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Characterization of the new osmotic mutants (*os*) which originated during genetic transformation in *Neurospora crassa*

By N. C. MISHRA

Genetics Laboratory, Department of Biology, University of South Carolina, Columbia, S. C. 29208

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SUMMARY

Inositol independent (*inl*$^+$) strains were obtained either as transformants following treatment of the inositol requiring (*inl*) strains of *Neurospora crassa* with the wild-type DNA or as revertants without any DNA treatment. A significant number of the inositol-independent transformants were also found to have acquired additional mutations called osmotics (*os*) which made them unable to grow on 1 M-NaCl medium. None of the inositol-independent revertants were found to possess such osmotic mutations and their growth remained unaffected by the presence of NaCl. Many of the osmotic mutants described here were found to be new alleles of the previously known *os–1* mutation on the linkage group I of *Neurospora crassa*. The remainder were found to map at two new genetic loci designated as *os-6* and *os-7*; these loci were found to be closely linked to *os–1*. Among the new osmotic mutants only *os-1* and *os-6* mutants showed intragenic complementation.The mechanism of DNA-induced mutation during transformation is discussed.

1. INTRODUCTION

Significant increases in the frequency of spontaneous mutations have been reported to occur in bacteria and in blue-green algae following genetic transformation or phage infection (Yoshikawa, 1966; Herdman, 1973; Taylor, 1966). Here we describe similar occurrence of mutation following genetic transformation in *Neurospora crassa*. We have recently described the transformation of an inositol requiring mutant (*inl*) of *Neurospora* into the inositol independent (*inl*$^+$) strain by treatment of the mutant strain with a wild-type DNA (*inl*$^+$) preparation (Mishra & Tatum, 1973; Mishra, Szabo & Tatum, 1973). During the transformation study, new mutations were found to appear in the transformed strain which made them unable to grow on 1 M-NaCl medium. Such osmotic mutations have been described earlier in *Neurospora* (Perkins, 1959; Emerson, 1963; Garnjobst & Tatum, 1967; Mays, 1969). In this paper, we describe the occurrence and characterization of additional osmotic mutants of *Neurospora* which appeared following genetic transformation.

2. MATERIALS AND METHODS

(i) *Strains*

The wild-type strains (RL3-8A, RL21a) and the osmotic mutant strains *os-1* (R2473 A/a, B135A), *os-2* (ALS10A), *os-3* (S-1A), *os-4* (L-5) and *os-5* (WM 216A) were used in the present study. These mutants are unable to grow in the presence of 1 M-NaCl; their growth characteristics and linkage relationship have been described previously (Perkins, 1959; Hamilton & Calvet, 1964; Mays, 1969). Twenty-two new osmotic mutants were also used in this study; most of them are of independent origin obtained from separate experiments involving DNA treatment as described below. However, a number of mutants (171-1, 171-3, 171-4 and 171-7) has a common origin from a single experiment; also, the strains 56-4 and 56-6 have originated together from another common experiment. These osmotic mutants have been divided into three groups (group I, group II and group III) on the basis of their inferred genotypes (*os-1*, *os-6* and *os-7* respectively) as discussed later in this paper. The mutants characterized here included group I (1, 2, 4, 8, 9, 27, 34, 171-1, 171-4, 185, 185-1 and 217); group II (14-3, 56-4, 56-6, 56-B-1, 171-3, 171-7 and 187) and group III (m-3, 17-5, 183, 185 and 185-3). The other strains used were 89601 (an inositol requiring (*inl*) mutant) and R2506-6-1 carrying mutations for colonial morphology (*rg*) and for inositol requirement (*inl*); these strains have been described elsewhere (Garnjobst & Tatum, 1967). All *Neurospora* strains except those genetically characterized during the present study were obtained from the collection of the Rockefeller University; all strains were maintained on appropriate medium as described elsewhere (Mishra, 1971).

(ii) *DNA preparation, treatment and isolation of the new osmotic mutants*

For transformation studies, the allo-DNA (inl^+) was prepared from the wild-type strain (RL3-8A) and iso-DNA (*inl*) from the mutant strain (R2506-6-1) by previously described methods (Marmur, 1961). A growing culture of the recipient strain (*rg*, *inl*, *a*) was treated with DNA (50 μg/ml). After 48 h of incubation the treated cultures were plated on minimal agar plates and the growing colonies were isolated as inositol-independent (inl^+) transformants. The inositol-independent (inl^+) revertants were similarly obtained by plating the recipient culture without any DNA treatment. The inl^+ strains (transformants or revertants) thus obtained were then examined for their ability (or inability) to grow on 1 M-NaCl medium; those unable to grow on such a medium were scored as osmotic (*os*) mutants. The new osmotic mutants were obtained in the wild type (rg^+) background by additional back crosses. The osmotic mutants were also identified by visual inspection; the mutants were found to have pale sticky conidia which is characteristic of the previously known osmotic mutants (Mays, 1969).

(iii) *Genetic analysis*

(*a*) *Genetic crosses*

The appropriate crosses between the wild-type and the mutant strains or between the two specific mutant strains were performed by analysis of random spores or individual asci as described earlier (Mishra & Threlkeld, 1967). A large sample of ascospores from a cross between two osmotic strains was analysed as described earlier (Giles, 1951). An aliquot of the heat-shocked (30 min at 60 °C) spores was added to the liquefied sorbose agar medium maintained at 48 °C. This ascospore suspension was then distributed into sterile Petri plates and incubated at 25 °C for 2–3 days. Afterwards, the liquefied agar medium containing 1 M-NaCl was overlayered on the top of growing colonies in each plate. These plates were further incubated for an additional 72 h and the growing colonies were scored on the basis of their growth pattern; those growing profusely with pink conidia were scored as the recombinant wild-type progeny whereas those having restricted growth were scored as mutant progeny (parental and the recombinant double mutants).

The map distance between the osmotic mutants was expressed as the percentage recombination between the loci involved. The recombination percentage was estimated as

$$\frac{2 \times (\text{no. of wild type})}{(\text{total no. of progeny})} \times 100.$$

This method (see also Mays, 1969) was adopted since the parental single mutant could not be distinguished from the recombinant double mutant among the progeny of a particular cross involving two mutant strains.

(*b*) *Complementation*

Complementation analysis was performed by heterokaryon test on a restrictive medium containing 1 M-NaCl. The pair-wise combinations showing growth comparable to that of the wild-type strain were scored as complementing mutants; whereas, those combinations which showed restricted growth or no growth on the NaCl medium were scored as non-complementing mutant combinations.

(*c*) *Conidial analysis*

Heterokaryosis at *os* locus was examined by plating a conidial suspension on NaCl medium as described by Mays (1969). In such tests, the pseudowild-type strain present was found to yield both the wild-type and mutant colonies as a result of the breakdown of the pseudowild type (Pittenger, 1964); whereas a true wild-type strain was found to yield only wild-type colonies.

3. RESULTS

(i) *Isolation of mutants and effect of DNA on their production*

Initially the osmotic mutants were identified by their pale sticky conidia among the progeny of a cross between the transformed and wild-type strains. Since none of the strains involved in transformation experiments were osmotic, these osmotic

mutations were assumed to have arisen as a result of DNA treatment. A systematic analysis of all the transformed strains was therefore undertaken in order to establish the relation of DNA treatment with the production of new mutations in the transformed strains. The data presented in Table 1 show that a significant number of the transformed strains were unable to grow on NaCl medium and, therefore, were carrying *os* mutations. In all 187 transformed strains were examined for their ability to grow on NaCl medium and 43 of them were found to be sensitive to the presence of NaCl in growth medium. As a control, the *inl*+ revertants

Table 1. *The occurrence of osmotic mutation* (os) *among the DNA induced transformants in* Neurospora crassa

Kinds of treatment	Total no. of *inl*+ transformants or revertants	Growth on NaCl medium (No. of colonies)		Frequency of *os* colonies
		Wild type (*os*+)	Osmotic (*os*)	
(1) Allo-DNA (18)*	187†	144	43	23%
(2) Control (without any DNA) (40)*	27†	27	0	0

* No. of experiments in parentheses

† Transformation frequency at *inl* locus was 1 per million whereas the reversion frequency in control cultures were 0·03 per million. The transformant or revertant colonies selected on a minimal medium by plating the recipient cultures with or without DNA treatment were tested for their ability to grow on the 1 M-NaCl medium; those able to grow were scored as *os*+; whereas those unable to grow on such a medium were scored as *os* (mutant strain.)

obtained without any DNA treatment were examined for their ability to grow on NaCl medium. All of the 27 such revertant strains were found to grow on the NaCl medium and were therefore of the same genotype as the wild-type strain (i.e. *os*+). In another control during our transformation experiments, the recipient culture was treated with its own DNA (i.e. iso-DNA); of 7 *inl*+ revertants obtained in this experiment only one was found to be restricted in its growth on the NaCl medium, indicating that this was carrying an *os* mutation. Thus among the different *inl*+ strains obtained after DNA treatment, a significant number were found to be osmotically sensitive whereas in case of the *inl*+ revertants (*obtained without any DNA treatment*) none were found to be sensitive to NaCl. These data clearly suggest that osmotic mutations (*os*) resulted from DNA treatment.

Growth characteristics of the new osmotic strains

The wild-type strains grows equally well on the medium with or without NaCl and shows a spreading filamentous morphology. The new osmotic mutants described in this paper were, however, found to show a restricted semi-colonial or colonial type of growth (see Garnjobst & Tatum, 1967) on the NaCl medium (see Fig. 1). This is in contrast to the full growth of the wild-type strain and to the complete inability of the previous osmotic mutants to grow on NaCl medium.

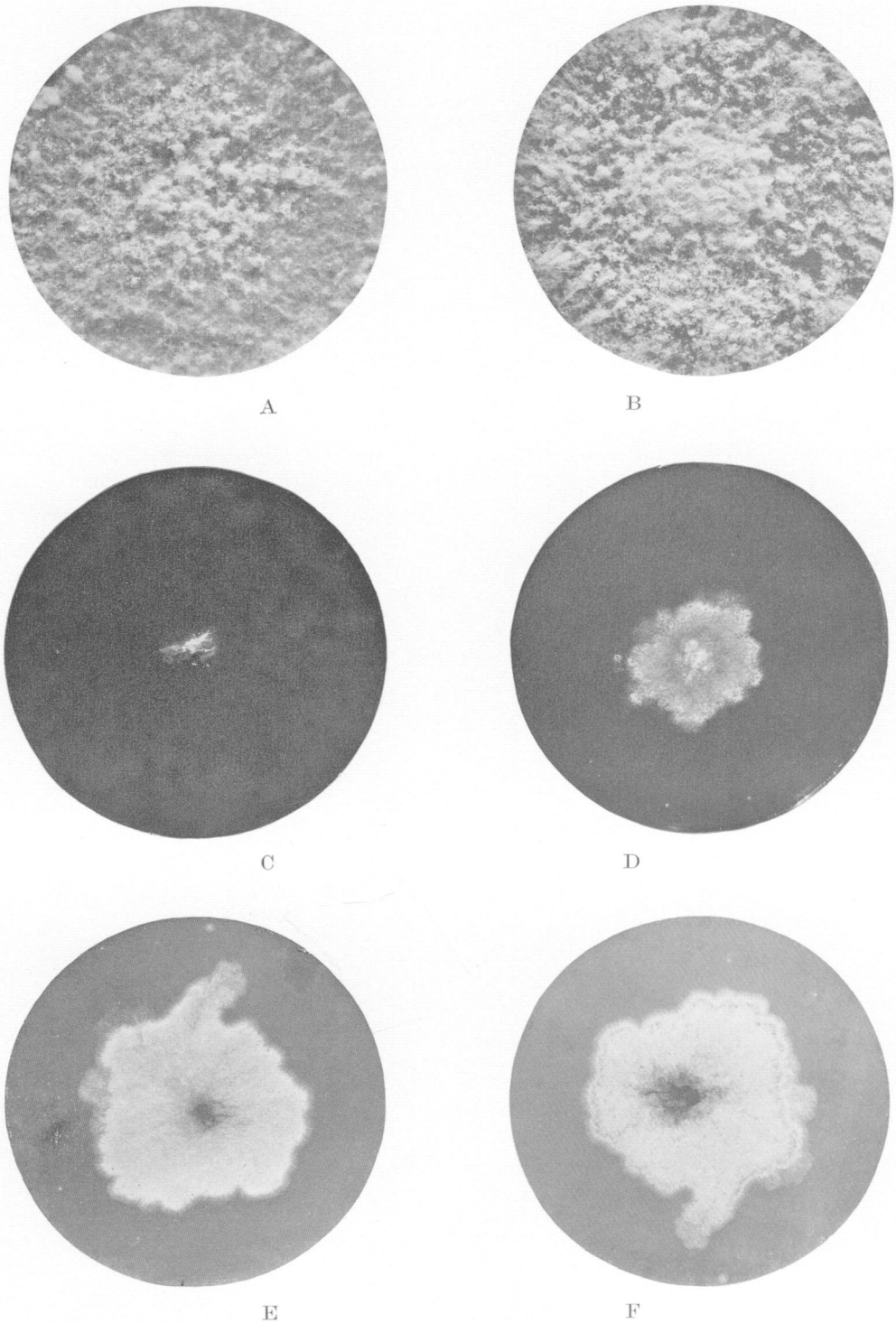

Fig. 1. Growth pattern of the different *Neurospora* strains on the minimal and the NaCl media. (A) Growth of the wild-type strain on 1 M-NaCl medium. (B) and (C) Growth of the *os-1* (R2473) on the minimal and the NaCl media respectively. (D), (E) and (F) Growth of new *os-1*, *os-6* and *os-7* mutants on the NaCl medium respectively.

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Furthermore, the growth of the osmotic mutants was found to be adversely affected by increasing the concentration of sugar in the medium. Their growth was severely reduced in 0·2 M sucrose medium; mutant cells growing in 0·4 M sucrose medium were very fragile and a great majority of them existed as protoplasts. However, such an increase in sucrose concentration had no adverse effect on the growth characteristics of the wild-type strain; the latter showed a full filamentous type of growth at the various sucrose concentrations. The new osmotic mutants were also examined for their ability to grow on galactose medium, since certain osmotic mutants have been recently reported to grow better on galactose medium than the wild-type strain (Rand, 1975). In general, all the osmotic strains (171-1, 171-4, 171-7, 56-6, 14-3 and m-3) showed better growth on galactose medium than the wild-type strain.

Genetic analyses

Twenty-two new osmotic mutants (*os*) were crossed to the known wild-type strain (os^+) in order to determine the mode of their inheritance. The progeny obtained by tetrad analysis were examined for their wild-type and mutant phenotypes by their growth pattern on the NaCl medium. Among the progeny of these crosses the two phenotypes were found to occur in the Mendelian ratio of 1:1. Furthermore, the different growth characteristics of the osmotic strain (see above) were found to be inherited together by the mutant progeny in such crosses. All crosses (except one involving mutant strain 185) yielded asci each with 4 wild-type and 4 mutant ascospores, indicating that all these mutant strains were carrying a single mutation. However, the cross between strains 185 × wild type yielded three (PD, NPD and T) types of asci which suggested that the strain 185, as isolated originally after the transformation, was a double mutant for the osmotic loci. One of the progeny (185-1) of the strain 185 contained a mutant which was allelic to R2473 (*os-1*) whereas another of the progeny (185-3) contained a mutant at a new locus, now designated as *os-7*. The computation of the data from tetrad analysis for the cross 185 × wild type showing 10 PD, 1 NPD and 1 T type asci suggest that in the double mutant strain 185 the two loci (*os-1* and *os-7*) are linked.

Allelism and linkage relationship among the osmotic mutants

Appropriate genetic crosses were constructed to test for the allelism among the new osmotic mutants and also to determine their allelism to previously described osmotic mutants. Results of allelic crosses are presented below:

os-1 × *os-1*. A number of group I mutants (nos. 1, 3, 4, 8, 9, 27, 34, 171-4, 185-1 and 217) were crossed to the previously known *os-1* mutant (R2473); approximately 10000 spores were analysed from each of these crosses and all of them were found to have phenotype characteristics of the two mutant parents involved in such a cross. No wild-type recombinants progeny were obtained from these crosses suggesting that these new osmotic strains were allelic to the previously known *os-1* mutant (R2473). Other crosses within group I (such as 171-1 × 171-4, 171-1 × 9 and 171-4 × 185-1) also yielded only mutant progeny. However, a particular cross

(R2473 × 171-1) was found to produce 2 wild-type progeny out of 10000 analysed; these were presumably the products of an intragenic recombination as discussed later in this paper. These data suggest that the group I mutants are new alleles of *os-1* (R2473) described earlier.

Table 2. *Results of non-allelic crosses among the osmotic mutants*

	Crosses	No. and phenotype of progeny: Mutant	Wild type	Total	Recombination (%)		Average map distance between loci
I	*os-1* × *os-6*						
	171-1 × 56-6	5849	98	5947	3·295		
	171-4 × 56-6	693	14	707	3·960		
	171-1 × 14-3	546	10	556	3·597		
	171-1 × 56-4	3606	46	3652	2·519	3·5	(*os-1* and *os-6*)
	R2473 × 56-1	840	17	857	3·967		
	R2473 × 56-4	527	10	537	3·720		
	R2473 × 14-3	2030	38	2068	3·670		
II	*os-6* × *os-7*						
	171-3 × m-3	1482	43	1525	5·639		
	171-7 × m-3	611	20	6131	6·525	5·7	(*os-6* and *os-7*)
	56-4 × m-3	1078	25	1103	4·533		
III	*os-1* × *os-7*						
	171-1 × m-3	132	8	140	11·42		
	171-4 × m-3	97	6	103	11·65	11·3	(*os-1* and *os-7*)
	R2473 × m-3	102	6	108	11·11		

os-6 × *os-6*. Among the new osmotic mutants, strain 56-6 when crossed with each of the strains 14-3, 56-4, 56-B-1 and 171-7 was found to yield only mutant progeny. Also the strain 14-3 in cross with strains 171-3, 171-7 and 56-4 yielded only mutant progeny. Similarly the cross 56-4 × 189 was found to yield only the mutant progeny. Approximately 10000 spores were analysed from each of these crosses but no wild-type recombinant progeny were found to occur among them. Absence of the wild-type progeny in these crosses suggested that these mutants were allelic among themselves and these were classed as group II mutants. Data presented in Table 2 suggest that the group II mutants are non-allelic to osmotic mutants at other loci. The group II mutants were, therefore, considered alleles of a new locus designated here as *os-6*.

os-7 × *os-7*. The remaining osmotic mutants were found to be allelic among themselves since no wild-type progeny were recovered among 10000 spores analysed from each of the following crosses (m-3 × 175 or 183 or 185-3 and 17-5 × 183). Therefore, these strains were classed as group III mutants. On subsequent genetic analysis (see Table 2) group III mutants were found to be non-allelic to group I and group II mutants; thus the group III mutants seem to be alleles of a new locus now designated as *os-7*.

The new osmotic mutants can be classed into three groups on the basis of their

allelism; members of each group are allelic among themselves. The data presented in Table 2 show that group II and group III mutants (now designated as *os-6* and *os-7* respectively) are non-allelic to each other and to *os-1* mutants although closely linked to *os-1*, thus suggesting their location to the linkage group I of *Neurospora crassa*. The data regarding linkage relationship of these three loci (*os-1*, *os-6* and *os-7*) are presented in Table 2. The new loci *os-6* and *os-7* are approxi-

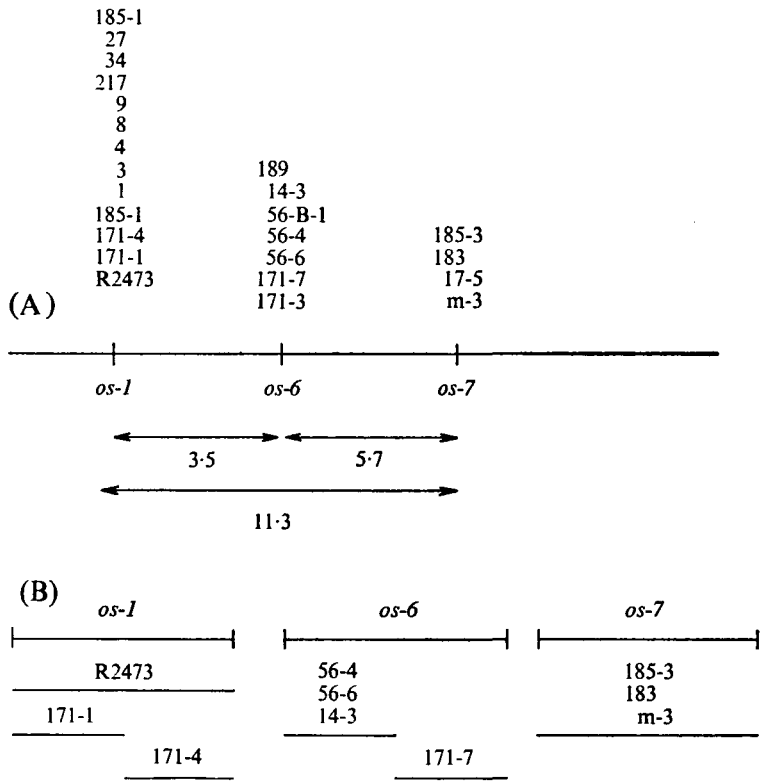

Fig. 2A, Genetic map of the osmotic mutants. Location of group I (*os–1*), group II (*os-6*) and group III (*os-6*) mutants on the linkage group I of *Neurospora crassa*. Allelic mutants are arranged in vertical rows on the top of the line. B, Complementation map of the alleles of *os-1*, *os-6* and *os-7* mutants. Complementing and non-complementing mutants are indicated by the non-overlapping and overlapping horizontal bars.

mately 3·5 and 11·5 map units away from *os-1*. The distance between *os-6* and *os-7* is approximately 5·6 map units (see Table 2). The linkage value of 11·5 map units between *os-1* and *os-7* loci is also suggested by the tetrad data from the cross 185 (*os-1 os-7*) × wild type which gave 10 PD, 1 NPD and 1 T type asci. The allelic and linkage relationship of the different *os-1*, *os-6* and *os-7* mutants are depicted in Fig. 2A.

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Absence of the pseudowild type among the progeny of cross os × os

There are two obvious sources for the presence of the progeny with wild-type phenotype from the crosses involving two osmotic parents; firstly, these may arise as a result of inter- or intragenic recombination, or secondly from complementation of mutants in pseudowild types arising by rare non-disjunction of chromosome (linkage group I) during meiosis. The distinction between a wild-type recombinant and pseudowild type can be made by conidial analysis. On such conidial analysis pseudowild type will yield both mutant and wild-type progeny (Pittenger, 1964), whereas a wild-type recombinant will yield only wild-type progeny. Five or less wild-type progeny from each cross (*os-1* × *os-1* and *os-1* × *os-6*) were examined by conidial analysis and none of them yielded any mutant isolate suggesting that all wild-type progeny examined were true wild-type recombinants (and never a pseudowild type).

Complementation among osmotic mutants

On the basis of genetic data presented in Table 2 it is obvious that the new mutants *os-1* and *os-6* and *os-7* are non-allelic to each other and should also be non-allelic to previously described osmotic mutants (Mays, 1969). The non-allelism of the mutants belonging to *os-1*, *os-6* and *os-7* loci was further examined by complementation analysis and the results are depicted in Fig. 2B.

A majority of the *os-1* mutants showed restricted growth on 1 M-NaCl medium when tested in pair-wise combination among themselves, suggesting that these were non-complementing. However, the strain 171-4 was found to complement with 171-1 and R2473 and B135, thus suggesting that there are at least two complementing groups among the *os-1* mutants. Likewise *os-6* mutants were found to have two complementing groups. However, no mutant has yet been found which is unable to complement with the two complementing groups of *os-6* mutants. All of the *os-7* mutants were found to show mutant phenotype in pair-wise combinations on 1 M-NaCl medium and thus belonged to the same complementing group. These data show the presence of at least five complementing groups among the osmotic mutants belonging to *os-1*, *os-6* and *os-7* loci (see Fig. 2B). The new osmotic mutants (*os-1*, *os-6* and *os-7*) were also tested with previously known osmotic mutants (*os-1*, *os-2*, *os-3*, *os-4* and *os-5*) in order to determine their complementation relationship. In such tests, the new *os-1* mutants did not complement with the previously known *os-1* (B135, R2473) mutants although they were able to complement with all the mutants at 6 different *os* loci. The *os-6* mutants were found to complement with all the other mutants (except *os-6*); likewise, *os-7* mutants were found to complement with all the other mutants (except *os-7*).

4. DISCUSSION

The data presented in this paper suggest the occurrence of DNA induced mutations following genetic transformation in *Neurospora crassa*. Such effects of DNA treatment in the production of mutation has been well established in bacteria (Yoshikawa, 1966) and in blue-green algae (Herdman, 1973). Several mechanisms have been proposed to explain the increased mutability of genes following DNA treatment. Some of these hypotheses are: (1) the incorporated DNA may act as a mutagenic agent (Demerec, 1963); (2) the degradation-product of the incorporated DNA may stimulate DNA replication thus producing a higher mutation frequency (see Yoshikawa, 1966); (3) insertion of the foreign nucleotide sequences into recipient chromosomes (Taylor, 1963). The molecular mechanism of nucleotide insertion has been elucidated in the case of mutation arising after infection of bacteria by phage Mu (Bukhari & Zipser, 1972; Bukhari & Taylor, 1972; Howe & Bade, 1975). At present it is difficult to distinguish among the different mechanisms of induced mutation following DNA treatment in *Neurospora*. Results of our preliminary experiments (Mishra, unpublished) in which calf thymus DNA or *Neurospora* RNA and DNase-treated DNA was ineffective in the induction of mutation in *Neurospora*, point against the first two possible mechanisms. The probability of the third mechanism (i.e. insertion of nucleotide) is, however, complicated by the fact that most of the mutants described in this paper were leaky (as indicated by their colonial growth pattern on the restrictive medium.) Approximately 30% of the mutants obtained in bacteria following transformation were also found to be leaky (Yoshikawa, 1966). The occurrence of leaky mutants does seem improbable since aberrant recombination (which might accompany the incorporation of the foreign nucleotide sequence) would lead to a deletion or insertion type of frame shift-mutation and not the base-substitution type of mutation. However, the repair of a heteroduplex during recombination may cause a substitution type of mutation (Whitehouse, 1963) which might be leaky. Furthermore, the nature of mutation would also be determined by the extent and the position of such frame shift as suggested earlier (Crick *et al.* 1961). Therefore, it is conceivable that insertion of a sizeable nucleotide sequence may lead to the production of a leaky mutation.

In addition to the osmotic mutations described here, the transformed strains were also found to possess other mutations which caused morphological changes or sterility. However, no mutation leading to a specific biochemical requirement for growth was found among the transformed strains since such mutations with additional nutritional requirement could not have survived on a minimal medium which was used to select the *inl*$^+$ transformants.

The data from genetic and complementation analyses clearly suggest that the new osmotic mutants belong to 3 loci (*os-1*, *os-6* and *os-7*); of these, *os-6* and *os-7* are new but *os-1* has been previously described (Perkins, 1959; Mays, 1969). Linkage data also show that *os-6* and *os-7* are closely linked to *os-1*; therefore, these (*os-6* and *os-7*) should be non-allelic to other osmotic loci; this is supported

by the fact that *os-2* is located on the linkage group IV whereas *os-3*, *os-4* and *os-5*, although on linkage group I, are very distantly linked to *os-1* (Mays, 1969). On the basis of recombination and complementation data, the *os-6* genetic region may be assumed to consist of two closely clustered loci (corresponding to the two groups of complementing *os-6* mutants). Such a complex nature of the *os-6* genetic region is supported by the fact that no mutant unable to complement with the two groups of *os-6* mutant has yet been found and also that *os-6* mutants were unable to show any recombination in crosses. However, further work is required to elucidate the precise nature of the *os-6* genetic region.

The data presented here thus increase the number of osmotic loci to 7 or more in *Neurospora crassa*. This suggests the involvement of a large number of proteins in the determinations of the osmotic phenotype; at least some of these proteins must be homomultimers as suggested by the intragenic complementation between *os-1* mutants. The involvement of such a large number of proteins in the determination of a particular phenotype can best be explained in terms of a multienzyme complex which can function as a unit in specifying the wild-type phenotype; presumably a single mutation can distort the architecture of such an enzyme complex leading to a mutant phenotype (sensitivity to NaCl). Evidence for the occurrence of an enzyme complex has been well established in *Neurospora* (Giles *et al.* 1973). However, in the absence of the knowledge regarding the nature of macromolecules which determine osmotic phenotype in *Neurospora* it is difficult to test the validity of this hypothesis. However, the results of a recent study by Rand (1975) may soon provide a clue to the understanding of the biochemical bases of osmotic sensitivity in *Neurospora*. According to Rand (1975) the wild-type strain of *Neurospora* has a colonial morphology on the medium containing galactose as the sole carbon-source; whereas the *os-1* mutants when grown on galactose medium has a filamentous morphology. Thus the osmotic sensitivity of *Neurospora* seems to be inversely related to a better utilization of an unusual sugar like galactose. It is known that the enzymes of sugar transport are also affected by Na^+ ions (Scarborough, 1973). Therefore, it appears that the osmotic phenotype may be determined by the components of the *Neurospora* cell-wall membrane complex involved in the transport and metabolism of sugars. The role of the cell-wall membrane complex in determining the morphology of *Neurospora* has recently been discussed (Mishra, 1977). The fact that the osmotic mutants described in this paper show morphogenetic changes on the NaCl medium suggests a possible damage in the cell-wall membrane complex as a result of the genetic mutation. Significant changes in the cell surface of the osmotic mutants have been observed by scanning electron microscopy (Mishra, 1976); however, the biochemical basis of the morphological changes in osmotic mutants remains to be elucidated.

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REFERENCES

BUKHARI, A. & ZIPSER, D. (1972). Random insertion of Mu-1 DNA within a single gene. *Nature New Biology* **236**, 240–243.

BUKHARI, A. & TAYLOR, A. L. (1975). Influence of insertions on packaging of host sequences covalently linked to bacteriophage Mu DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 4399–4403.

CRICK, F. H. C., BARNETT, L., BRENNER, S. & WALLS-TOBIN, R. J. (1961). General nature of the genetic code for proteins. *Nature* **192**, 1227–1232.

DEMEREC, M. (1963). Selfer mutants of *Salmonella typhimurium*. *Genetics* **48**, 1519–1531.

EMERSON, S. (1963). Slime: A plasmoid variant of *Neurospora crassa*. *Genetics* **34**, 162–182.

GARNJOBST, L. & TATUM, E. L. (1967). A survey of new morphological mutants in *Neurospora*. *Genetics*, **57**, 579–603.

GILES, N. H. (1951). Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. *Cold Spring Harbor Symposia on Quantitative Biology* **16**, 283–313.

GILES, N. H., CASE, M. E., PARTRIDGE, C. W. H. & AHMED, S. I. (1967). A gene cluster coding for an aggregate of five aromatic synthetic enzymes. *Proceedings of the National Academy of Sciences of the United States of America* **58**, 1453–1460.

HAMILTON, J. G. & CALVET, J. (1964). Production of protoplast in an osmotic mutant of *Neurospora crassa* without added enzyme. *Journal of Bacteriology* **88**, 1084–1086.

HERDMAN, M. (1973). Mutations arising during transformations in the blue green alga *Anacystis nidulans*. *Molecular and General Genetics* **120**, 369–378.

HOWE, M. M. & BADE, E. G. (1975). Molecular Biology of Bacteriophage Mu. *Science* **190**, 624–632.

MAYS, L. L. (1969). Isolation, characterization and genetic analysis of osmotic mutants of *Neurospora crassa*. *Genetics* **63**, 781–794.

MISHRA, N. C. (1971). Heterokaryosis in *Neurospora sitophila*. *Genetics* **67**, 55–59.

MISHRA, N. C. (1976). Surface architecture of the different strains of *Neurospora crassa* as revealed by scanning electron microscopy and their relation to morphology. *Journal de Microscopie et de Biologie Cellulaire* **26**, 151–159.

MISHRA, N. C. (1977). Genetics and Biochemistry of morphogenesis in *Neurospora*. In *Advance in Genetics*, vol 19 (ed. E. Caspari). New York: Academic Press (in the Press).

MISHRA, N. C., SZABO, G. & TATUM, E. L. (1973). Nucleic acid-mediated genetic changes in *Neurospora*. In *The role of RNA in Reproduction and Development* (ed. M. C. Niu and S. Segal), pp. 259–268. Amsterdam: North Holland Publ. Co.

MISHRA, N. C. and TATUM, E. L. (1973). Non-medelian inheritance of DNA induced inositol independence in *Neurospora*. *Proceedings of the National Academy of Sciences of the United States of America* **70**, 3875–3879.

MISHRA, N. C. & THRELKELD, S. F. H. (1967). Variation in the expression of *ragged* mutants in *Neurospora*. *Genetics* **55**, 113–121.

PERKINS, D. D. (1959). New markers and multiple point linkage data in *Neurospora*. *Genetics* **44**, 1185–1208.

PITTENGER, T. (1964). The general incidence of pseudowild type in *Neurospora crassa*. *Genetics* **39**, 326–342.

RAND, J. (1975). The regulation of sugar transport and galactose metabolism in *Neurospora crassa*. Ph.D. thesis, The Rockefeller University, New York, N.Y. pp. 1–229.

SCARBOROUGH, G. A. (1973). Transport in *Neurospora*. *International Review of Cytology* **30**, 103–122.

TATUM, E. L., BARRATT, R. W. & CUTTER, V. M. (1940). Paramorphogenic effects of L-Sorbose on *Neurospora* and *Syncephalastrum*. *Science* **109**, 509–511.

TAYLOR, A. L. (1963). Bacteriophage-induced mutation in *E. coli*. *Proceedings of the National Academy of Sciences of the United States of America* **50**, 1043–1051.

VOGEL, H. J. (1964). Evolution of lysine pathways. I. *Ann. Naturalist* **98**, 435–469.

WHITEHOUSE, H. L. K. (1965). *Towards an Understanding of the Mechanism of Heredity*, 3rd edition, pp. 327–371. New York: St Martin's Publisher.

YOSHIKAWA, H. (1966). Mutations resulting from the transformation of *Bacillus subtilis*. *Genetics* **54**, 1201.