酵母菌のサプレツシィブプチ変異株に対する紫外線とアクリアラビンの影響

誌名	The Japanese journal of genetics
ISSN	0021504X
巻/号	473
掲載ページ	p. 159-163
発行年月	1972年6月

JAPAN. J. GENETICS Vol. 47, No. 3: 159-163 (1972)

EFFECT OF ACRIFLAVINE AND ULTRAVIOLET-LIGHT IRRADIATION ON SUPPRESSIVE PETITES OF *SACCHAROMYCES CEREVISIAE*

AKIRA UCHIDA

Biology Division, College of General Education, Kobe University, Nada, Kobe 657

Received September 10, 1971

In *Saccharomyces cerevisiae*, extrachromosomal-gene mutations (vegetative-petite mutations) which result in respiratory deficiency, have been extensively studied. Ephrussi *et al.* (1955) first reported that there exist at least two types of vegetative petites, namely, the neutral petite, and the suppressive petite which retains the suppressive factor in cytoplasm. When a cross was made of a grande with a suppressive petite, a certain proportion of zygotes gave rise to petite colonies and the proportion of the petite colonies was characteristic of the suppressive petite used (Ephrussi and Grandchamp 1965). Since mitochondrial DNA was demonstrated in a suppressive petite (Mounolou *et al.* 1966), the mitochondrial DNA has been presumed as a molecular basis of the suppressive factor.

Ephrussi *et al.* (1955) described that, although suppressiveness was relatively stable, a decrease of the degree of suppressiveness could be experimentally induced. But no detail was given of experimental procedures and of the mutagens they used.

We report here preliminary results concerning the stability of suppressiveness of highly suppressive petites after acriflavine treatment or ultraviolet-light irradiation.

MATERIALS AND METHODS

Strains; A suppressive-petite strain, 6C3 (@), was spontaneously issued from an ascus of a diploid strain, *a* (Uchida and Suda 1970). This suppressive petite was a temperature-sensitive (*ts*) mutant concerning the growth, that is, the petite could not form colonies at 30°C when plated on complete or minimal agar medium but at 20°C the growth was almost normal. The genetic analysis showed that a recessive-gene mutation was involved in the temperature sensitivity. The *ts*+ revertants from 6C3 showed almost the same degree of suppressiveness indicating that the *ts* mutation was genetically independent to the suppressive nature of 6C3. 6C33 was a derivative of 6C3 with a slightly higher degree of suppressiveness. A grande strain, 133 (*a*, *ade*1, *ura*3) which was respiratory competent, was used as a tester strain.

Media: A complete medium contained 10 g each of peptone and yeast extract and 25 g of glucose in 1,000 ml water. A selective medium for zygotes was Wickerham's minimal medium (Wickerham 1946) supplemented with 0.1% casamino acid.

Mating procedures and determination of degree of suppressiveness: The suppressive petites and the grande were grown in complete medium at 20°C for 4 days. Before

mating cells of the suppressive petites were washed twice with M/15 KH_2PO_4, and resuspended in the same volume of the solution. One part of the petite-cell suspension was mixed with 100 parts of the grande culture to which were added final 2% glucose. The mixture was then centrifuged (3,000 rpm, 3 min) with sharpless tubes and the supernatant was discarded. The cells, left pelleted at the bottom of the tube, were incubated at 20°C for 1 day for zygote formation. The cells were plated on minimal agar medium and incubated at 30°C which permit only zygotic clones to grow. Routinely the petites and the grande were plated separately on minimal agar and incubated at 30°C to check revertants, if any. After 4 days' incubation petite and grande colonies were scored by TTC overlay technique (Ogur *et al.* 1957). The calculation of the degree of suppressiveness (% suppressiveness) was done after Sherman and Ephrussi (1962).

Mutagenic treatments: The suppressive petite used here had 10 times higher sensitivity to acriflavine than the grande. If cells were inoculated in complete medium with 2 μg/ml of the drug, the growth was almost completely inhibited. Therefore, for acriflavine treatment the petite cells were incubated for 4 days at 20°C in the presence of 0.5 μg/ml of acriflavine, the concentration of which allowed the growth with a slight inhibition. For ultraviolet-light (UV) irradiation the petite cells were washed twice with M/15 KH_2PO_4 and suspended to get 10^7 cells per ml of the solution. The cell suspension being agitated by a magnetic stirrer was irradiated for 2 min. with a Toshiba Germicidal Lamp (15W) located 73 cm above. This dose gave rise to 5.1% survival and the efficiency of zygote formation was 1.04% of the survivors, while the efficiency was 0.50% in the case of the non-irradiated cells.

RESULTS AND DISCUSSION

Acriflavine and UV irradiation are known to be potential inducers for extrachromosomal-gene mutation. The effects of these mutagens on highly suppressive petites were investigated. A suppressive petite, 6C3, grown in the presence of acriflavine exhibited a decrease of the degree of suppressiveness. Whereas, an increase of the degree of suppressiveness was observed when the petite cells were mated immediately after UV irradiation (Table 1). To see whether or not the changes of the degree of suppressiveness observed after the above mutagenic treatments were temporary, about one hundred colonies which were derived from the cells grown in the presence of acriflavine

Table 1. Effect of acriflavine and ultraviolet-light irradiation on suppressiveness of 6C3

Treatment	Number of colonies		% suppressiveness
	total	petite	
none	11,377	9,685	85.1
acriflavine	4,034	2,660	65.9
ultraviolet-light	1,869	1,756	94.0

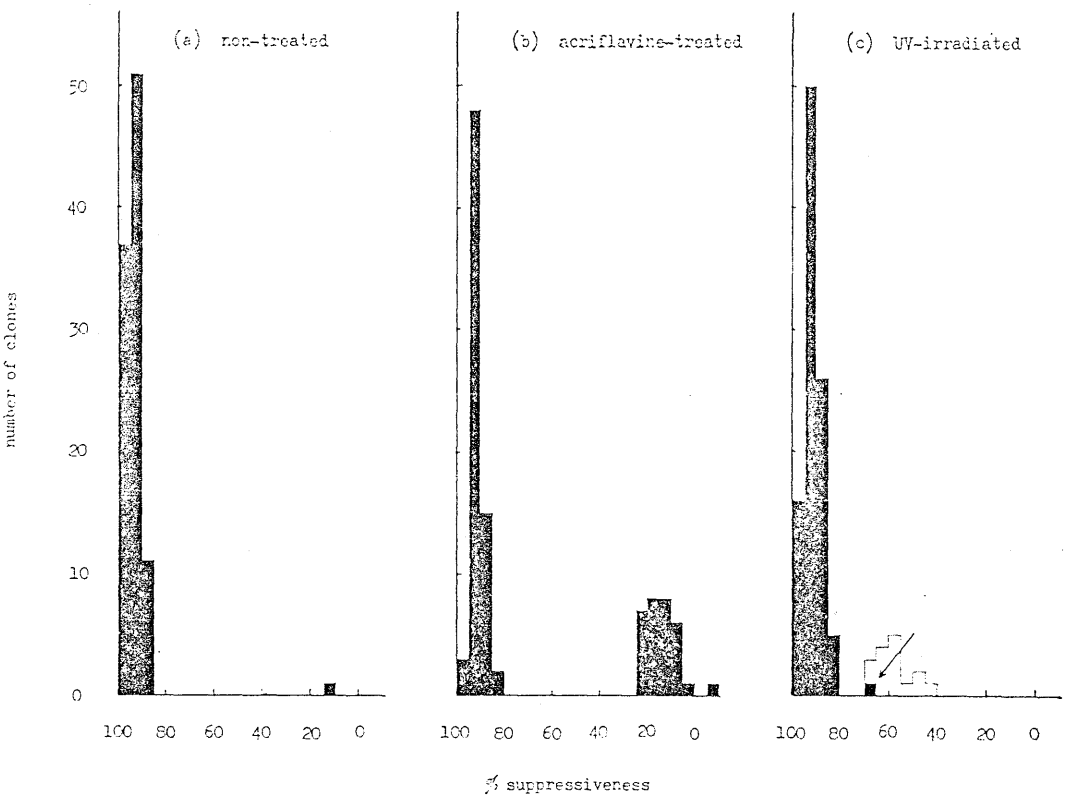

Fig. 1. Distributions of suppressiveness of the clones derived from (b) acriflavine-treated and (c) ultraviolet-light irradiated cultures in comparison with (a) non-treated culture of 6C33. The averages of % suppressiveness of (b), (c) and (a) were 69.2, 90.3 and 93.7, respectively. Open squares in (c) indicate the distribution of suppressiveness of the subclones derived from the petite with intermediate suppressiveness (shown by an arrow).

and from the UV-irradiated cells of 6C33 were randomly picked up and the degree of suppressiveness of each clone was examined (Fig. 1).

Fig. 1b shows that petites with neutral and low suppressiveness (below 25% suppressiveness) were induced by acriflavine. Thus, it could be concluded that the decrease of suppressiveness by acriflavine would be a result from, if not all, the induction of petites with neutral and low suppressiveness. The increment of suppressiveness by UV irradiation was demonstrated as a temporary effect of UV, since the clones derived from UV-irradiated cells did not exhibit the increment of suppressiveness (Fig. 1c). Such enhancement of suppressiveness was observed when a UV-irradiated grande was crossed to another grande (Williamson 1970). Fig. 1c also shows that a petite with intermediate suppressiveness (68.2% suppressiveness; shown by an arrow) was induced by UV irradiation. To examine whether the petite clone was a mixture of cells with neutral and high suppressiveness, fifteen subclones of the petite were isolated and the degree of suppressiveness of each subclone was determined. They were all revealed to be of intermediate suppressiveness (open squares in Fig. 1c).

The intercalating dyes, euflavine and ethidium bromide, could induce neutral petites and petites with low suppressiveness from a grande strain (Saunders *et al.* 1970) and mitochondrial DNA deficient petites, induced by ethidium bromide, were neutral (Nagley and Linnane 1970). It seems, therefore, probable that acriflavine would interact with the mitochondrial DNA leading to an elimination of the suppressive factor during the growth in the presence of the intercalating dye. In contrast to the effect of acriflavine, UV irradiation could induce neither neutral petites nor petites with low suppressiveness. Instead, UV irradiation induced a petite with intermediate suppressiveness from a highly suppressive petite. Saunders *et al.* (1970), using spontaneously arisen petites, examined the retention of an erythromycin-resistance factor and its relation to the degree of suppressiveness of each petite. From the results they proposed that the mitochondrial DNA of neutral petites and of highly suppressive petites would carry no or little information, but that petites with intermediate suppressiveness would have less damaged mitochondrial DNA. However, the fact that a petite with intermediate suppressiveness was emergent from a highly suppressive petite suggests that only the structural alterations or the changes of information content in the mitochondrial DNA would not be sufficient to explain the phenomenon of high suppressiveness.

SUMMARY

The stability of suppressiveness of highly suppressive petites of *Saccharomyces cerevisiae* was examined. The results obtained were; 1). Acriflavine induced neutral and low-suppressive petites with a high frequency. 2). A transient increment of suppressiveness was observed when the suppressive petite was mated immediately after ultraviolet-light irradiation. 3). In contrast to acriflavine, ultraviolet-light irradiation induced neither neutral nor low-suppressive petites, but it did induce a petite with intermediate suppressiveness.

ACKNOWLEDGMENTS

The author wishes to express his tanks to Dr. Y. Tsubo, Kobe University and to Dr. K. Wakabayashi, University of Tokyo for their valuable criticisms during the preparation of the manuscript.

LITERATURE CITED

Ephrussi, B., H. de Margerie-Hottinguer, and H. Roman, 1955 Suppressiveness: A new factor in the genetic determinism of the synthesis of respiratory enzymes in yeast. Proc. Natl. Acad. Sci. **41**: 1065-1071.

Ephrussi, B., and S. Grandchamp, 1965 Études sur la suppressivité des mutants a deficience respiratoire de la levure. I. Existence au niveau cellulaire de diverse 'Degrés de suppressivité'. Heredity **20**: 1-7.

Mounolou, J. C., H. Jakob, and P. P. Slonimski, 1966 Mitochondrial DNA from yeast "petite" mutants: Specific changes of buoyant density corresponding to different cytoplasmic mutations. Biochem. Biophys. Res. Commun. **24**: 218-224.

Nagley, P., and A. W. Linnane, 1970 Mitochondrial DNA deficient petite mutants of yeast. Biochem. Biophys. Res. Commun. 39: 989-996.

Ogur, M., R. St John, and S. Nagai, 1957 Tetrazolium overlay technique for population studies of respiratory deficiency in yeast. Science **125**: 928.

Saunders, G. W., E. B. Gingold, M. K. Trembath, H. B. Lukins, and A. W. Linnane, 1970 Mitochondrial genetics in yeast: Segregation of a cytoplasmic determinant in crosses and its loss or retention in the petite. In "Autonomy and biogenesis mitochondria and chloroplasts" (Boardman, N. K., A. W. Linnane, and R. M. Smilie, ed.) pp. 185-193. North-Holland Publ. Comp.

Sherman, F., and B. Ephrussi, 1962 The relationship between respiratory deficiency and suppressiveness in yeast as determined with segregational mutants. Genetics **47**: 695-700.

Uchida, A., and K. Suda, 1970 A mutant of yeast with temperature sensitive respiration. Japan. J. Genetics **45**: 261-268.

Wickerham, L. J., 1946 A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeast. J. Bacteriol. **52**: 293-301.

Williamson, D. H., 1970 The effect of environmental and genetic factors on the replication of mitochondrial DNA in yeast. In "Control of organelle development" (Miller, P. L., ed.) pp. 247-276. Cambridge Univ. Press.