Rs 3-8
Price Rs. 1-12-0] [Vol. 32 A, Part VI, pp. 91 to 111

JOURNAL

OF THE

INDIAN INSTITUTE OF SCIENCE

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STUDIES ON THE MUTAGENIC ACTION OF CHEMICAL AND PHYSICAL AGENCIES ON YEASTS

Part II. Mutations Induced in Yeast by Temperature Shocks

BY

B. RANGANATHAN, M.Sc., PH.D

1950

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CONTENTS

INTRODUCTION

In a previous contribution (Subramaniam and Ranganathan, 1947), some preliminary observations on the effect of low temperature on the control 2-chromosome brewery yeast were recorded. Propagation of the yeast in the cold room was shown to have induced three distinct morphological variations. It was not possible, however, to classify these mutants on the basis of chromosomal or genic changes, in view of the limited knowledge then at our disposal. The rapid advances since made have rendered possible a more extensive study of the problem.

Cytological investigations have enabled a classification of the mutants obtained after treatment with acenaphthene as due either to a chromosomal duplication (Subramaniam, 1945, 1947) or a chromosomal breakage (Subramaniam and Ranganathan, 1946) the latter resulting in the production of a top yeast. On exposure of the control strain to low temperatures, top yeasts and cells resembling morphologically the acenaphthene-induced tetraploid, were isolated. When the giant colony characteristics of the

strains obtained after exposure to cold were identical with those of the acenaphthene-induced mutants, it was logical to suggest that there is a correlation between chromosomal constitution and the characteristics of the giant colonies (Subramaniam and Ranganathan, 1948). It was possible therefore to identify chromosomal mutations by diverse agencies employing the nature of sculpturing of the giant colonies as a criterion.

The top yeast obtained after acenaphthene treatment showed two unequal chromosomes (Subramaniam and Ranganathan, 1946). It was initially concluded that this may be the result of a chromosomal deletion. Such an explanation, however, had to be given up, since investigations on the nutritional requirements of some of the strains (Prema Bai, 1947) indicated that the top yeasts were relatively more auto-trophic than the diploid control, BY 1, or the auto-tetraploid, BY 3. In view of the above discovery, it became apparent that the actual change is a chromosomal translocation and not a deletion. Since a distinction of a translocation mutant from a tetraploid was feasible, it was easy to identify gene mutations on the basis of observed spontaneous reversions (Subramaniam, Ranganathan and Krishna Murthy, 1948; Krishna Murthy and Subramaniam, 1950). Reverse mutations were also induced by exposure to ultra-violet irradiation (Subramaniam and Krishna Murthy, 1948).

The validity of the use of giant colony characteristics to identify the various types of mutants had to be tested extensively (Ranganathan and Subramaniam, 1950). This was done by simultaneous investigations on the cytology and the changes in the sculpturing of the giant colony of the acenaphthene-induced autotetraploid. The confirmation obtained has enabled us to classify the different mutants using morphology alone. It is possible to differentiate tetraploids from translocation mutants and both from gene mutations. Evidence on similar lines has also been offered from a study of the seasonal variation observed in the giant colonies of the acenaphthene-induced top yeast BY 2 (Ranganathan and Subramaniam, 1950).

In the preliminary contribution on the effect of cold (Subramaniam and Ranganathan, 1947), it was indicated that even after their isolation the " big cells " were still mutating and throwing out the smaller types which, unlike the parent, were top-fermenting ones. The changes in the cultures initially isolated were systematically followed up till the different strains became relatively stable. Though this work was completed by the middle of 1947, the results had to be held up in order to clearly evaluate the changes produced by culturing the yeast in an ice-room at temperatures ranging from 5 to 10° C.

The investigations on the effect of cold shock on the control diploid strain have assumed an added importance, since it was demonstrated very recently in this laboratory, that the acenaphthene-induced top yeast isolated in 1945, has not only lost its fermentative ability but excretes also an appreciable quantity of riboflavin (Mitra, 1949, 1950).

Historical Resume

Temperature shocks have been known to induce mutations in plants and animals. Heat or cold shocks, like colchicine, acenaphthene and other polyploidogens, disturb the spindle mechanism and inhibit the normal separation of the chromosomes. Lundegardh (1914), Sakamura (1926) and Koschuchow (1928) found that seedlings of some plants, when immersed in water kept at high temperatures, gave rise to tetraploid cells. Lundegardh even noticed irregular division and fragmentation of chromosomes after such treatment. Randolph (1932, 1935) working with *Zea*, records that exposure of the seedlings to temperatures ranging from 40 to 45° C. for periods varying from 15 minutes to 2 hours induced tetraploidy. Some chromosomal translocations were also observed. The actual treatment was given during the early division of the zygote.

Many other workers have induced chromosomal mutations in plants by adopting Randolph's method. Peto (1935), for instance, induced the formation of tetraploid and even octoploid nuclei in the root-tip cells of *Pisum* by heat as well as by chloral hydrate. He observed chromosome associations in root-tip cells of *Hordeum vulgare* subjected to a temperature of 35 to 36° C. Interchange and crossing-over occurred between homologous and non-homologous chromosomes.

The effective use of temperature shocks in inducing polyploidy in economically important plants has been reported by several workers. Dorsey (1936) found that seeds of wheat and rye subjected to a temperature of 42 to 43° C. produced plants having 28, 56 and 84 chromosomes. Tetraploid barley plants were obtained by Muntzing, *et al.* (1937), using a similar method. Ichijima (1934) claimed the production of triploid and tetraploid plants of rice after soaking seeds or young seedlings in water and treating them at 28° C. for 24 to 48 hours. Similar mutations were induced by X-rays and ultra-violet irradiation. Matusima (1935) treated the same material at 42·5 to 45·5° C. and noticed the occurrence of tetraploid cells; but he did not regard the plants so obtained as tetraploids. The useful nature of both high and low temperature shocks in the induction of polyploidy has been indicated by Beachell and Jones (1945). In their experiments on *Oryza sativa*,

the panicles were subjected to both low (0–4° C.) and high (38–48° C.) temperature shocks. The treatment given during the early divisional stages of the embryo was for a duration of 19 to 24 hours. Tetraploid sectors as well as tetraploid plants were obtained. The authors found the low temperatures more effective than heat shocks in the production of tetraploids.

Cases are on record where attempts to induce polyploidy by temperature shocks resulted in sterile progeny. Mention may be made of sterile tetraploid individuals obtained by Atwood (1936) in *Melilotus alba* and Cooper (1939) in alfalfa.

Sax and Dermen have carried out independent investigations on the cytological effects of temperature in plants (Sax, 1936, 1937; Dermen, 1938). Dermen showed the occurrence of tetraploid cells in seedlings of peach, lima bean, apple and cherry subjected to both low (1–3° C.) and high (35° C.) temperatures. Further, he found that the microsporocytes exposed to high temperatures at the time of the first meiotic division, gave rise to tetraploid microspores. In a later paper, Dermen (1940) has compared the cytological effects of colchicine and temperature shocks. During colchicine treatment, the growth of the cells is altered but not stopped. However, normal separation of the parent into daughter cells is prevented. Splitting of the chromosomes takes place resulting in a cell with double the number of chromosomes. If the drug is in a sufficiently concentrated solution, cell division is inhibited for a long time. The effect of temperature on the other hand, is said to be limited and temporary. Growth is completely arrested during treatment and the chromosomes remain in the prophase condition both in mitotic and meiotic phases. When the temperature-treated material is transferred to the normal environment, growth is resumed but doubling of the chromosomes would be noticed only in those cells that were in the division stages during treatment.

A great deal of work has been done on the effect of temperature on animals. Rostand (1933, 1934 and 1936) found that freezing of frog's eggs resulted in the stoppage of the second maturation division. Diploid unreduced eggs were produced. Gross (1932, 1935) obtained tetraploid races from the refrigerated parthenogenetic eggs of a diploid race of *Artemia salina*. The polar body is said to be retained and tetraploid individuals appear.

Extensive observations on the effects of cold shock on animals, especially Amphibia, have been recorded in a series of papers by Fankhauser and collaborators (Fankhauser, 1945). Refrigeration of eggs of *Triturus viridescens* during the first cleavage mitosis at a temperature ranging from 0·5°

and 3° C. for 5 to 24 hours, and subsequent transfer to the room temperature resulted in the appearance of haploid and triploid individuals (Griffiths, 1941; Fankhauser and Griffiths, 1939). The percentages of triploids so produced are stated to vary with different animals. While Griffiths recorded 100 per cent. triploids in experiments on *Triturus viridescens*, Fankhauser and Humphrey (1942) obtained only 80 per cent. triploid individuals in *Axolotl* larvæ. Variations in temperature and the duration of treatment were found to have little or no influence in the production of triploids (Fankhauser, Crotta and Perrot, 1942). Higher polyploids and aneuploids are said to occur only rarely.

Polyploidy has been induced in animals by short heat treatments also. Hasimoto (1933) reported the occurrence of tetraploidy in the eggs of silkworms. Astauvrov (1940) immersed the eggs of silk moths for 18 minutes in water at a temperature of 46° C. and obtained tetraploid and parthenogenetic individuals. Fankhauser and Watson (1942) record triploids in fertilised eggs of *Triturus viridescens* exposed for 5 minutes at 37° C. and for 8 minutes at 36° C.

Reports on the effect of temperature shocks on yeasts are rather scanty. Fabian and McCullough (1934) have, however, recorded some interesting observations in their temperature experiments. They describe dissociative changes in a strain of *Saccharomyces cerevisiæ* Saaz, when grown in broth cultures at 9°, 17°, 23°, 29° and 37° C. respectively. At temperatures ranging from 29° to 37° C. the normal smooth form was found to change after two weeks into the 'R' type. The 'G' form persisted at 37° C. but the 'S' and 'R' forms had disappeared. On the other hand, at 29° C. all the three types were noticed in plates after four weeks. Serial transfers of cultures in lithium chloride or brilliant green also induced such dissociative changes. Fabian and McCullough have not carried out any experiments on the effect of low temperature on yeasts. The recovery of the haploid from the diploid, and the diploid from the autotetraploid has enabled Duraiswami and Subramaniam (1950) to re-interpret the observations of Fabian and McCullough (1934). The so-called gonidial or 'G' forms appear to be the haploids which transform into the 'S' or 'R' forms by a somatic doubling of the chromosome complement. The change from the 'S' form to 'R' type and *vice versa* appear merely to be caused by mutations at a specific locus.

It was Hansen (1906) who first reported the effect of low temperature on yeast. He obtained a top yeast when the bottom strain, *Saccharomyces turbidans*, was brought to the normal environment after exposure to 0·5° C.

for 3 to 5 months. *Saccharomyces ellipsoideus* and the yeast *Johannisberg II* also gave rise to top yeasts under similar experimental conditions.

The stimulus for this series of investigations was the remarkable observations of Hansen at the beginning of the present century. An advance was possible. The results presented are on a strain of knowa chromosomal and genetic constitution.

Material and Methods

The experiment was commenced in July 1945, and was continued till July 1947. On the 13th of July 1945, a 100 ml. flask half full of wort was inoculated with a loop from an agar-slant culture of the control 2-chromosome brewery yeast (BY 1) and was transferred to the cold room where it remained for 90 days. At intervals of 30 days, it was removed from the ice-room, the contents were well shaken, most of it discarded and replaced with fresh sterile wort. The flask was then transferred back to the cold room where the temperature varied from 5–10° C. Even at the end of the first month, scum formation was observed. After 90 days, it was brought to room temperature, agitated well and a loop was inoculated into a sterile tube of wort. The culture should therefore have been exposed to the following conditions. The transfer to the cold room should have produced a sudden cold shock; the low temperature in the ice-room may quite likely have acted as a selective environment and a return to the laboratory conditions may have produced another temperature shock.

At the end of 24 hours, the wort tube inoculated with the treated culture showed two distinct layers. As the cells from the bottom and top layers were distinct morphologically these were isolated by serial dilution plates, using in each case, three dilutions.

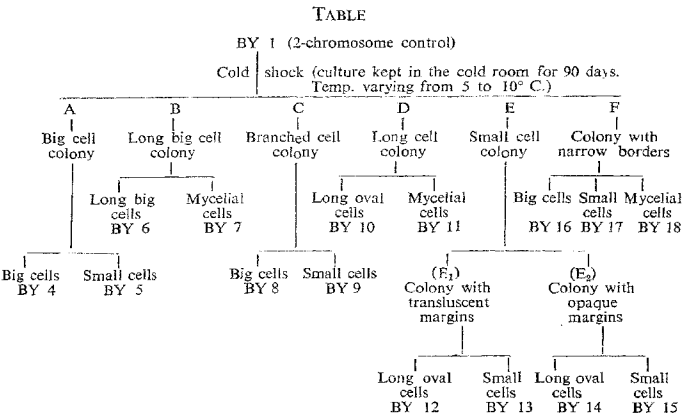
As large numbers of cultures were being handled, methods were standardised to get not more than half a dozen colonies in the third dilution plate. A loop of material was introduced initially into a sterile tube of wort. This was well shaken and three loops from the above were transferred to the molten agar. The contents of the inoculated wort-agar tube were well shaken and poured into the first sterile petri-dish. Since most of the wort-agar had been poured out, two successive additions of fresh molten agar to the above, gave the second and third dilution. Under the experimental conditions employed in this laboratory, the last plate usually showed only a few growing colonies. It need hardly be emphasised that absolutely sterile conditions were maintained.

Observations

The highly mutable nature of the culture, immediately after removal from the cold room, necessitated the use of arbitrary criteria for identification. The morphology of the cells as well as the appearance of the colonies were utilised for a rough and ready classification. Just as the mutants differed in size, shape and mode of budding of cells, the colonies developing in serial dilution plates also showed morphological differences. Some were transluscent and others opaque and some opaque colonies had transluscent margins. Though these are the criteria employed by most investigators it was felt that a clearer idea may be obtained from the sculpturing of the giant colonies (Subramaniam and Ranganathan, 1948). The seasonal changes in the giant colony characteristics of the control strain has been under observation from 1946. An idea of the range of variations due to gene mutations has already been given elsewhere (Subramaniam, Ranganathan and Krishna Murthy, 1948).

Six colonies were isolated from the agar plates on the basis of cell morphology. The accompanying table gives the history of the different strains isolated. The six colonies isolated were arbitrarily classified as A.

TABLE



Big Cell colony; B. Long Big Cell colony; C. Branched Cell colony; D. Long Cell colony; E. Small Cell colony and F. Colony with narrow borders.

The Big cell colony (A), after 24-hour growth in wort showed top and bottom layers. The shape and mode of budding of the cells in the wort-agar

plates, before isolation, were reminiscent of the acenaphthene-induced tetraploid, BY 3. Even after isolation, the colony was still mutating as evidenced by the formation of the big and small cells. By serial dilution both types occurring in a 24-hour wort culture were isolated. These were given the numbers BY 4 and BY 5.

In February 1947, the strain BY 4 gave a giant colony which was smooth and had radial thickenings in its outer half (Photo 1). In September 1947, a *Rough II* colony (Photo 2) was obtained from the strain. In a previous contribution (Subramaniam, Ranganathan and Krishna Murthy, 1948) the changes in the giant colony characteristics of the strain BY 4 have already been described and discussed. It was indicated that the strain BY 4 is a diploid and that after 90 days' exposure to cold, an unmodified diploid could still be isolated.

The colony of BY 5 is illustrated in Photo 3. It is indistinguishable from that of BY 2, isolated after treatment of the control with acenaphthene (*cf.* Photo 3. Subramaniam and Ranganathan, 1948).

The Long Big Cell colony (B) gave rise to Long Big Cells and a Mycelial type of cells. These have been designated as BY 6 and BY 7. The strain BY 6 exhibits a smooth and uniform texture (Photo 4). Its similarity to the acenaphthene-induced tetraploid is striking (*cf.* Photo 2. Subramaniam and Ranganathan, 1948). The strain BY 7, is a top-fermenting yeast. It shows a well-defined rim (Photo 5).

Both the Branched Cell colony (C) and the Long Cell colony (D) gave rise to bottom and top yeasts. These are given the numbers BY 8, BY 9, BY 10 and BY 11.

The first giant colony of the bottom strain BY 8, made in February 1947 and photographed after growth for 18 days, is illustrated in Photo 6. It has a smooth appearance with almost indistinguishable radial lines. Dispersed in the colony are found a few island-sectors shaped like lenses. One such is almost near the margin. The fact that these sectors have been completely closed in on all sides by the growth of the main body of the colony indicates that the cells composing such sectors have a relatively slower growth rate. Photo 7 illustrates the giant colony of the same strain after an interval of two months. It will be noticed that there is a complete absence of sectors. Apparently, those cells which in the earlier giant colony had given rise to the island-sectors, were eliminated in the interval from the culture by the more vigorous and faster growing ones.

Among all the top yeasts described in this paper, the strain BY 9 shows a pronounced rim formation (Photo 8). The central core of the colony is smooth and slimy and appears elevated. The rim is broad and smooth but shows irregularly distributed knob-like projections.

The bottom yeast obtained from the Long Cell colony is the one designated BY 10. The giant colony of the latter is illustrated in Photo 9. It is smooth and except for a slightly greater diameter is indistinguishable from that of BY 14 (Photo 13) isolated from the Small Cell colony (E–E_2). The giant colony of BY 11 (Photo 10), which was also isolated from the Long Cell colony, resembles more closely that of BY 7 (Photo 5).

The Small Cell colony (E) gave rise to a pronounced top and a scanty bottom layer. Two colonies were thus obtained one with the transluscent (E_1) and the other with opaque margins (E_2) (see Table). Each of these colonies on further culturing in wort and plating produced stable types, isolated and numbered BY 12, BY 13, BY 14 and BY 15. The first and the third are bottom-fermenting types while the second and the last are top yeasts.

The giant colonies of the bottom yeasts, BY 12 (Photo 11) and BY 14 (Photo 13) are smooth and uniform in appearance and closely resemble the strain BY 10 (Photo 9). The top yeasts, BY 13 and BY 15, differed only in the width and sculpturing of their outer margin. The rim is rough and narrow in BY 13 (Photo 12) but is relatively broad with fewer granulations in BY 15 (Photo 14). Apart from this slight difference the colonies resemble in general the other top yeasts.

The colony with Narrow Borders (F) gave rise to three distinct cultures. These were (i) Big cells, (ii) Small cells and (iii) Mycelial cells. These three were numbered as BY 16, BY 17 and BY 18. The sector present in the colony of BY 16 (Photo 15) is different in appearance from that observed in BY 8 (Photo 6). Located as it is on the outer half, it should have originated during the growth of the colony. Its wedge shape suggests that the cells composing it should have a slightly accelerated growth rate. However, it has been the experience in this laboratory that sector formation is very rare in tetraploids.

Among the top yeasts BY 17 and BY 18, the latter (Photo 17) shows a close resemblance to BY 15 (Photo 14). Both the colonies inoculated simultaneously showed the same diameter after growth for 24 days and were photographed on the same date. The main difference appears to be the slightly rougher texture of the rim of the colony of BY 18. Both, however, show sectors.

The colony of BY 17 (Photo 16) has a very narrow rim. From the series of photographs of top yeasts (Photos, 3, 5, 8, 10, 12, 14, 16 and 17) it would be found that the size of the rim as also its sculpturing is a variable factor. This difference has since been shown to be exhibited by the same strain during different seasons of the year (Ranganathan and Subramaniam, 1950).

Discussion

The variations in shape and size of the cells described in this paper appear merely to be a confirmation of similar observations by Lepeschkin (1903) and Guilliermond (1920). They noticed elongated mycelial types in *Saccharomyces Carlsbergensis* kept at 7° C. and claim to have maintained such types unchanged over a period of time.

The extended series of investigations on the control 2-chromosome yeast strain and the mutants produced from it (Subramaniam, 1945, 1946, 1947, 1948 *a*, *b*; Subramaniam and Ranganathan, 1945, 1946, 1947, 1948, 1949; Ranganathan and Subramaniam, 1947, 1950; Subramaniam and Krishna Murthy, 1948, 1949; Krishna Murthy and Subramaniam, 1950, Subramaniam, Ranganathan and Krishna Murthy, 1948; Prema Bai, 1947; Mallya and Subramaniam, 1949; Mitra, 1948; Mitra and Subramaniam, 1949; Duraiswami and Subramaniam, 1950; Subramaniam and Sreepathi Rao, 1950) has enabled us to evaluate clearly the changes produced by culturing the control in the cold room. The various strains described in this paper fall into three categories: They are (*a*) unmodified diploids (BY 4, Photos 1 and 2); (*b*) autotetraploids (BY 6, Photo 4; BY 8, Photos 6 and 7; BY 10, Photo 9; BY 12, Photo 11; BY 14, Photo 13; and BY 16, Photo 15), and (*c*) top yeasts resulting from chromosomal translocations (BY 5, Photo 3; BY 7, Photo 5; BY 9, Photo 8; BY 11, Photo 10; BY 13, Photo 12; BY 15, Photo 14; BY 17, Photo 16; and BY 18, Photo 17). It has been the experience of several workers that even after long treatment with colchicine, tetraploidy may not be induced. In another paper (Ranganathan and Subramaniam, 1950) the isolation of an unmodified diploid, BY COL 2 (Photo 8 in that paper), has already been discussed. The production of a similar unmodified diploid strain after culturing the control for 90 days in the ice-room, thus, does not at all appear to be surprising.

Neither is it strange that tetraploids should be isolated after culturing in the cold room. That, like acenaphthene, temperature shocks also produce what are apparently chromosomal translocations resulting in the appearance of top yeasts is an observation worth special consideration.

(a) Temperature and Mutability

Muller (1928) has shown that the mutation rate can be accelerated by merely increasing the temperature. Similar results have been recorded by a host of other workers. Temperature shocks were shown to produce a similar increase in mutability (Gottschewski, 1934). Independent investigations by Birkina (1938) and Kerkis (1939) indicated that cold shocks increase the mutability to the same degree as heat shocks. According to Gottschewski (1934) the effect of temperature is non-specific and it merely enhances the normal mutational ability of the organism. Timofeef-Ressovsky (1935), and Plough (1942) consider that temperature accelerates the mutation rate. The influence of temperature on mutation rate appears to depend on whether the genes involved are stable or unstable. The mutation rate of stable genes is accelerated by increasing the temperature (Muller, 1928), while that of unstable genes remains unaltered (Demerec, 1932), or even shows a decrease (Faberge and Beale, 1942). Plough (1942) observes: "Temperature is not the cause of mutability but it is important in determining its rate" (p. 15). He continues: "In relation to evolution the increase in mutability due to shocks would be essentially the same as the temperature effect, namely to furnish more genetically determined variations to be screened out by selection." (p. 16.)

Storing the control culture of yeast in the cold room should have produced initially a cold shock. The conditions in the ice-room may have formed a selective environment. It was shown by Hansen that when *Saccharomyces Carlsbergensis* was cultured at 27° C., it was normal in appearance, while at 7° C., it produced curious colonies "made up of elongated cells forming a sort of mycelium" (Guilliermond. 1920, p. 179). On the other hand Hansen (1906) obtained top yeasts in cultures of *Saccharomyces turbidans* cultured at 0·5° C. The production of top yeasts as a result of a cold shock on the control strain is reminiscent of similar observations by Hansen. In view of the limited knowledge of the genetics of even higher organisms available at that time, he could only surmise that the appearance of the top yeast was the result of a spontaneous change comparable to the "mutations" of De Vries. Hansen believed that the low temperature merely formed a selective environment for the top yeast.

This speculation of Hansen has to be modified in the light of recent advances. Though mutations are spontaneous they are accelerated by temperature shocks. When after a cold shock new mutants do appear, the low temperature may selectively eliminate those unfit for survival.

(b) Gene Mutation and Polyploidy

An analysis of the above observations indicates that three types of mutations have been induced by temperature shocks. It is true that an unmodified diploid BY 4 was isolated. But it had a curious type of sculpturing which has not since been observed. The identification of BY 4 as an unmodified diploid was based on reverse mutations. Spontaneous mutability has already been demonstrated in the control strain (Subramaniam, Ranganathan and Krishna Murthy, 1948). The new type of sculpturing observed in BY 4, ought to be a gene mutation. The origin of polyploids falls under an entirely different category. At the time the temperature shock experiments were carried out, the main angle of approach was whether polyploidy could be induced. Recent investigations on the factors responsible for an induction of polyploidy have suggested that a doubling of the chromosome complement should be preceded by a gene mutation (Subramaniam and Sreepathi Rao, 1950). The occurrence of an unmodified diploid in the temperature shock experiments is not strange, since such an unmodified diploid was isolated even after long treatment with colchicine. The peculiar kind of sculpturing exhibited by BY 4, indicated that the temperature shock has brought to light even a rare allele. Induction of tetraploidy does not consist in a mere duplication of the chromosome complement. One has to remember that the tetraploid complement should be capable of functioning harmoniously. Levan's interesting observations (1939) on *Petunia* and *Beta* offer indirect evidence that a gene mutation should precede any induction of autotetraploidy. If that is so, the difference between *Petunia* and *Beta* may merely depend on the mutation rate at the locus governing polyploidy. Once it is assumed that the action of agencies producing tetraploidy is merely a directed gene mutation, it stands to reason that the above agencies should also accelerate the mutation rate in other loci. Acceptance of the above suggestion alone would explain the appearance of the rare type of allele in BY 4.

Further evidence for the above contention is offered by temperature experiments in *Drosophila* and higher plants. The gene mutation rate is accelerated by temperature shocks in the former, while in the latter, the identical agency produces an inhibition of cell division with the resultant formation of tetraploid cells, sectors or seedlings. When the same agency has different effects on different organisms, a clear evaluation of the conditions conducive to the formation of tetraploid cells becomes a necessity. If temperature shocks merely produce an inhibition of cell division in yeasts, it follows that the exposed culture should become a pure tetraploid. The

fact that along with the tetraploids, gene and chromosomal translocation mutants appeared, suggests that mere inhibition of cell division may not result in an induction of polyploidy. Evidence from an entirely different direction that a gene mutation should precede any duplication of the chromosome complement has recently been offered from this laboratory (Subramaniam and Sreepathi Rao, 1950).

The spontaneous but rare occurrence of tetraploid sectors under laboratory conditions indicates that mutations at the loci responsible for the appearance of tetraploid cells are sporadic. The results reported above could legitimately be explained as due to an acceleration of the mutation rate by temperature shocks. When normal occurrence of tetraploid sectors is rare, it has to be surmised that mutations at the locus should have a low frequency. Acceleration due to temperature shocks need not therefore affect all the cells. Thus the unmodified diploid, BY 4 (*cf.*, Col. 2, Photo 8; Ranganathan and Subramaniam, 1950) may have persisted as a diploid owing to the non-occurrence of a gene mutation enabling the cell to become a tetraploid. A systematic series of investigations on the effect of camphor on the control diploid strain has brought forth interesting evidence in this direction (Subramaniam and Sreepathi Rao, 1950). Though the tetraploids isolated after culturing in the cold room are indistinguishable morphologically from one another and from those induced by chemical agencies, they were numbered separately in the belief that they may differ in their genic constitution. If temperature shocks accelerate gene mutation rate, it appears likely that the different strains may not after all be identical in their genic constitution.

(*c*) *Chromosomal translocations*

In the year 1945, top yeasts were produced by treatment with acenaphthene and by temperature shocks. Further, it was demonstrated by a series of investigations that the top yeast obtained after the treatment with acenaphthene is the result of a chromosomal translocation. A repetition of the experiment in 1947 gave only tetraploids (Subramaniam and Krishna Murthy, 1949). The suggestion was offered that the appearance of top yeasts on treatment of the control strain with acenaphthene may be conditioned by particular genic constitution. The occurrence of chromosomal translocations on exposure of the control to temperature shocks cannot, therefore, be explained merely as due to the rupture of the chromosome as a result of the abnormal conditions during division. The suspicion has been engendered that even the chromosome breakage should be preceded by gene mutations at one or more loci. When top yeasts obtained after exposure to

acenaphthene and to cold shock are mixed together there is no sector formation indicating their morphological identity (Photo 8, Ranganathan and Subramaniam, 1950). Since the change has been brought about by two entirely different agencies the probability that gene mutations should precede chromosomal translocations becomes feasible. Cold shock inhibits cell division but accelerates the rate of gene mutation. This indicates that the effect of temperature shocks may be unpredictable. Further, just as gene mutations have to precede any origin of stable tetraploid cells, the production of a top yeast may itself be dependent on similar directed mutations at specific loci.

Chemical mutagens have also been known to produce chromosomal translocations (Auerbach, 1949). Using ethyl urethane with potassium chloride, Oehlkers (1943, 1946, 1949) could induce a very high percentage of chromosomal translocations in plants. Several phenolic derivatives have been known to produce chromosomal breakages in Allium (Levan and Tjio, 1948 *a*, *b*), but mere breakage of chromosomes does not result in translocations. We have to distinguish between ordinary chromosome breakages and those which are followed by the production of viable translocation mutants. When the above distinction is made, Oehlkers's results differ from those of Levan and Tjio. Vogt (1948) has shown that urethane produced mutations in *Drosophila*. When the same chemical accelerates the gene mutation rate and also produces chromosomal translocations it is reasonable to suspect that viable chromosomal translocations should be preceded by specific gene mutations. The fact that in 1947 the same chromosomal translocations did not occur on treatment with acenaphthene (Subramaniam and Krishna Murthy, 1949) leads to the legitimate conclusion that even a specific mutation leading to such translocation is possible only when particular allelic sequences occur.

(d) Polyploidy in animals

Workers investigating the effect of temperature on Amphibian development (Fankhauser, 1945) seem to have proceeded on the assumption that the phenomena they observed in Amphibia are identical with those recorded in plants. Fankhauser states: "On the one hand, many aspects of polyploidy that have been thoroughly investigated in plants require a comparative study of animal material, since the conditions and consequences of such unusual changes in the genetic system are not the same in all details, in spite of the general validity of cytological and genetic principles for both plants and animals" (Fankhauser, 1945, p. 21).

Colchicine inhibits cell division in animals but does not induce polyploidy. The suggestion has been offered (Subramaniam and Sreepathi Rao, 1950) that animals may not have a specific locus rendering polyploidy possible. The recorded results indicate that the effect of cold and heat on Amphibia are not comparable to the results obtained after similar treatments in plants. It would be seen from Table III given by Fankhauser (1945, p. 53), that in *Triturus viridescens*, the spontaneous changes resulting in the production of haploid individuals is 0·16 per cent., pentaploid animals 0·28 per cent. and triploid ones 1·8 per cent. Among the total number of 2,448 larvæ examined he found only one tetraploid. It is known that Amphibian eggs could be made to develop parthenogenetically. Unlike in frogs, the majority of the eggs in the Salamanders are stated to be polyspermic. However, the accessory sperms do not seem to disturb the normal process of cleavage. The very rare occurrence of really tetraploid animals either spontaneously or after temperature treatments, gives the clue that the origin of triploids and pentaploids in Amphibia may be the result of an entirely different mechanism. Unreduced germ cells as a result of meiotic aberration ought to be as common in Amphibia as in higher plants. Somatic doubling has been known to occur in eggs stimulated to develop parthenogenetically. From the above facts it would be legitimate to suggest that the normal tendency for somatic doubling in haploid Amphibia works in conjunction with the possibility of fertilisation of the unreduced egg by one or more sperms. As in other animals, even those Amphibia investigated may not have a locus for tetraploidy.

(*e*) *Lyophilisation and mutation*

It would have become apparent from the results obtained by temperature shocks, that lyophilisation as a method of preservation may be unsuitable for yeasts. In lyophilisation, one of the major steps is to cool suddenly the culture to a very low temperature. When mutations occur as a result of the preservation of the control strain of yeast in the cold room the sudden cooling prior to dehydration may produce drastic changes. While even in Bacteria, Morton and Pulaski (1938) observed the so-called, 'dissociation', similar changes should be expected in lyophilised yeast cultures. In fact, Atkin, *et al.* (1949) noticed more than 50 per cent. of variants in rejuvenated lyophilised cultures. In their studies on the bios pattern of some of the revitalised cultures, they found a definite departure in their biochemical behaviour, and suggest that the altered strains have to be classified separately.

Lindegren (1945) has used in his genetical investigations, cultures dehydrated in a colloid suspension. That this method of preservation itself produces genetical changes is suggested by his statement that on revitalisation, the so-called 'haplophase' cultures are composed of 'genetically and morphologically different forms (p. 129)'. It becomes questionable therefore whether his rather surprising results may not themselves be caused by the method of preservation.

SUMMARY

1. Storage of the 2-chromosome control brewery yeast strain in the cold room for 90 days, resulted in the production of top and bottom yeasts which resembled in their morphology and giant colony characteristics, the acenaphthene-induced tetraploid and top yeasts.

2. The effect of temperature shocks on plants and animals is briefly reviewed.

3. The experimental details are given and the method of isolation of the various strains described.

4. The morphology and giant colony characteristics of the individual strains are dealt with elaborately.

5. The transfer of the control culture to the cold room should have produced initially a cold shock, as a result of which mutations may have occurred. It is suggested that mutations that occur spontaneously are merely accelerated by temperature shocks.

6. The fact that an unmodified diploid, chromosomal translocation mutants and tetraploids are isolated indicates that mere inhibition of cell division may not be responsible for induction of tetraploidy. The presence of the unmodified diploid is probably caused by the non-occurrence of a gene mutation enabling the cell to become a stable tetraploid.

7. The production of chromosomal translocation mutants by acenaphthene as well as by temperature shocks, leads to the suspicion that such changes should be preceded by a gene mutation. It is further suggested that any such specific gene mutation leading to translocation is possible only when particular allelic sequences occur.

8. The very rare occurrence of tetraploid animals either spontaneously or as a result of temperature shocks may be due to the absence of a specific locus for polyploidy.

9. When temperature shocks accelerate the rate of mutation, the sudden cooling prior to dehydration during the process of lyophilisation

may produce drastic changes rendering the above method unsuitable for the preservation of yeast cultures.

ACKNOWLEDGMENT

I am very grateful to Dr. M. K. Subramaniam for his active interest and help during the progress of these investigations and to the Council of Scientific and Industrial Research for generous financial assistance.

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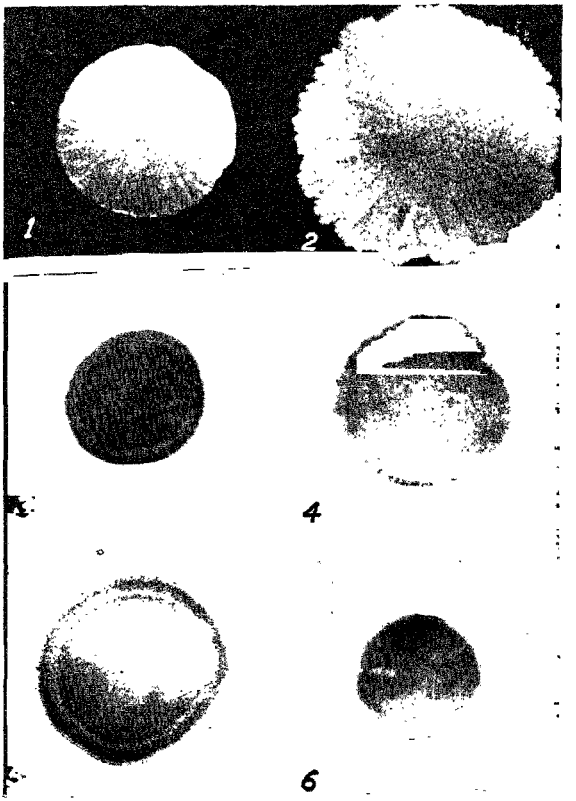
Description of Photographs

(All the colonies were grown in Barley-malt agar)

Photo	1, BY	4,	3·6 cm.	18	Day	Growth,	28–2–1947.
Photo	2, BY	4,	2 8 cm.	14	,,	,,	30–9–1947.
Photo	3, BY	5,	2·4 cm.	18	,,	,,	28–2–1947.
Photo	4, BY	6,	2·8 cm.	18	,,	,,	28–2–1947.
Photo	5, BY	7,	2 8 cm.	22	,,	,,	19–3–1947.
Photo	6, BY	8,	2 6 cm.	18	,,	,,	28–2–1947.
Photo	7, BY	8,	2·7 cm.	23	,,	,,	10–4–1947.
Photo	8, BY	9,	—	14	,,	,,	27–6–1947.
Photo	9, BY	10,	3·3 cm.	25	,,	,,	18–3–1947.
Photo	10, BY	11,	2·8 cm.	25	,,	,,	18–3–1947.
Photo	11, BY	12,	2·8 cm.	22	,,	,,	19–3–1947.
Photo	12, BY	13,	2·5 cm.	25	,,	,,	18–3–1947.
Photo	13, BY	14,	3·0 cm.	25	,,	,,	18–3–1947.
Photo	14, BY	15,	2·8 cm.	24	,,	,,	7–7–1947.
Photo	15, BY	16,	3·3 cm.	26	,,	,,	19–3–1947.
Photo	16, BY	17,	2·9 cm.	26	,,	,,	19–3–1947.
Photo	17, BY	18,	2·8 cm.	24	,,	,,	7–7–1947.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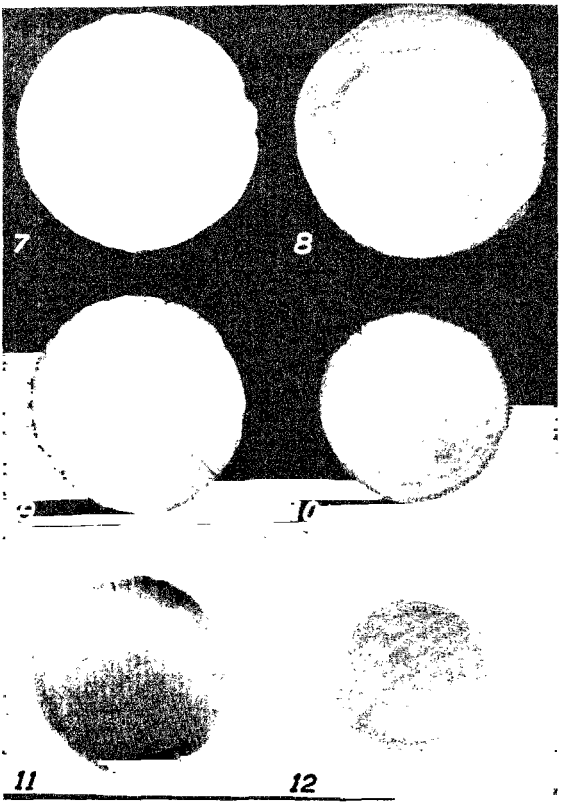
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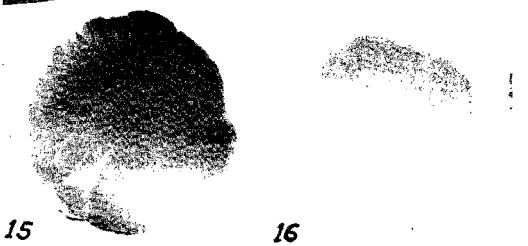
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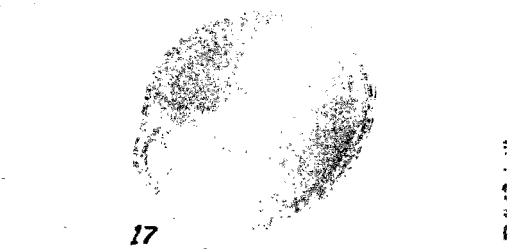
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