 | asco A247<br>.338 | |
| A247 ad-8 (6th) Ex-6A x 259 A | $d_1$(4781) | | 947 | 17 | 10 | 7 | 0 | | | | | ad-8 A247 A259<br>3.527 | A247 A259<br>.592 | |
| A249 Ext-1a x (asco (8th) ad-8 (6th)) 3rd Ext-30A | $d_1$(29725) | | 5947 | 46 | 15 | 2 | 29 | | | | | ad-8 asco A249<br>.567 | asco A249<br>.095 | |
| A252a x asco (8th) ad-8 (6th)) 5th Ext-1A | $d_1$(18302) | | 3653 | 37 | 7 | 4 | 26 | | | | | ad-8 asco A252<br>.596 | asco A252<br>.100 | |
| A257 x asco (8th) ad-8 (6th) 5th Ext30A | $D_1$(10203) | | 1999 | 60 | 19 | 2 | 39 | | | | | ad-8 asco A257<br>2.04 | asco A257<br>.343 | |
| A259 ad-8 (6th) Ext-12a x asco (8th) Ext-1A | $d_1$(5168) | | 1030 | 12 | 0 | 4 | 8 | | | | | ad-8 asco A259<br>0.768 | asco A259<br>.129 | |
| A259a x A289 ad-8 (6th) Ext-3A | $d_1$(5396) | | 1080 | 8 | 7 | 1 | 0 | | | | | ad-8 A289 A259<br>1.471 | A289 A259<br>.262 | |
| A263 ad-8 (6th) Ext-1A x asco (8th) Ext-2A | $d_1$(22978) | | 4590 | 64 | 13 | 19 | 32 | | | | | ad-8 asco A263<br>1.375 | asco A263<br>.232 | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| †A263 ylo Ext-1A x asco (7th) Ext 11a | 5795 | 76% | 7605 | 11 | 3 | 0 | 8 | | | | | asco A263 ylo<br>.079 | asco A263<br>.045 | asco A263<br>.136 |
| †A263a x asco (7th) ylo Ext-2A | 2682 | 46% | 5795 | 12 | 3 | 0 | 9 | | | | | A263 asco ylo<br>.103 | A263 asco<br>.071 | |
| †A263 ylo Ext-3a x asco (7th) Ext-4A | 4485 | 49% | 9188 | 15 | 3 | 2 | 10 | | | | | Asco A263 ylo<br>.109 | asco A263<br>.0731 | |
| A227-ylo Ext 1a x asco (7th) Ext-4A | 3811 | 66% | 5781 | 17 | 3 | 1 | 13 | | | | | asco A277 ylo<br>.138 | asco A277<br>.083 | |
| A284 ylo (mk1) Ext-1A x asco (8th) ad-8 (6th) km 2 Ext-2a | 2515 | 484% | 498 | 22 | | | 4 | 8 | 2 | 8 | 0 | ad-8 asco A284 ylo<br>6.154 | asco A284<br>1.186 | asco A284<br>.810 |
| A284 ylo Ext-1A x asco (8th) Ext-2a | 2660 | 366% | 710 | 16 | 6 | 2 | 8 | | | | | asco A284 ylo<br>2.204 | asco A284<br>.413 | |
| A287 ad-8 (6th) Ext-1A x asco (7th) Ext-11a | $d_1$(11603) | | 2523 | 18 | 8 | 6 | 4 | | | | | ad-8 asco A287<br>1.102 | asco A287<br>.198 | |
| A287 cys-1 Ext-1A x asco | – | | 18408 | 17 | 10 | 6 | 1 | | | | | asco A287 cys-1<br>.174 | | |
| A290 Ext-1a x (asco (8th) ad-8 (6th)) 3rd Ext-2A. | $d_1$(29562) | | 5939 | 21 | 11 | 2 | 8 | | | | | ad-8 asco A290<br>0.436 | asco A290<br>.073 | |
| A916-4A x asco (9th) ad-8 (6th)) 2nd Ext-16a | $d_1$(13268) | | 2626 | 49 | 16 | 2 | 31 | | | | | ad-8 asco A916<br>1.3458 | asco A916<br>.226 | |
| A923a asco (8th) ad-8 *6th) 3rd Ext-2A | $d_1$(10535) | | 2105 | 19 | 10 | 2 | 7 | | | | | ad-8 asco A923<br>1.1299 | asco A923<br>.1896 | |
| A924a x asco (8th) ad-8 (6th)) 3rd Ext-2A | $d_1$(18352) | | 3677 | 23 | 5 | 5 | 13 | | | | | ad-8 asco A924<br>.5405 | asco A924<br>.0907 | |
| A934a x asco (8th) ad-8 (6th)) 3rd Ext-30A | $d_1$(24249) | | 4934 | 55 | 12 | 6 | 37 | | | | | ad-8 asco A934<br>.7216 | asco A934<br>.124 | |
| A935 ad-8 (6th) ad-1A x asco (86h) 1A | $d_1$(4627) | | 944 | 8 | 3 | 1 | 4 | | | | | ad-8 A935 asco<br>8403 | A935 asco<br>.1432 | |
| A945a x asco (8th) ad-8 (6th)) 3rd Ext-2A | $d_1$(50508) | | 10121 | 62 | 42 | 2 | 18 | | | | | ad-8 asco A945<br>0.864 | asco A945<br>.14499 | |
| A946 x asco (8th) ad-8 (6th)) 3rd Ext-30A | $d_1$(12772) | | 2485 | 88 | 18 | 2 | 66 | | | | | ad-8 asco A946<br>1.5546 | asco A946<br>.261 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A947-1A x asco (8th) ad-8 (6th) 5th Ext-8A | $d_1$(11144) | 2212 | 55 | 14 | 2 | 39 | ad-8 asco A947<br>1.4116 | asco A947<br>.239 |
| A950-1A x asco (8th) ad-8 (6th)) 5th Ext-8a | $d_1$(11482) | 2273 | 43 | 10 | 3 | 30 | ad-8 asco A950<br>1.1231 | asco A950<br>.1899 |
| A973-2A x asco (8th) ad-8 (6th)) 5th Ext-8a | $d_1$(12445) | 2480 | 29 | 5 | 3 | 21 | ad-8 asco A973<br>0.6377 | asco A973<br>.107 |
| A975a x asco (8th) ad-8 (6th)) 3rd Ext-2A | $d_1$(10748) | 2149 | 18 | 9 | 9 | 7 | ad-8 asco A975<br>1.7301 | asco A975<br>.290 |
| A1032a x asco (8th) ad-8 (6th)) 3rd Ext-2A | $d_1$(21392) | 4271 | 42 | 26 | 4 | 12 | ad-8 asco A1032<br>1.391 | asco A1032<br>.233 |
| A1043a x asco (8th ad-8 (6th)) 3rd Ext-2A | $d_1$(27067) | 5376 | 81 | 44 | 0 | 37 | ad-8 asco A1043<br>1.612 | asco A1043<br>.271 |
| A1048a x asco (8th) ad-8 (6th) 3rd Ext-30A | $d_1$(10902) | 2158 | 40 | 11 | 3 | 26 | ad-8 asco A1048<br>1.6700 | asco A1048<br>.214 |
| A1075a x asco (8th ad-8 (6th)) 5th Ext-1A | $d_1$(12861) | 2557 | 36 | 6 | 2 | 28 | ad-8 asco A1075<br>0.6170 | asco A1075<br>.104 |
| STL-ad-8 (6th) Ext-1A x asco (7th) Ext-11a | $d_1$(50270) | 10106 | 29 | 5 | 13 | 11 | ad-8 asco STL<br>0.355 | asco STL<br>.0596 |

well as the new lys-5 mutants. It also suffered from high lethality amongst the progeny. Its fertility improved with back crossing with Em. Reasonable fertility was achieved in the 7th generation, it further improved in the 8th and 9th generation back crossed progeny. Mostly 8th generation back cross isolates were used in the study of fine structure of lys-5 (Table-3).

Improvement in fertility of asco *(8th) + ad-8 *(6th) double mutants could be further effected by continuing the back crossing of the double mutants with Em upto 5 generations (Table 3). New lys-5 mutants which were sterile with asco (8th) + ad-8 (6th) double mutants became fertile with the successiveback crossed generations of these double mutants (Table 3).

### *Improvement in the fertility of lys-5 mutants through back crossing*

When the lys-5 mutants were back crossed with Em, some of the isolates became fertile with asco + ad-8 double mutants with which the original mutants were sterile. Thus A916 and A947 which were sterile with asco + ad-8 double mutants became fertile with them when their isolates, A916-4A and A947-1A were crossed (Table-3).

### *Effect of changes in the concentration of supplements on the fertility of lys-5 mutants.*

Increase in the lysine supplement from 20mg to 40mg, 50mg and 60mg per 100ml in the crossing medium, improved fertility of crosses. Concentrations lower than 20mg lysine, decreased fertility of the crosses.

Adenine gave its maximum beneficial effect at 20 to 30mg per 100ml of the medium. Higher concentrations of adenine had either no effect or an adverse effect on perithecia formation. Eighty mg adenine per 100ml of the medium also seemed to have a retarding effect on mycelial growth and was thus apparently toxic. When the concentration of adenine was raised to 50mg or more per 100ml of the medium, there was an accumulation of a yellowish to orange red pigment in the crosses of some lys-5 mutants with ad-8, for example crosses of A203, A254 and A268 with ad-8.

### *Crossing media*

The efficiency of the two crossing media, Westergaard's (Westergaard & Mitchell, 1947) and Suyama's (Suyama, Woodward & Sarachek, 1958) differed with the strains. Suyama's medium proved better in crosses of lys-5 mutants with EmA, while Westergaard's medium gave better results in crosses of lys-5 mutants with ad-8. Fertility of triple

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* (8th), (6th) indicate the number of generations for which a mutant was backcrossed with Em.

and quadruple point crosses with ad-8 as a marker was found to be better on the whole in Westergaard's medium than in Suyama's medium. Suyama's medium showed less growth of mycleia which led to the deposition of the liberated spores mostly on the walls of the crosssing tube instead of being caught in the meshes of mycelia as often happens in Westergaard's medium. This helped in collecting spores for plating.

### *Fine structure map of locus lysine-5:*

Mapping of 38 lys-5 mutants was attempted with the help of successful triple point and quadruple point crosses (Table 3, Fig. 1). Distances of 35 new lys-5 mutants have been obtained from their direct crosses with asco. A226 has been placed distal to asco on the basis of its distance with A28 which has mapped proximal to asco at a distance of 0.198 centimorgan. A289 has been mapped with the help of its cross with A259. The lack of excess of any recombinant class as single cross-over between A224 and asco, made difficult the positioning of A224 with respect to asco. However, when one considers the expected double and triple crossovers, the data fits in better with the placing of A224 proximal to asco. A924 could not be placed on the map as the expected single and double crossovers in its triple point cross with asco were equal in frequency (Table 3). Based on total spore count, its distance from asco was found to be about .091. The length occupied by the 38 mutants comes to 1.315 centimorgan.

Due to the high inviability of the lys-5 ascospores in the progeny from the crosses, distances between mutants have been calculated on the basis of total of viable and inviable spores. This was found necessary because the calculation of distances, taking viable spores only into account, gave very abnormal distances. Thus the distance between asco and A284 came to 6.154 centimorgan and between A226 and A287 to 4.189 centimorgans (Table 3). These distances are obviously too abnormal as one could not expect a locus to be 6.154 or 4.189 map units long.

## Discussion

Lys-5 mutants suffer from a high degree of sterility in crosses with other mutants. They also suffer from a very high degree of inviability of ascospores, for a mutation at this locus results in lethality of ascospores (Stadler, 1956; Ahmad et al. 1960). To achieve a successful cross between two mutants of poor fertility the mixing of a third strain was utilised by Beadle & Coonradt (1944) and Lein & Lein (1952). Stadler (1956) used it in achieving a cross between the lys-5 mutant asco and cytosine mutant. He inoculated ad-1 and asco having same mating type on unsuplemented crossing medium. When the heterocaryotic growth produced protoperithecia, he fertilized it with the cytosine strain having opposite mating type.

For improving fertility of lys-5 mutants in addition to the previously reported

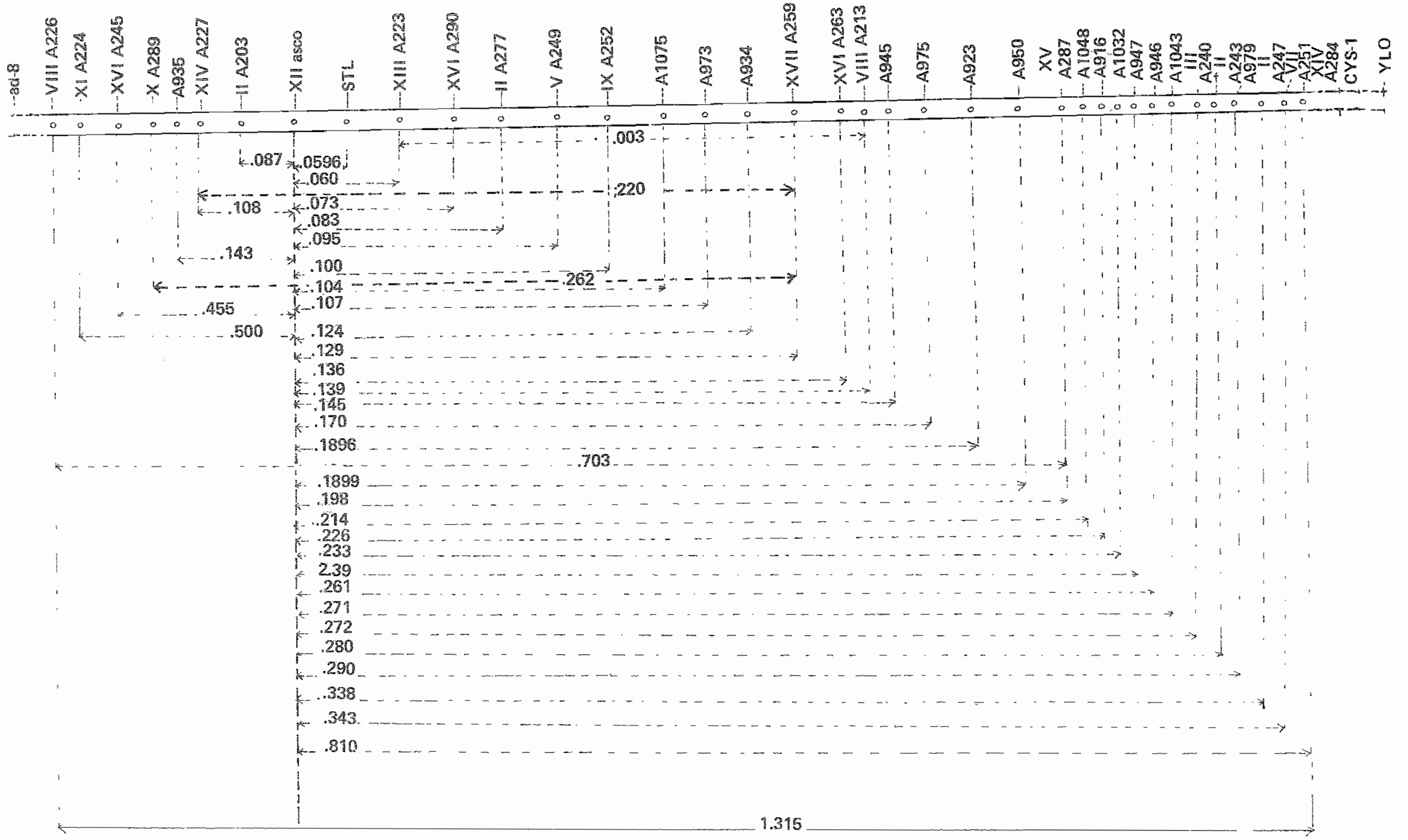

Fig. 1. Genetic map of lys-5 locus. The distances obtained directly from the experimental data have been inidicated by dotted lines. The length of the locus obtained by adding individual linear distances between the mutants has been indicated by a continuous line.

A 224? : Probable position. Roman numerals II-XVII on top of mutants give their complementation group.

techniques by Ahmad et al (1967) and Ahmad & Rahman (1969), back crossing of lys-5 mutants, of markers, as well as of double mutants with the parental wild type Emerson stocks has been found to be effective. Back crossing to parental wild type is, therefore, recommended as a successful means of achieving fertility between two poorly fertile or even sterile mutants.

Genetical studies showed that due to high inviability of lys-5 mutant spores, distances between different lys-5 alleles were abnormally high when they were calculated on the basis of viable progeny only. Thus distance between A226 and A287 was 4.189 centimorgans and between asco and A284 it was 6.154 centimorgans. This abnormal swelling up of distances followed from very high inviability of lys-5 mutant spores, since mutation at locus lys-5 gives ascospore lethal mutant strains. In order to overcome this difficulty distances between mutants were calculated on the basis of total ascospore progeny produced by their crosses.

Taking total ascospore progeny into account the genetic map length of locus lys-5 came to about 1.315. This map length is considerably larger than the map length of 0.2 units for pyrimidine-3 (Woodward, 1962), of 0.322 units for trp-3 (Ahmad et al, 1966) and of 0.338 units for pantothenic-2 (Case & Giles, 1960). Three isoleucine, valine, (iv), loci together have been reported, to occupy a segment of not more than four subunits (Wagner, Somers & Bergquist, 1960). Likewise Ahmad et al (1976b) have found the genetic map length of locus leu-2 to be about 1.0872 centimorgans. The map length of locus lys-5 compares favourably with the map lengths of iv and leu-2.

Ahmad et al (1980) have also studied interallelic complementation at locus lys-5. When the position of mutants belonging to different complementation groups is examined on the genetic map (Fig. 1) it is found that they are interspersed along the locus. Thus, of the mutants falling in complementation group II, A203 lies distal to asco at a distance of .087 centimorgan and A277, A243, and A247 lie proximal to asco at distances of .083, .280 and 0.338, resepctively. Similarly, of the three mutants belonging to complementation group XVI which have been mapped, A245 lies distal to asco at a distance of 0.455 centimorgan. While A290 and A263 lie proximal to asco at distances of 0.073 and 0.136 centimorgan. In between the different members of group II and the different members of group XVI lie members of other complementation groups as shown in Fig. 1. These findings seem to point out that a complementation group may comprise an assembly of mutants whose location sites have similar configurational effects on the three dimensional structure of the enzyme controlled by the locus.

## References

Ahmad, M., Md. Khalil, N.A. Khan and A. Mozamdar, 1964. Structural and functional complexity at the tryptophan-l locus in *Neurospora crassa*. Genetics, 49: 925-933.

Ahmad, M., Mahtābuddin Ahmad and A. Zaman. 1960. Differentiation within a gene. Proc. Fourth Pan Indian Ocean Sci. Assoc., Section B, 43-50.

Ahmad, M., B.U. Ahmed, A. Rahman and Md. M. Rahman. 1966. Studies on the organization of locus tryptophan-3 in *Neurospora crassa*. Proc. Pak. Acad. Sci., 3. 1-12.

Ahmad, M., A. Das, M.R. Khan and M.H. Huda. 967. Improving fertility in crosses of *Neurospora crassa* lys-5 mutants. Neurospora News Letter, 11. 19.

Ahmad, M., Md. K.U. Choudhry and S.M. Islam.1969. Complementation and recombination between indole utilizing tryptophan-3 mutants of *Neurospora crassa*. Heredity, **24**: 656-660.

Ahmad, M. and A. Rahman 1969: Utilization of mammalian sex hormones for improving fertility in crosses of *Neurospora crassa* lysine-5 mutants. Neurospora News Letter, **15**: 11.

Ahmad, M., A. Mozamdar, A. Baset. Md. Fayaz, A. Badrul, Md. A. Rahman and B.C. Shah. 1979. Studies on the organization of genes controlling lysine biosynthesis in *Neurospora crassa*. II. Organization of locus lysine 1. Pak. J. Bot. **11**: 21-32.

Ahmad, M., S. Haque, Md. Z. Hoque, Ghulam Murshed, Akbar Hussain, S.C. Majumder and Obaidur Rahman 1976). Studies on the organization of genes controlling leucine biosynthesis in *Neurospora crassa*. III. Organization of locus leucine-2 in *Neurospora crassa*. Proc. Pak Acad. Sci. (In press).

Ahmad, M., S. Haque, Shamsuddin, R.K. Masud, A. Baset and N. Huda. 1981. Studies on the organization of genes controlling lysine biosynthesis in *Neurospora crassa*. VI. Interallelic complementation at the locus lys-5. Pak. J. Bot. (In press).

Beadle, G.W. and V.D. Coonradt. 1944. Heterocaryosis in *Neurospora crassa*. Genetics, **25**: 291-308.

Case, M.E. and N.H. Giles 1960. Comparative complementation and genetic maps of the pan-2 locus in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S., **46**: 459-676.

Fincham, J.R.S. and P.R. Day 1971 Fungal Genetics. Third Edition, Blackwell.

Good, N. 1951. Lysine metabolism in *Neurospora*. Galif. Inst. of Technology. Ph.D. thesis.

Lein, J. and P.S. Lein 1952. Studies on a suppressor of non-allelic acetate-requiring mutant of *Neurospora*. Proc. Natl. Acad. Sci., U.S., **38**: 44-48.

Stadler, D.R. 1956. A map of linkage group VI of *Neurospora crassa*. Genetics, **41**: 528-543.

Suyama, Y., V.M. Woodward and A. Sarachek. 1958. Nutrition and fertility in *Neurospora crassa*. Microbial Genetics Bull., **14**: 29-30.

Vogel, H.J. 1956. A convenient growth medium for *Neurospora* (Medium N). Microbial Genetics Bull., 13: 42-43.

Wagner, R.P., C.C. Somers, and Bergquist. 1960. Gene structure and function in *Neurospora*. Proc. Natl. Acad. Sci. U.S., 46: 708-717.

Westergaard, M., and H.K. Mitchell. 1947. *Neurospora* V.A. Synthetic medium favouring sexual reproduction. Am. J. Bot., 34: 573-577.

Woodward, V.W. 1962. Complementation and recombination among pyr-3 heteroalleles of *Neurospora crassa*. Proc. Nat. Acad. Sci., 48: 348-356.