EFFECT OF ACENAPHTHENE ON YEAST STRAINS OF DIFFERENT GENIC AND CHROMOSOMAL CONSTITUTIONS

BY M. K. SUBRAMANIAM, M.A., D.Sc.
AND
S. N. KRISHNA MURTHY, M.Sc.

(Cytogenetics Laboratory, Department of General Chemistry, Indian Institute of Science, Bangalore, 3)

Received November 27, 1948
(Communicated by Dr. B. Sanjiva Rao, F.A.Sc.)

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INTRODUCTION

Do yeast strains of differing genic and chromosomal constitutions react in an identical fashion when treated with acenaphthene and colchicine? A rational explanation of the contradictory results obtained by different investigators would be possible if it could be shown that strains of varying genic and chromosomal constitutions react differently when treated with the above chemicals. Richards (1938) found an acceleration in the rate of growth when the yeast strain used by him was grown in media containing colchicine. Levan and Sandwall (1943) on the other hand, could not find any such stimulation. The results recorded by Richards and Levan and Sandwall are based on experiments with different strains of yeasts. Their results are not strictly comparable and offer no evidence for any generalization. It is known that the genotype as a whole and occasionally even a specific gene (Rhoades, 1938; Dobzhansky, 1947) may control the mutability of one or more of the genes. The chromosomal constitution of the strains appears to be a much more important factor. While autopolyploidy could be induced in a diploid with known chemical and physical agencies, these may have no effect at all on a tetraploid or a higher polyploid.
A CRITICAL EVALUATION OF SOME RECENT PUBLICATIONS

During the past three years a brewery yeast strain, BY 1, has been under constant observation and most of our experiments on induction of polyploidy by diverse agencies have been carried out on this strain. The intensive study of a few selected strains has enabled us to evaluate critically not only the methods of approach, but also the significance of the divergent results obtained by workers investigating the effect of various polyploidizing agencies on yeasts.

Neither Richards (1938) nor Levan and Sandwall (1943) knew either the chromosome or the probable genic constitution of the strains they were experimenting on. From a study of the growth curves of the diploid and tetraploid it was demonstrated (Prema Bai and Subramaniam, 1947) that Richards' results are capable of a different interpretation. The similarity of the growth curve of our tetraploid, BY 3, to that of Richards' strain in media containing colchicine, led us to suggest that colchicine had in fact induced polyploidy.

Levan and Sandwall (1943) do not appear to have made a distinction between vegetatively growing cells and fermenting ones. The difference between these two is fundamental (Subramaniam, 1947b) as fermenting cells appear to have no genetical future at all. While Richards used actively growing cells for his experiments, Levan and Sandwall (1943) used a culture "which was in lively fermentation" (p. 165). While the majority of the cells were capable of active vegetative multiplication in Richards' experiments, only a very small percentage of cells were capable of doing so in Levan and Sandwall's experiments. Fermenting cultures contain only a very small percentage of cells capable of active vegetative multiplication, and the "rather considerable differences" observed by them between the different controls cannot therefore be due to "slight differences in treatment" as suggested by them, but is in all probability the result of different numbers of vegetative cells contained in the same quantity of inocula. In their controls for colchicine and acenaphthene series they introduced 280 cells per mm. At the end of 20 hours while the former contained 16,125 cells per mm. the latter had only 7,870 in the same volume. The fact that the same number of cells were introduced in each case, viz., 280 per mm., has little significance since the number of viable cells in the inocula may have been different. If we assume that the inoculum introduced into the control for the acenaphthene series contained only half the number of viable cells as that for the colchicine series then, the different cell numbers which they observed, at the end of 20 hours in the controls of the two series, appear
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probable. Since the percentage of viable cells in a fermenting culture is very small, the same aliquot from a well shaken liquid culture need not contain the same number of viable cells.

The number of cells capable of active vegetative multiplication introduced at the start of the experiment determines the yeast crop at the end of 20 hours. When wide variations in the yeast crop in the controls for the different series themselves were observed by Levan and Sandwall (1943) any evaluation of the effect of the various drugs, by estimating the number of yeast cells formed at the end of 20 and 48 hours, is bound to be inaccurate.

There appears to be also no justification for their conclusion that "neither colchicine nor acenaphthene, which both have a very pronounced c-mitotic effect on higher plants have any such effect in yeast, at any rate not by inducing c-mitoses". Even if we assume that all cells in the inocula used by them were capable of active vegetative multiplication, their claim that "during the first 20 hours the cell number is augmented from some hundred cells to about 10,000 cells per mm. 3" implies about seven cycles of multiplication by all the cells. Therefore, the time for each division is about three hours. Any lag due to the presence of the chemical in the medium should be apparent at the beginning of the experiment. Levan and Sandwall do not appear to have made any cell counts during this crucial period. Our experience indicates (Subramaniam, 1947) that after a single duplication of the chromosomes, acenaphthene has no further effect on the cells. While our control, BY 1, takes 21 hours to reach the end of the logarithmic phase, the auto-tetraploid, BY 3, takes only 16 hours. Therefore, even if acenaphthene or colchicine had induced a doubling of the chromosomes in Levan and Sandwall's (1943) experiments, the prolongation of the lag phase due to inhibition of cell division would be compensated by the shortened generation time of the new strain. Cell counts made at the end of 20 and 48 hours would give no indication of the changes in the population which had taken place in such cultures. There is also no evidence in published literature that the inhibition of cell division by colchicine and acenaphthene extends for such long periods during which the cells are capable of dividing several times, under normal conditions.

Most of the investigators do not seem to have envisaged the possibility that experiments on the rate of growth in media containing various chemicals will not give a correct picture of the effect. Such media form abnormal environments for yeasts, and if the chemicals induce mutations, the culture at different intervals would contain different numbers of mutants.
depending on (1) the rate of induction of mutations by the drug, (2) the viability, and (3) the rate of growth of the different mutants themselves. Before starting any quantitative experiments proof has to be adduced that the chemical employed does not produce gene or chromosomal mutations. This Levan and Sandwall (1943) have not done. There is no proof that the yeast crop obtained by them are not mixtures of gene and chromosomal mutants.

**Material and Methods**

A seasonal variation was observed in the characteristics of the giant colonies of our control brewery strain, BY1, and basing our conclusions on spontaneous (Subramaniam, Ranganathan and Krishna Murthy, 1948) and induced (Subramaniam and Krishna Murthy, 1948) reverse mutations, it was suggested that multiple alleles exist at the locus determining the nature of sculpturing of the colony. The discovery that changes in chromosome constitution do produce changes in the characteristics of the giant colonies (Subramaniam and Ranganathan, 1948) has provided a simple technique for the identification of a tetraploid. In 1945 it was the strain giving giant colonies of the *Smooth* type which was treated with acenaphthene. The procedure employed in making giant colonies has already been described elsewhere (Subramaniam and Ranganathan, 1948). Since during particular months both the *Smooth* and *Rough* types are available in our cultures, actively growing cells of both were again treated for 45 days with acenaphthene. There is also in our collection a strain of distillery yeast which has been shown to be a naturally occurring auto-tetraploid (Ranganathan and Subramaniam, 1948). Active cultures of the distillery yeast were also treated continuously for a year with acenaphthene. At the end of the treatment, the cultures were grown for 24 hours in wort, plated and the various types isolated. Giant colonies of the above were grown for a detailed study of the changes induced.

**Observations**

In 1945 when the *Smooth* type (Photo 1) was treated for 90 days with acenaphthene, the resultant culture contained two distinct types of cells. These were shown to be a "top yeast" (Photo 3) (Subramaniam and Ranganathan, 1946) and a tetraploid (Photo 2) (Subramaniam, 1945). Since higher polyploids did not appear even after treatment for a year with acenaphthene (Subramaniam, 1947) it was assumed that acenaphthene ceases to have any effect once a chromosome doubling had occurred. Periodically tetraploid sectors could be observed in the colonies of the control. Such normally occurring tetraploid sectors in *Rough I* and *Smooth I* types of colonies
are illustrated in Photos 5 and 4. When the identical Smooth I type of cells (Photo 6) were again treated in 1947 with acenaphthene for 45 days, only tetraploids were obtained (Photo 8). The treatment did not produce top yeasts. Since mutations at the locus determining the nature of sculpturing of the giant colony have been demonstrated, it is only reasonable to conclude that mutations at other loci should have also occurred. The production of top yeasts on treatment of the control with acenaphthene appears, therefore, to be conditioned by particular genic constitutions.

A classification of the various types of colonies observed in the control has been given elsewhere (Subramaniam, Ranganathan and Krishna Murthy, 1948). It was assumed that the Smooth I type of cells were homozygous for the Smooth gene, while the Rough I type was heterozygous for the Rough allele. The Rough I type of cells (Photo 5) because of their different genic constitution were also treated for 45 days with acenaphthene. Only a tetraploid could be isolated after treatment (Photo 9). The giant colony of this tetraploid was slightly different from that obtained from the Smooth I type in that it had faint striations radiating to the margin. Such a change in the characteristics of the tetraploid, BY 3, was observed (Photos 7, 8 and 9) during the summer of 1947, when the control was giving Rough I type of colonies. In January 1948, the colony of the tetraploid did not show the faint radial striations. Just like the reversibility of the Rough to Smooth, there appears to be a reversibility in the characteristics of the tetraploids also. But the changes in the characteristics of the tetraploid colony are so insignificant that they may be missed unless looked for.

The giant colony of the tetraploid distillery yeast (Photo 10) is indistinguishable in appearance from that of the tetraploid brewery yeast (Photos 7, 8 and 9). On treatment for one year with acenaphthene it also gave rise to a top yeast and a bottom one. The giant colony of the top yeast (Photo 12) had an appearance similar to that of the top yeast obtained from the brewery strain (Photo 3). But whether they have comparable chromosome constitutions could only be judged after careful cytological investigations. The giant colonies of the distillery yeast before and after treatment (Photos 10 and 11) were indistinguishable in appearance. If tetraploids and octoploids give rise to giant colonies having identical appearance, only cytological investigations would indicate the exact nature of the change that has resulted from such long treatment with acenaphthene.

DISCUSSION

As would be evident tetraploids could be obtained from the two chromosome brewery yeast strains of different genic constitutions by long treatment
with acenaphthene. On the other hand, the characteristics of the giant colonies of the control tetraploid distillery yeast as well as one of the strains isolated after one year's treatment of the above with acenaphthene are identical in appearance. At first sight it would appear that since both the treated and control colonies of the distillery yeast are similar in appearance, even one year's treatment has produced no change at all. The appearance of a top yeast on treatment of the tetraploid definitely indicates that acenaphthene has some action and hence any assertion that the bottom strain isolated after treatment is identical with the control should be substantiated by proof that the giant colonies of the tetraploid and octoploid are different in appearance. Since the cytology of the new strains has yet to be investigated, no conclusions could be drawn from the mere similarity in appearance of the control and treated strains. The above facts should dissuade workers from making hasty generalizations based on meagre data.

Our identification of the tetraploid was based purely on chromosome number. Further studies on the diploid and tetraploid showed (1) that their giant colony characteristics (Subramaniam and Ranganathan, 1948), (2) growth curves (Prema Bai and Subramaniam, 1947), (3) rate of fermentation (Mitra, 1948), and (4) nicotinic acid content (Duraiswami and Subramaniam, 1948) are entirely different. The tetraploid had a shorter generation time and showed only one single growth cycle and an accelerated rate of fermentation. It has also been demonstrated (Prema Bai Mallya, unpublished) that tetraploids produced by treatment of the control with chemical as well as physical agencies not only gave giant colonies of identical appearance, but had also similar growth curves. Thus as far as our control brewery yeast, BY 1, is concerned it is a simple matter to identify a tetraploid merely from the characteristics of its giant colonies.

It is surprising that most of the investigators on yeast (Dodge, Dodge and Johnson, 1941; Levan and Sandwall, 1943; and Richards, 1938) do not seem to have appreciated the importance of isolating stable types after various treatments by simple plating and then comparing their behaviour with that of the control in normal media. So long as qualitative investigations do not precede quantitative ones, much importance cannot be attached to the latter.

We have observed differences in viability and rate of growth of strains assumed to be homo- and heterozygous for a single allele (Prema Bai Mallya and Subramaniam, 1948) and since even in our control cultures grown at room temperature different gene mutants get established during the different seasons of the year, the legitimate conclusion is that even the same
mutant may have different growth rates during the varying seasons of the year. It is natural, therefore, to conclude that media containing specific chemicals may even form (Braun, 1947) selective environments for particular mutants quite apart from the mutagenic action exerted by the chemical itself on the cells growing in such media. Any investigation on the effect of various mutagenic agencies on yeasts should take into consideration the interaction of the host of factors involved for arriving at any generalization.

Quite apart from all this, the claim of Levan and Sandwall (1943) that both colchicine and acenaphthene have no c-mitotic effect on yeast is unwarranted as they did not investigate either the cytology of the control or of the cells at different stages of treatment with the drugs.

The tetraploids isolated after treatment of both Rough I and Smooth I types of the control give rise to giant colonies having a smooth surface and a faintly wavy margin. The faint radial striations observed in the colony of the tetraploid derived from the Rough I are so insignificant that they do not necessitate any consideration at all. If the nature of sculpturing of the colony is gene determined, the cumulative action of the duplicated genes should find an expression in the tetraploid. However, duplication of the genes, as happens in tetraploids, actually results in a loss of sculpturing. This result would not strike one as strange when it is remembered that the giant colony is not strictly comparable to tissues of higher organisms.

The doubling of the chromosomes alters usually the surface-volume and nuclear-cytoplasmic ratios and hence should affect the physiology of the cell as a whole. The major changes in the functioning of the cell caused by tetraploidy have to be taken into consideration when trying to evaluate the possible cumulative action of particular genes. The diploid and tetraploid yeasts in our collection differ from each other in cell shape and mode of budding, which appear to be two of the important factors determining the nature of sculpturing of the giant colony. The absence of any type of sculpturing in the tetraploid colony does not, under the circumstances, appear surprising.

The significance of auto- and allopolyplody in yeasts has been completely ignored by Lindegren (Lindegren and Lindegren, 1946) and Spiegelman (1946) in evaluating the "irregular" genetic behaviour of yeasts observed by them under particular environmental conditions. Their speculations seem to have very little experimental justification. A quotation from Lindegren and Lindegren (1946) given below may justify the above criticism. "We had previously reported that a recessive could be transformed into a fermenter if the recessive spore was cut out of a heterozygous cytoplasm
in the continued presence of the respective substrate. 'Masked' recessives, in which the character was induced by this technique of direct transfer of cytoplasm, could be unmasked by dissimilation in the absence of the respective substrate. *We recently made several attempts to duplicate this experiment, but were not able to produce more 'masked' recessives by continued exposure to substrate than were found in the untreated controls.* This does not necessarily invalidate our first experiment, but indicates at least, that the conditions which control the phenomenon have not been completely worked out" (p. 123). It is on such insufficient data that Lindegren tries to modify the fundamental Mendelian principle of independent assortment and speaks about "recessives acquiring the dominant character at meiosis ......by direct transfer from dominant chromogene to recessive chromogene" (p. 123). In tetraploids incomplete dominance is a common phenomenon owing to the number of genotypes possible. Little (1945) states: "On account of striking differences between the genetics of diploids and tetraploids, plant breeding procedures applicable to diploids must frequently be modified in dealing with tetraploids" (p. 82). While the probability of obtaining a triple recessive in a diploid is 1 in 64, in an auto-tetraploid, it may range anywhere between 1 in 8,000 to 1 in 46,656, depending upon the rate of incidence of 'double reduction'.

The "Cytogene" and "Plasmagene" concepts would be valid only if it is proved that Lindegren and Spiegelman are not dealing with polyploid segregation.

**SUMMARY**

1. The effect of acenaphthene on yeast strains of different genic and chromosomal constitutions was investigated.

2. A critical evaluation of the methods of approach as well as the significance of the divergent results obtained by workers investigating the effect of various polyploidizing agencies on yeast is given.

3. In 1945, when the *Smooth I* type was treated for 90 days with acenaphthene, the resultant culture contained a top yeast and a tetraploid. When the identical *Smooth I* type was again treated in 1947 with acenaphthene for 45 days, only tetraploids were obtained. Since mutations at the locus determining the nature of sculpturing of the colony have been demonstrated it is only reasonable to conclude that mutations at other loci should also have occurred. The production of top yeasts on treatment with acenaphthene appear, therefore, to be conditioned by particular genic constitutions.

* Italics are ours.
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4. Normally occurring tetraploid sectors in Rough I and Smooth I types of colonies are illustrated. The Rough I type of cells, which have a different genic constitution also gave rise to a tetraploid.

5. A tetraploid distillery yeast when treated for one year gave rise to a top yeast and a bottom one. The giant colonies of the control, and the bottom strain isolated after treatment, were indistinguishable in appearance. Entire reliance should not be placed on mere similarity in appearance of giant colonies and the invalidity of hasty generalizations based on meagre data is emphasized.

6. The absence of sculpturing in the tetraploid colony is not surprising since doubling of the chromosomes alters usually the surface-volume and nuclear-cytoplasmic ratios and hence should affect the physiology of the cell as a whole.

7. The “Cytogene” and “Plasmagene” concepts would be valid only if it is proved that Lindegren and Spiegelman are not dealing with polyploid segregation.

REFERENCES


**DESCRIPTION OF PHOTOGRAPHS**

Photo 1.  BY 1. Diploid control. 2·4 cm. 12-day growth. 27-9-1946.

Photo 2.  BY 3. Tetraploid. 2·1 cm. 12-day growth. 27-9-1946.

Photo 3.  BY 2. Top Yeast. 2·2 cm. 12-day growth. 27-9-1946.

Photo 4.  BY 1. Diploid control grown in Ragi malt agar. 2·4 cm., 20-day growth. 5-3-1948.

Photo 5.  BY 1. Diploid control. 2·5 cm, 10-day growth. 30-6-1947.


Photo 7.  BY 3. Tetraploid. 14-day growth. 27-6-1947.

Photo 8.  BY 3. Tetraploid. 2·3 cm., 9-day growth. 28-7-1948.

Photo 9.  Tetraploid obtained by treating the Rough 1 type of cells. 2·7 cm., 13-day growth. 3-11-1947.

Photo. 10.  DY Tetraploid-control. 2·7 cm., 15-day growth. 10-4-1947.

Photo. 11.  DY Bottom Yeast obtained after one year's treatment with acenaphthene. 3·0 cm., 23-day growth. 10-4-1947.

Photo. 12.  DY Top Yeast obtained after one year's treatment. 2·9 cm., 31-day growth 21-4-1947.

Except in the case of Photo 4 all the colonies were grown in Barley-Malt-agar. The measurement of the longest diameter is given.
and S. N. Krishna Murthy