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

IV. Segregation patterns, maturity and viability of ascospores and conidiation of some lys-5 mutants.

MAJEED AHMAD AND S. HAQUE,

Institute of Biological Sciences, University of Islamabad

and

M.U. AHMAD, A. ZAMAN, M. MOHIUDDIN, Y.A. SAEED AND A. KHAIRUL.

Department of Botany The University, Dacca, Bangladesh.

Abstract

Studies on 39 U.V. induced lys-5 mutants have shown that a mutation at this locus not only results in a requirement for lysine but also retards the maturation of spores. The mutant spores lack pigment at a stage of development when the wild type spores have acquired it. Mutant spores are mostly inviable, the inviability of mutant acospores has been found to range from about 70 to 95%. Both the pigment and viability of ascospores generally improves with time.

In addition to the expected first and second division, segregation patterns of coloured and colourless spores, 98 different variations in the number and distribution of coloured and colurless spores have been seen. Increase in the concentration of lysine from 20 mg to 80 mg per 100 ml of the medium, has been found to be generally helpful in the induction as well as production of abundant conidia by mutants.

Introduction

Locus lys-5, in *Neurospora crassa* was discovered by Good (1951) as mutant strain 37402. It was named asco by Stadler (1956) as he found it to be an ascospore lethal mutant. On inducing lysine mutants in *N. crassa*, Ahmad *et al* (1960; 1977), came across the situation that out of 189 mutants 113 belonged to a single locus. This locus on linkage tests proved to be different to the four known lysine metabolism controlling loci in this fungus. Further search showed that it was not a new locus, but one already named as asco. Due to the involvement of this locus in lysine biosynthesis, Ahmad *et al* (1960) renamed it as lys-5 to restore it to its rightful place amongst loci controlling lysine metabolism in *N. crassa.*

As a large number of new lys-5 mutants were available, it was decided to study the effect of mutation at this locus on maturity and viability of ascospores and also to find the optimal concentration of lysine for inducing abundant conidiation in these mutants.

Materials and Methods

New lysine-5 mutants used were A 201, A202, A203, A207, A209, A213, A218, A223, A224, A226, A227, A230, A240, A242, A243, A245, A247, A248, A249, A252, A254, A256, A257, A259, A260, A261, A262, A263, A264, A268, A275, A277, A281, A284, A286, A287, A289, A290, A301, A301, = 39.

A916, A923, A924, A934, A935, A945, A946, A947, A950, A973, A975, A979, A1032, A1043, A1048, A1075 = 16. Lysine-5 mutants from Dr. Stadler: Asco (37402) a, DS. 6.85 lys a, STL-7-A.

Parental wild types Emerson (Em) A (5296) and Emerson (Em) a (5297). Mutants of linkage group VI tested and used in conducting these studies have been mentioned in Table 1.

Media and methods used were the same as followed by Ahmad *et al.* (1977).

Table 1. Mutants of linkage group VI tested and used.

Mutant	Mutant Symbol	Isolation number	Phenotype	Person who kindly sent it.
Ascospore	asco	37402	delayed ascospore maturation.	Dr. D.G. Catcheside and Dr. D.R. Stadler
Cytochrome	cyt	C117	slow growth, altered cytochrome system	Dr. D.R. Stadler
Adenine-E or Adenine-8	ad-8		requires adenine	,, ,, ,, ,,
Cysteine-2	cys-2	38401	requires cysteine	,, ,, ,, ,,
Cysteine-1	cys-1		requires cysteine	Dr. M.B. Mitchell
Yellow	y10	Y30539Y	Yellow conidia	,, ,, ,, ,,

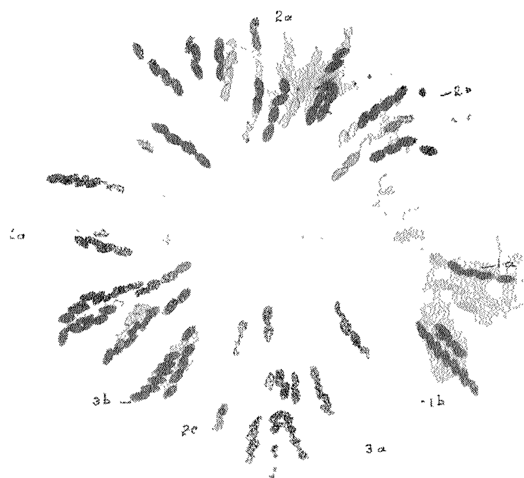

Fig. 1. Segregation of colourless (lys-5) and coloured (wild type) spores in crosses of lys-5 mutant A248a with EmA.

1a and 1b show the two types of 1st div. segregation.
2a,b,c and d show 4 types of 2nd div. segregations.
3a and b show two departures from 4 colourless:
4 coloured spores per ascus.

Results

1. *Effect of mutation at locus lys-5 on maturation of spores and departure from expected patterns of segregation of mutant and wild type spores.*

Nine new lys-5 mutants were crossed with the parental wild type strain EmA to study the effect of mutation at locus lys-5 as compared to wild type and to study the segregation patterns of the mutants and wild type spores. An incubation of the crosses at 25°C for 15 to 17 days was found most suitable for classifying colourless mutant and black wild type spores.

Majority of asci showed the presence of 4 colourless mutant spores and 4 black wild type spores which followed an arrangement distinguishable as first or second division segregation of the mutant and wild type alleles of locus lys-5 (Fig. 1). In addition it was found that many asci showed patterns of distribution of ascospores which could not be classified either as first or second division segregations. Leaving aside the asci which showed either all 8 colourless or all 8 black spores, there were 98 variations from the expected first and second division, segregation patterns (Table 2). All the variations were not realised in the crosses of each individual mutant with EmA. It was also seen that percentage of asci containing all black spores were significantly high in the cross of A 223 with EmA.

Table 2. Different exceptional patterns of segregation of colourless and black ascospores in crosses of 9 lysine-5 mutants with the parental wild-type, EmA.

Pattern of segregation in ascus	Proportion of black and white spores in ascus.	247a	248a	254a	256a	224a	281a	249a	301a	223a
7B+1W	7B.1W	3			6	1		3	17	1
1W+7B	1W.7B				2					
7W+1B	7W:1B			1		1		1	4	
1B+7W	1B:7W				1					
6B+2W	6B:2W	12		3	2	4			12	6
2W+6B	6B:2W	1		2	2	1		4	2	
6W+2B	6W.2B		3					3	2	
2B+6W	2B 6W		8	12	5	4	2	3	11	2
6B+1W+1B	7B:1W	2	2	1	1				4	
1B+1W+6B	7B:1W		1	1						
6W+1B+1W	1B:7W		1		1				1	
5B+3W	5B:3W			1	1		2	1	4	6
5W+3B	3B+5W				1					
3B1+5W	3B·5W					1	1	1	10	
5B+1W+2B	3B·1W								3	3
2B+1W+5B	7B·1W		1						1	2
5B+1W+1B+1W	6B:2W	3			2			3	2	1
1W+1B++1W+5B	6B·2W				1		1			
2B+5W+1B	3B 5W	1			1					
1W+2B+5W	2B.6W								2	1
4B+3W+1B	5B:3W			2					2	
1B+3W+4B	5B:3W			2	3					1
4B+2W+4B	6B:2W		1	2	3			1	10	1
2B+2W+4B	6B:2W	5			1	2		2	3	1
4B+2W+1B+1W	5B·3W	1	1		1	2	1	2		
1W+1B+2W+4B	5B 3W		1		1		2	1		1
4B+1W+3B	7B·1W	2	2	2	1		2	1	5	1
3B+1W+4B	7B:1W	1	2			1	1		1	
4B+1W+2B+1W	6B.2W	1						2	4	
1W+2B+1W+4B	6B·2W	1			1					
4B+1W+1B+2W	5B·3W	1		1		1		2		1
2W+1B+1W+4B	5B:3W				1			1		
4B+1W+1B+1W+1B	6B:2W	1	2		1				2	1
4W+3B+1W	3B.5W					2	5			
1W+3B+4W	3B:5W	2					2	1	4	
4W+2B+2W	2B:6W	4	3	4		1	6		3	
2W+2B+4W	2B 6W	3	5	9	2	5	3	3	9	1
4W+1B+3W	1B:7W					1	2			3
3W+1B+4W	1B+7W	1					1	1	1	
4W+1B+1W+1B+1W	2B+6W	1								1
1W+1B+1W+1B+4W	2B+6W	1								
3B+3W+2B	5B+3W	-	-	1	2				5	
2B+3W+3B	5B+3W	1	-					1	2	
3B+2W+3B	6B+2W	-			1				1	1
3B+2W+1B+2W	4B+4W								1	1

3B+1W+2B+2W	5B+3W	–	1		–	–	–	–	3	1
2W+2B+1W+3B	5B+3W	–	–	–		–	–	1	–	
3B+1W+1B+1W+2B	6B+2W	–	–	–	–	–	–	–		1
3B+1W+3B+1W	6B+2W	1	–			–			1	
3B+1W+2B+1W+1B	6B+2W	–	–	–	–	–	–	–	1	–
3W+3B+2W	3B+5W	–	–	–	–	–		–	2	2
2W+3B+3W	3B+5W	1	–		–	1	–			1
2B+5W+1B	3B+5W	–	–	1	–	–	–		–	–
2B+4W+1B+1W	3B+5W	–		–	–	–	–		3	–
1W+1B+4W+2B	3B+5W	–	–	–		1		–	–	1
2B+3W+1B+2W	3B+5W	–	–	–	–		–	–	2	
2B+3W+2B+1W	4B+4W	–	–	–	–	–		–	2	
2B+3W+1B+1W+1B	4B+4W	–	2	–	–	–	–	–	–	
2B+2W+3B+1W	5B+3W	1	–	–	–	1	–			–
1W+3B+2W+2B	5B+3W	–	–	–	1	–	–	1	1	–
2B+2W+1B+3W	3B+5W	–	–	–		–	–	1		
2B+2W+1B+1W+2B	5B+3W	–	–	–	–	–	–		1	–
2B+1W+1B+2W+2B	5B+3W	–	1	–	–	–	–	–	–	
2B+2W+2B+1W+1B	5B+3W	–	–	–	–	–	–	–	1	
2B+2W+1B+1W+1B+1W	4B+4W	–	–	–	–	–	–	–	1	–
2B+1W+3B+2W	3B+5W	–	–	–	–	–	–	1	–	
2W+3B+1W+2B	5B+3W	–	–	1	–	2	–		2	
2B+1W+2B+3W	4B+4W	–	–	–	–	–	–	1		–
2B+1W+1B+4W	3B+5W	–	3	–	1	–	–	–	2	
2B+1W+2B+1W+2B	6B+2W	–		–	–	–	–	–	1	
2B+1W+1B+1W+1B+1W+1B	5B+3W	–	–	–	–	–	–	–	1	
1B+1W+1B+1W+1B+1W+1B+1W	4B+4W	–	–	–	–	–	–	1	–	
2B+1W+1B+1W+2B+1W	5B+3W	1	–	–	–	–	–			–
2B+1W+1B+1W+1B+2W	4B+4W	–	–	–	–			–	2	–
2W+2B+3W+1B	3B+5W	–	–	–	–	–		–	1	–
1B+3W+2B+2W	3B+5W	–	–	–	–	1	–	–		
2W+2B+2W+1B+1W	3B+5W	–	–	–	–		1	–	3	
1W+1B+2W+2B+2W	3B+5W	1	–	–	1		–	1	–	
2W+2B+1W+1B+2W	3B+5W	–	–	–	–		–	–	1	
2W+2B+1W+1B+1W+1B	4B+4W	–	–	–	–	–	1	–	2	–
2W+5B+1W	5B+3W	1	–	–	–		–			
1W+5B+2W	5B+3W	–	1	–	–	–	–	1	1	1
2W+1B+5W	1B+7W	–	1	1		–	–	–	1	–
2W+1B+1W+3B+1W	4B+4W	1	–	–	–	–	–	–		–
1W+3B+1W+1B+2W	4B+4W	–	–	–	–	–		–	1	
2W+1B+4W+1B	2B+6W	–		–	–			–	1	–
1B+6W+1B	2B+6W	–	–	–	–	1	–			
1B+2W+5B	6B+2W	–	–	–	1		–			
1B+1W+4B+2W	5B+3W	1	–	1	–		1	–	1	–
1B+1W+2B++4W	3B+5W	1	1	–	1	2				
1W+6B+1W	6B+2W	1	–	–	–			–	3	1
1W+4B+3W	4B+4W		–	–	–	–	–	1		1
1W+4B+1W+2B	6B+2W	–	–	–	–	–			1	
1W+3B+2W+1B+1W	4B+4W			–	–	–	–	–		1
1W+3B+1W+2B+1W	5B+3W	–	–	–		–	–		–	1
1W+2B+2W+2B+1W	4B+4W			–	–	–	–		2	
1W+2B+1W+3B+1W	5B+3W	–	–		–	1		–		
1W+1B+1W+1B+3W+1B	3B+5W	–			–	–	–			1

In order to study the degree of lethality amongst strains in which a mutation had occured at locus lys-5,6 new lys-5 mutants were crossed with the parental wild type EmA. Seven to 11 asci from about 20 days old crosses were dissected in each case. The spores which proved viable were classified into mutant and wild type by testing each surviving single spore culture; 70 to 95% of the expected mutant progeny proved to be inviable (Table 3).

2. *Effect of period of incubation of the crosses on viability of mutant spores*

Since mutation at locus lys-5 delays the maturity of spores and affects the viability of spores it was decided to study the effect of increase in incubation time of crosses on maturity and viability of spores. Therefore, 9 lys-5 mutants were crossed with EmA and incubated at 25°C. Fifty to eighty single spore cultures were isolated at different periods of incubation ranging from about 20 to 76 days and the proporation of mutants was ascertained in each set of isolates.

In the majority of cases the frequency of the viable mutant spores amongst the progeny increased with the period of incubation of the cross (Table 4). For example in the cross A 223XEmA the frequency of mutants amongst 50 isolates was found to be zero at 30 days, 1 at 36 days, 3 at 46 days and 8 at 60 days incubation. However, in some cases no improvement in the recovery of viable mutant single spore cultures was found with the increase in the period of incubation. The cross A290XEmA yielded no viable mutant single spore culture when isolations were made after 30, 37 or 54 days. In one case, A226XEmA, one mutant spore was found amongst the 50 isolates of 30 days old cross but no mutant single spore culture was recovered from each of 50 isolates made after 50 and 56 days incubation of the same cross.

Examination of younger perithecia showed more asci with 8 colourless spores whereas of older perithecia showed more asci with 8 coloured spores. With the increase in time of incubation, proportion of dark coloured spores increased. These observations demonstrated that the mutant spores mature and synthesise pigment much more slowly as compared to the wild type spores.

Isolation of lighter black or grey coloured growing spores from lysine supplemented media increased the chances of recovery of lys-5 mutant isolates from their crosses with Em. This finding helped later in preparing isolates of individual mutants from their crosses with Em.

3. *Determination of the amount of lysine required by lysine-5 mutants for maximum conidiation*

For the study of interallelic complementation through heterocaryon formation and also for the study of genetic fine structure of a locus it is helpful if the mutants in question are provided with optimal concentration of lysine, for the production of abundant conidia by lys-5 mutants within 72 hours.

Table 3. High lethality amongst lys-5 mutant spores, 70 to 95% of which proved inviable.

Cross	No. of asci dissected	ASCOSPORES								Total
		Inviable		Wild			Mutant			
		Expected	Observed	Expected	Observed	Percent inviable	Expected	Observed	Percent inviable	
A248 x EmA	8	0	31	32	30	6.25	32	3	90.64	64
A202 x EmA	10	0	55	40	23	42.05	40	2	95.00	80
A289 x EmA	7	0	41	28	13	53.57	28	2	92.87	56
A201 x EmA	11	0	56	44	25	43.18	44	7	84.09	88
A245 x EmA	7	0	27	28	22	22.43	28	7	75.00	56
A223 x EmA	10	0	41	40	27	32.5	40	12	70.00	80

Table 4. Improvement in viability of mutant spores with increase in period of their incubation.

Cross	Age of spores in days	Number of spores isolated and tested	Number of mutants amongst isolates.
A201 x EmA	33	50	2
	45	50	5
A202 x EmA	32	80	0
	42	50	0
	50	50	0
A207 x EmA	20	70	0
	38	70	4
	50	50	7
A213 x EmA	27	50	2
	46	50	7
A223 x EmA	30	50	0
	36	50	1
	46	50	3
	60	50	8
A226 x EmA	30	50	1
	50	50	-
	56	50	
A227 x EmA	39	50	0
	49	50	5
A230 x EmA	25	80	0
	32	80	0
	37	80	0
	45	80	0
A259 x EmA	27	80	0
	35	80	1
	76	50	2
A268 x EmA	28	50	-
	34	50	2
A290 x EmA	30	50	0
	37	50	0
	54	50	0

Thirtynine lys-5 mutants were inoculated in duplicate in tubes containing Vogel's minimal medium (Vogel, 1956) supplemented with 20, 40, 60 and 80 mg lysine per 100 ml. The tubes were incubated at 25°C for 72 hours. Conidiation of the strains at 25°C improved as the concentration of lysine was increased from 20 mg to 80 mg (Table 5). This can be seen in two ways. First of all, number of strains which did not form conidia declined with the increase in concentration. Thus, 25 lys-5 mutants out of 39 failed to form conidia at 20 mg, 4 failed to form conidia at 40 mg, 1 failed to form conidia at 60 mg and none failed to form conidia at 80 mg lysine per 100 ml. Secondly, it is seen that 22 out of 39 strains formed very good conidia when grown on medium with 80 mg and only 1 strain formed very good conidia with a supplement of 20 mg lysine per 100 ml.

Discussion

Good (1951) observed that lysine requiring mutant, 37402, also has delayed ascospore maturation. Stadler (1956) reported that lysine requirement and delayed ascospore maturation in this mutant could not be separated by crossing it with wild type. The acsi which contained any mature black spores had two pairs of black wild type and two pairs of colourless mutant spores. If these crosses were allowed to age, occasionally a fifth mature spore could be found in an ascus; when such a spore was grown it always proved to be mutant for lysine as well as ascospore maturation.

These characteristics of the mutant spores as well as segregation of 4 colourless mutant spores to 4 black coloured wild type spores per ascus in the crosses of lys-5 mutant with wild type reported by Stadler (1956), were observed during our studies as well. However, in addition to these regular expected patterns, all kinds of variations in the number and distribution of colourless white and black spores in asci were seen. Leaving aside the cases where an ascus contained all 8 colourless white spores or all 8 coloured black spores, there were 98 additional patterns of segregation of white and black spores in asci (Table 2). The causes of variations in the number and distribution of colourless and coloured spores in indiviual asci are not understood except that some variations could be explained thrugh overlapping of spindles during nuclear divisions.

Furthermore, not only the slow maturation of lys-5 mutant spores but their lethality was confirmed. As shown in Table 3, the lethality of mutant spores ranged from about 70 to 95 percent of the mutant progeny. Mutation at the locus thus results in partial and not complete lethality of ascospores.

Studies on the effect of period of incubation of the crosses on viability of ascospores have shown that their viability increases with time in most cases. Out of crosses of 11 mutants with Em, 7 showed no recovery of mutant spores amongst isolates varying in age and one showed the presence of one mutant spore in 30 days old spores but none amongst 50 and 60 days old ascospores. These results can be best explained on the hypothesis that viability of mutant spores increases with time in general, however, the proportion of viable mutant spores is so low that none may be picked up in a sample of 50 to 80 spores.

Table 5. Stimulation of conidia formation with increase in the concentration of lysine in the medium.

Lysine per 100 ml	DESIGNATION OF LYSINE - 5 MUTANTS					
	FORMATION OF CONIDIA					
	None	Very Poor	Poor	Fair	Good	Very Good
20 mg	A203,A207,A218,A224,A226,A227, A240,A242,A247,A249,A254,A257, A259,A260,A261,A262,A263,A264, A268,A277,A281,A284,A286,A289, A301 = 25	A223,A252,A290 = 3	A275 = 1	A209,A213,A230,A245 = 4	A201,A243,A248 A256,A287 = 5	A202 =1
40 mg	A227,A249,A286,A289 = 4	A224,A262,A268, A277,A301 = 5	A203,A207,A218,A213 A240,A242,A247,A252, A259,A260,A261,A264, A275,A281,A284,A290 = 16	A201,A209,A213 A226,A230,A245, A254,A257,A263 = 9	A243,A248,A256, A287 = 4	A202 = 1
60 mg	A89 = 1	–	A224 = 1	A201,A226,A227, A249,A259,A263, A268,A275,A277, A281,A284 = 11	A218,A223,A240 A242,A243,A245, A247,A257,A260, A261,A262,A264, A286,A287,A290, A301 = 16	A202,A203,A207, A209,A213,A230, A248,A252,A254, A256 = 10
80 mg	–	-	–	A201,A224 = 2	A207,A209,A226, A227,A230,A243, A249,A259,A262, A268,A275,A277, A281,A284,A286, = 15	A202,A203,A213,A218, A223,A240,A245,A247, A248,A252,A254,A256, A257,A260,A261,A263, A264,A287,A289,A290, A301 = 21

Experiments on the effect of lysine concentration on the formation of conidia by mutant strains have demonstrated that the capacity of mutant strains to form conidia generally increases with the increase in the lysine concentration from 20 mg to 80 mg per 100 ml (Table 5). Thus 25 out of 39 mutants failed to form conidia at 25°C when the V.M. was supplemented with 20 mg lysine per 100 ml but when the medium was supplemented with 80 mg lysine all the 39 strains formed condia. It was also found that 22 out of 39 strains formed abundant conidia when V.M. was supplemented with 80 mg lysine per 100 ml while only 1 strain produced abundant conidia when V.M. was supplemented with 20 or 40 mg lysine per 100 ml.

Additionally it was noticed that individual mutants in a few cases differed from the general mass of mutants. Thus mutant A 202 showed formation of abundant conidia at all the four concentrations of 20 mg, 40 mg, 60 mg, and 80 mg lysine per 100 ml of the medium.

References

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

Ahmad, M.,A. Mozmadar, A. Baset, M. Fayyaz, A. Badrul, A. Rahman, and B.C. Saha. 1977. Studies on the organization of genes controlling lysine bio-synthesis in *Neurospora crassa.* I. Isolation and characterization of lysine mutants belonging to 4 loci. Pak. J. Bot., 9: 99-106.

Good, N. 1951. Lysine metabolism in *Neurospora:* Calif. Inst. of Technology, Ph.D. Thesis.

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

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