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Genetic Linkage of Biochemical Mutants of *Neurospora crassa* Induced with Ultraviolet Radiation

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Abstract: *Neurospora crassa is a haploid organism and its haploid chromosome number is seven. It has also seven linkage groups. 2 biochemical mutants such as leu 299a and trp 101a of N. crassa were obtained by mutation with UV radiation and were used for the study of genetic linkage. Mutant leucine (leu 299a) was found to be linked with leu-3(R 156)A of linkage group I and tryptophan (trp 101a) was found to be linked with trp-2 (75001)A of linkage group VI. Genetic map of leu 299a with the marker leu-3(R 156)A gave a distance of 16.16 CM and distance of trp 101a mutant with the marker trp-2 (75001)A is 18.63 CM.*
Keywords: *Linkage, Mutants, Neurospora crassa, UV radiation*

I. Introduction

At the initial stage of twentieth century, Bateson and Punnett discovered Genetic linkage. Sturtevant used this phenomenon to show that genes are arranged linearly on the chromosome [1]. Linkage occurs when genetic loci or alleles for genes are inherited jointly. Genetic loci on the same chromosome are physically connected and tend to segregate together during meiosis and are thus genetically linked. Due to independent assortment of chromosomes during meiosis alleles for genes on different chromosomes are usually not linked. Sturtevant proposed that the greater the distance between linked genes, the greater the chance that non-sister chromatids would cross over in the region between the genes. A genetic map is constructed based on the frequencies of recombination between markers during crossing over of homologous chromosomes. When the frequency of recombination between the markers is low, the physical distance between them is also low and vice-versa.

In *Neurospora crassa,* genetic linkage was first demonstrated by Lindegren [2]. He published linkage maps of linkage group I and II subsequently [3-4]. Haploid chromosome number of *N. crassa* is seven and seven linkage groups have been identified. Each chromosome of *N. crassa* corresponds to a linkage group. In this study, an attempt was taken to produce biochemical mutants of *N. crassa* Ema using UV radiation of 254 nm. The mutants were then used for study of genetic linkage.

II. Materials and Methods

2.1 Induction of mutation: UV radiation of 254 nm wave length was used as physical mutagen for induction of mutation in *N. crassa* Ema using filtration enrichment method [5]. This method is very important for induction of mutation in *N. crassa* for biochemical mutants. The wild type strain of *N. crassa* (Ema) was obtained from Microbial Genetics Stock, Department of Botany, University of Dhaka. UV ray was passed through a small Petri dish containing suspension of fresh Ema conidia at a distance of 15 cm with two different treatment times (45s and 2m). Vogel's minimal medium was used for culturing *N. crassa* in the test tube [6]. Sorbose minimal medium (SM) was used for single colony isolation. Westergaard crossing medium was used for crossing [7].

2.2 Crossing: *N. crassa* strains are heterothallic with two mating types 'A' and 'a'. Sexual fruiting bodies called perithecia are produced only when two opposite mating types are brought together on a suitable crossing media [8-9]. Before the crosses, mating type and fertility of the mutants were determined. For determination of mating type, the mutants were crossed with both the two wild opposite mating types (Ema & EmA). Crosses were made in 1"×6" tubes. Each tube carried 5 ml of Westergaard's crossing medium and a folded filter paper strip. One strain was inoculated into one trough of the filter paper and other strain was inoculated into the other trough of the folded filter paper. The crosses were then incubated at 25^0C. Where the mutants were fertile, perithecia were formed in a week. Normally the appearance of perithecia was found in 4-5 days, very rarely after 10-12 days. The perithecia generally liberate spores in the second week after making the cross but occasionally in the third week.

2.3 Detection of linkage: For detection of linkage, the mutants were crossed with seven markers of seven linkage groups in Westergaard crossing medium with required supplements. After cross, 4 types of progeny were expected. These are: i) mutant, ii) marker, iii) mutant + marker and iv) wild.

The linkage was studied by analyzing the percentage of recombinant and parental types.

III. Results and Discussion

To obtain biochemical mutants, the UV ray induced isolates were tested for growth on SM plates. Isolates those did not grow on SM plates, were further tested on SM supplemented plates separately with adenine, arginine, histidine, leucine, lysine and tryptophan to classify them into biochemical mutants.

UV ray induced biochemical mutants (Table1) were designated as tryptophan (trp 101) and leucine (leu 299). These 2 biochemical mutants of *N. crassa* induced with UV radiation were used for linkage study.

Table 1. UV ray induced biochemical mutants of *N. crassa*

Sl.	Culture no.	SM + adenine	SM + arginine	SM + histidine	SM + lysine	SM + leucine	SM + tryptophan	Inference of mutants
1	101	○	○	○	○	○	●	tryptophan
2	299	○	○	○	○	●	○	leucine

SM= Sorbose Minimal Medium
●= Growth ○= No growth

All the mutants formed perithecia crossing with EmA (Table2). So it is revealed that mating type of all the mutants is 'a' and is not changed due to mutation. Besides, both mutants showed fertility with maximum of the markers of seven linkage groups by formation of perithecia and spreading of spores which are essential for linkage study.

Table 2: Mating type of mutants of *N. crassa*

Sl.	Designation of the cross	Whether perithecia formed (Yes/No)	Days of initiation of perithecia	Frequency of perithecia	size of perithecia	Spore shedding days	Fertility	Mating type
1	leu 299 ×EmA leu 299 ×Ema	Yes No	13	Many	Medium	21	Fertile	a
2	trp101 ×EmA trp101 ×Ema	Yes No	12	Many	Large	18	Fertile	a

After successful crosses, leucine (leu 299a) was found to be linked with leu-3(R 156)A of linkage group I and tryptophan (trp 101a) was found to be linked with trp-2 (75001)A of linkage group VI (Table 3 & Table 4). But Rahim [10] and Akter [11] reported different linkage groups of some tryptophan and leucine mutants. The reason is that same type of mutant can be obtained by mutation in different chromosome of same strain.

Table3: Determination of Linkage of leucine (leu 299a) mutant

Sl.	Marker used	Linkage group	Name of the cross	Total isolates	Progenies and their number	No. & % of mutant and wild	Inference of linkage
1	leu-3 (R156) A	I	leu 299a × leu-3A	99	leu =45 leu-3 =38 leu+leu =14 wild =02	mutant=97 (97.98%) wild =02 (**2.02%**)	**Linked with leu-3 of linkage group I**
2	arg-5 (27947)A	II	leu 299a × arg-5A	95	leu =28 arg-5 =29 leu+arg =14 wild =24	mutant =71 (74.74%) wild =24 (25.26.%)	Not linked
3	ade-2 (STL2)A	III	leu 299a × ade-2A	88	leu =23 ade-2 =27 leu+ade=13 wild =25	mutant =63 (71.59%) wild =25 (28.41%)	Not linked
4	trp-4 (Y2198)A	IV	leu 299a × trp-4A	93	leu =19 trp -4 =25 leu+trp =16 wild =33	mutant =60 (64.52%) wild =33 (35.48%)	Not linked
5	trp-5 (A420)A	V	leu 299a × trp-5A	82	leu =22 trp -5 =25 leu+trp =13 wild =22	mutant =60 (73.17%) wild =22 (26.83%)	Not linked
6	trp-2 (75001)A	VI	leu 299a × trp-2A	79	leu =26 trp -2 =18 leu+trp =12 wild =23	mutant =56 (70.89%) wild =23 (29.11%)	Not linked

7	arg-10 (B317) A	VII	leu 299a × arg-10A	104	leu =31 arg-10 =18 leu+arg=16 wild =39	mutant =65 (62.5%) wild =39 (37.5%)	Not linked

From this data, value of probability (serial no.1) is higher than tabular chi-square value at 5% (.05) and is significant. So, the data is not acceptable for free segregation in the ratio 3:1 which is hampered due to linkage between mutant leu 299a & marker leu-3 (R156)A.

Table 4: Determination of Linkage of tryptophan (trp 101a) mutant

Sl.	Marker used	Linkage group	Name of the cross	Total isolates	Progenies and their number	No. & % of mutant and wild	Inference of linkage
1	leu-3 (R156) A	I	trp 101a × leu-3A	87	trp =25 leu-3 =22 trp+leu =16 wild =24	mutant =63 (72.41%) wild =24 (27.59%)	Not linked
2	arg-5 (27947)A	II	trp 101a × arg-5A	91	trp =30 arg-5 =20 trp+arg =14 wild =27	mutant =64 (70.33%) wild =27 (29.67%)	Not linked
3	ade-2 (STL2)A	III	trp 101a × ade-2A	103	trp =23 ade-2 =26 trp+ade =12 wild =32	mutant =71 (68.93%) wild =32 (31.07%)	Not linked
4	trp-4 (Y2198)A	IV	trp 101a × trp-4A	99	trp =29 trp -4 =25 trp+trp =16 wild =29	mutant =70 (70.71%) wild =29 (29.29%)	Not linked
5	trp-5 (A420)A	V	trp 101a × trp-5A	78	trp =22 trp -5 =20 trp+trp =13 wild =23	mutant =55 (70.51%) wild =23 (29.49%)	Not linked
6	trp-2 (75001)A	VI	trp 101a × trp-2A	102	trp =36 trp -2 =47 trp+trp =15 wild =4	mutant =98 (96.08%) wild =4 (**3.92%**)	**Linked with trp-2 of linkage group VI**
7	arg-10 (B317) A	VII	trp 101a × arg-10A	82	trp =31 arg-10 =16 trp+arg=14 wild =21	mutant =61 (74.39%) wild =21 (25.61%)	Not linked

From this data, value of probability (serial no.6) is higher than tabular chi-square value at 5% (.05) and is significant. So, the data is not acceptable for free segregation in the ratio 3:1 which is hampered due to linkage between mutant trp 101a & marker trp-2 (75001)A.

Genetic map of the mutants with their marker was also constructed. By working out the number of recombinants it is possible to obtain a measure for the distance between the genes. This distance is called a genetic map unit or a centimorgan (CM) and is defined as the distance between genes for which one product of meiosis in 100 is recombinant.

Genetic map of leu 299a with the marker leu-3(R 156)A of linkage group I gave a distance of 16.16 CM (Fig. 1). Genetic map of trp 101a mutant with the marker trp-2 (75001)A gave a distance of 18.63 CM (Fig 2).

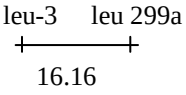

Fig 1. Genetic map of leu 299a auxotroph

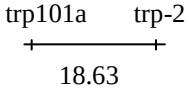

Fig 2. Genetic map of trp 101a auxotroph

IV. Conclusion

The present study revealed that UV radiation is a suitable mutagen to produce biochemical mutants in *N. crassa.* After mutation in *N. crassa* using UV radiation, biochemical mutants of *N. crassa* were successfully obtained. Genetic linkage of two biochemical mutants is also studied and it confirmed that it is the exception of Mendel's laws. To understand whether the genes are linked or independently assort from parents to offspring in microorganism, plant or animal; linkage study would be a helpful tool for such research.

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