Saccharomyccs cerevisiae におけるホモタリズム遺伝子の胞子形成に及ぼす影響

誌名	The Japanese journal of genetics
ISSN	0021504X
著者	壷井, 基夫 高橋, 俊明 柳島, 直彦
巻/号	47巻3号
掲載ページ	p. 185-192
発行年月	1972年6月

JAPAN. J. GENETICS Vol. 47, No. 3: 185-192 (1972)

EFFECT OF THE HOMOTHALLISM-CONTROLLING GENE, *D* ON SPORULATION IN *SACCHAROMYCES CEREVISIAE*

MICHIO TSUBOI[1], TOSHIAKI TAKAHASHI[2]
AND NAOHIKO YANAGISHIMA[1]

Department of Biology, Faculty of Science, Osaka City University, Osaka 558[1]
and
Suita Laboratory, Brewing Science Research Institute, Suita 564[2]

Received February 8, 1972

Homothallism-controlling gene, *D* is thought to be a mutator gene which causes mutations in mating type alleles, a to α and α to a, resulting in homothallic phenomenon (Hawthorne 1963). Thus, genotype of homothallic diploid cells concerning mating type alleles is thought to be a/α. From the above assumption, homothallism-controlling genes should exhibit their effect mainly prior to cell conjugation. On the other hand, the sporulation ability of diploid cells appears to be controlled by mating type alleles, a and α, since a/α diploid can sporulate, while a/a or α/α diploid cannot.

Recently, Takahashi (1970) studied effect of *D* gene on the spore-forming ability and assumed that complementary action of heterozygosity of mating type locus (a/α) and homozygosity (D/D or d/d) or heterozygosity (D/d) of homothallism-controlling gene is required for the sporulation of *Saccharomyces*. Yanagishima *et al.* (1970) showed that diploid cells carrying homothallism-controlling genes expanded in response to yeast sexual hormones and also to a plant hormone, auxin, while heterothallic diploid cells did not. Hence it is suggested that homothallism-controlling genes have some effects on cellular activity even after diploidization.

We attempted to find effect of a homothallism-controlling gene on sporulation, using strains isogenic except the homothallism-controlling gene, *D*.

MATERIALS AND METHODS

Preparation of strains used

Genetic markers, except *D* gene, involved in the strains used were derived from breeding stocks of Drs. D. C. Hawthorne and R. K. Mortimer. Homothallism-controlling gene *D* was derived from a homothallic yeast *Saccharomyces chevalieri* kindly supplied by Dr. C. Roberts. To prepare isogenic strains carrying or not carrying *D* gene, dissected spores were conjugated under microscope.

Media

The presporulation culture medium (VHG-BII) contained 5 g peptone, 5 g KH_2PO_4, 2 g $MgSO_4 \cdot 7H_2O$, 50 g glucose, 40 mg adenine sulfate, 40 mg uracil and trace of vitamin mixture in 1,000 ml of distilled water. The sporulation medium was 1% potassium acetate solution.

Sporulation test

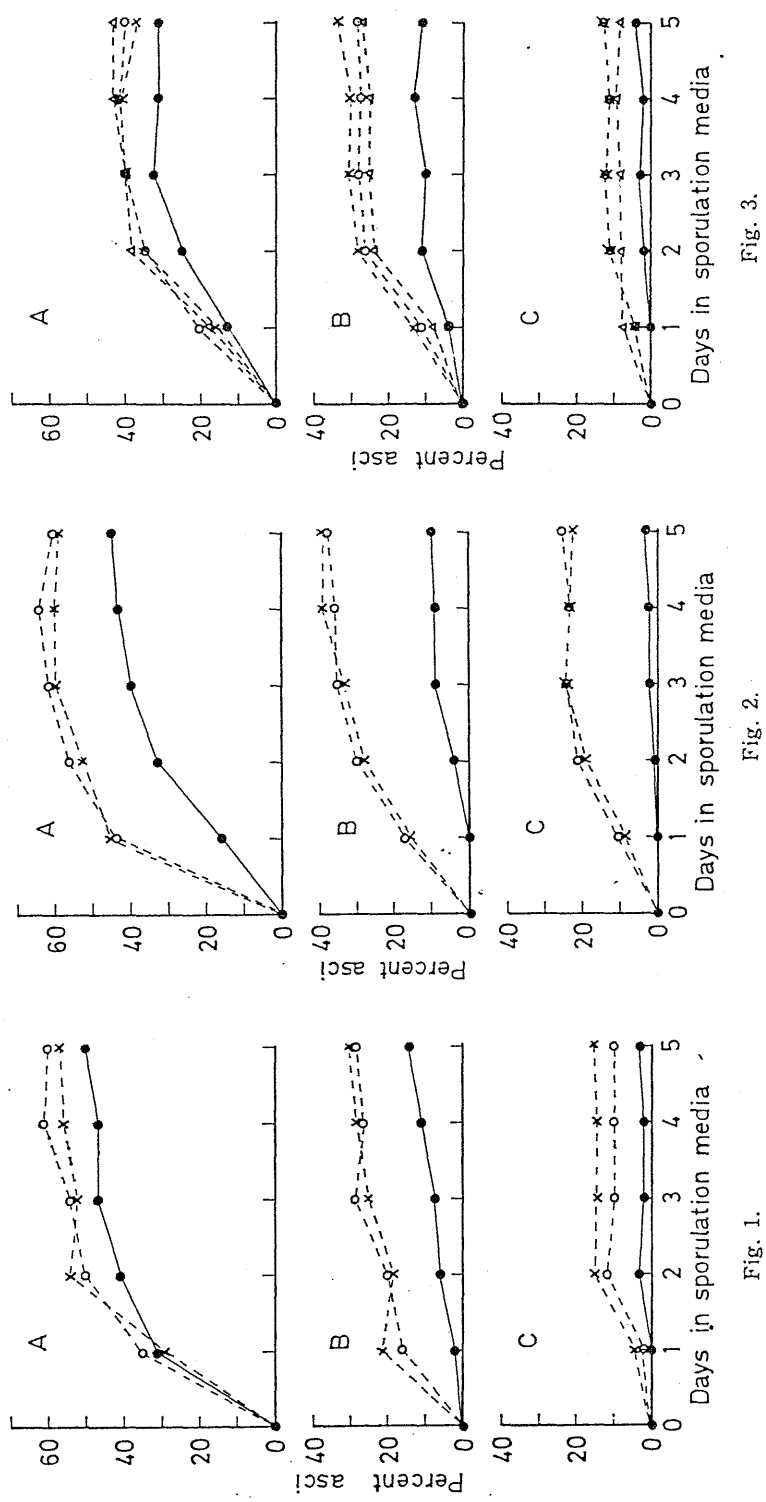
One day-old cells in VHG-BII were inoculated into VHG-BII for presporulation culture. After shaking for 16, 24 or 48 hrs at 26°C, cells were harvested by centrifugation. They were suspended in distilled water, sonicated, washed several times with distilled water and inoculated into sporulation medium at cell density of 2×10^7 cells per ml. Each 100 ml conical flask containing 10 ml of the cell suspension was shaken on a reciprocating shaker. Cell number was counted with Thoma's haemocytometer, all buds being counted as cells. No increase in cell number was observed during the incubation in the sporulation medium.

RESULTS

Three groups of strains differing only in *D* gene were prepared (Table 1). Namely, in each group, a heterothallic diploid strain which had not homothallism-controlling gene *D*, a/α (d/d), a homothallic diploid strain, D/D, and one or two hybrid(s) between above two strains, D/d, were contained, respectively. Each strain was inoculated into VHG-BII at a cell density of 2×10^5 cells/ml. After 16, 24 and 48 hrs' incubation, cells were harvested, washed and transferred to the sporulation medium. Percentage of asci was counted daily. The results are showh in Figs. 1, 2 and 3. In each group homothallic strains sporulated better than the heterothallic strain. No significant differ-

Table 1. Yeast strains (*Saccharomyces cerevisiae*) used

Group	Strain	Genotype
I	C2212	$a\ ad_6\ ar_4\ met_3$
		$\alpha\ ad_6\ ar_4\ met_3$
	C2182-18A	$D\ ad_6\ ar_4\ met_3$
		$D\ ad_6\ ar_4\ met_3$
	C2231	$D\ ad_6\ ar_4\ met_3$
		$d\ ad_6\ ar_4\ met_3$
II	C2213	$a\ ad_6\ met_3\ ur_3$
		$\alpha\ ad_6\ met_3\ ur_3$
	C2180-5C	$D\ ad_6\ met_3\ ur_3$
		$D\ ad_6\ met_3\ ur_3$
	C2232	$D\ ad_6\ met_3\ ur_3$
		$d\ ad_6\ met_3\ ur_3$
III	C2222	$a\ ad_6\ le_2\ met_3$
		$\alpha\ ad_6\ le_2\ met_3$
	C2181-3A	$D\ ad_6\ le_2\ met_3$
		$D\ ad_6\ le_2\ met_3$
	C2235a and b	$D\ ad_6\ le_2\ met_3$
		$d\ ad_6\ le_2\ met_3$



Figs. 1, 2 and 3. Sporulation of each strain listed in Table 1.
Period of presporulation culture: 16 hr (A), 24 hr (B) and 48 hr (C). Figs. 1, 2 and 3; groups I, II and III in Table 1, respectively. For experimental procedures see Materials and Methods.

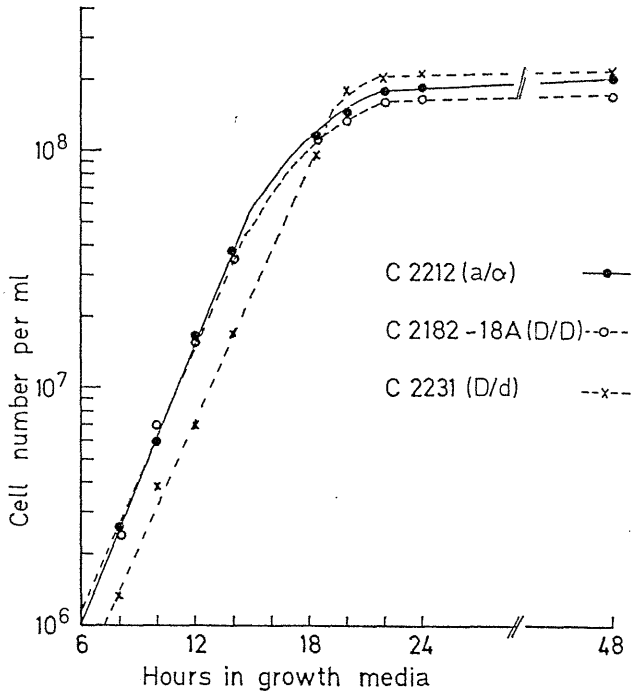

Cell number per ml
10^8
10^7
10^6
6
12
18
24
48
Hours in growth media
C 2212 (a/α)
C 2182-18A (D/D)
C 2231 (D/d)

Fig. 4.

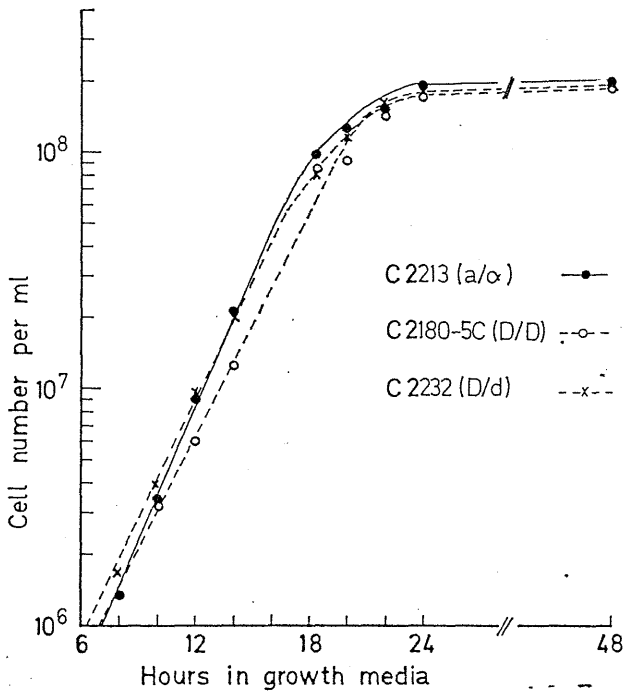

Cell number per ml
10^8
10^7
10^6
6
12
18
24
48
Hours in growth media
C 2213 (a/α)
C 2180-5C (D/D)
C 2232 (D/d)

Fig. 5.

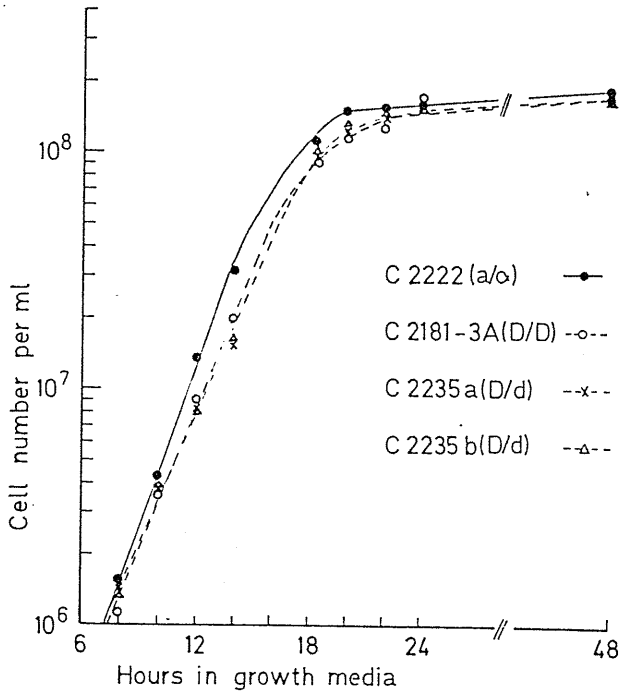


Fig. 6.

Figs. 4, 5 and 6. Growth of strains listed in Table 1 in VHG-BII media. Each strain was shaken in VHG-BII medium at 26°C. Initial cell density, 2×10^5 cells/ml. Figs. 4, 5 and 6; groups I, II and III in Table 1, respectively.

ence in sporulation ability was detected between strains carrying *D/D* and *D/d*. In general, sporulation ability decreased with the age of presporulation culture.

It is known that the sporulation ability is influenced by the culture age (Croes, 1967). Hence we compared homothallic strains with the heterothallic one for their growth patterns. Cells were inoculated in VHG-BII medium at density of ca. 2×10^5 cells/ml and cell number was counted with Thoma's haemocytometer at intervals.

As shown in Figs. 4, 5 and 6, no significant difference in growth pattern was observed between homothallic and heterothallic strains in each group. In each strain, the doubling time was ca. 100 min. and the cell number in the stationary phase was ca. 2×10^8 cells/ml. It is thus clear that homothallic strains carrying *D* gene, either *D/D* or *D/d*, can sporulate better than heterothallic strains, not as a result of difference in cell number increase during the preculture.

Whether or not *D* gene affects the sporulation ability may be tested by comparing homothallic segregants with the heterothallic segregant from *D/d* strains. Tetrads of strains, C2231, C2235a and b, were dissected with micromanipulator. Among them, C2231-1, C2235b-2 and C2235b-4 showed $2D : a : \alpha$ segregation, and C2235a-4 showed $D : D'^{*} : a : \alpha$ segregation. From these tetrads, four groups were obtained each com-

* *D'*: *2n* but sporulation negative segregant

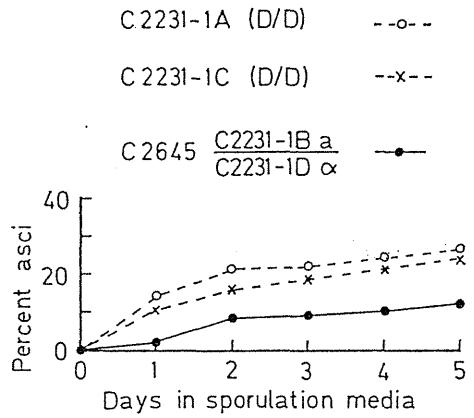


Fig. 7.

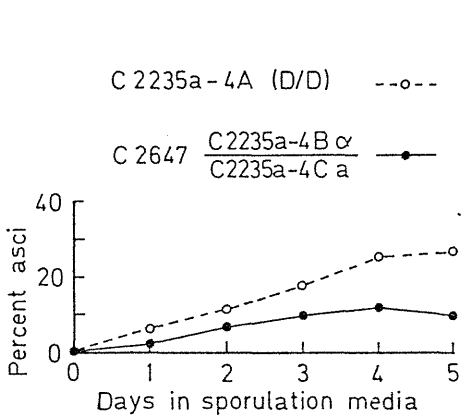


Fig. 8

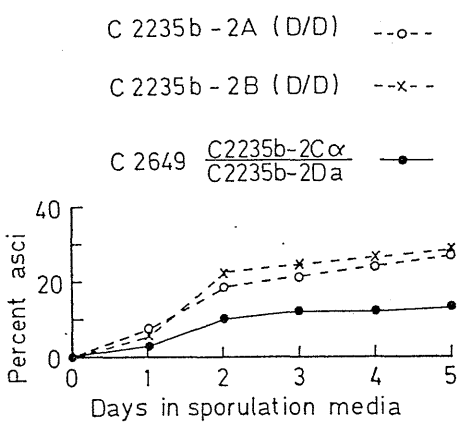


Fig. 9.

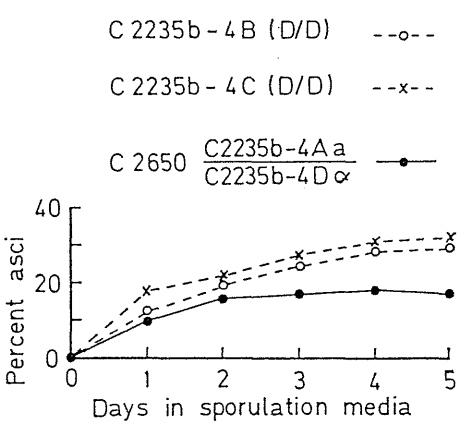


Fig. 10.

Figs. 7, 8, 9 and 10. Sporulation of diploid strains derived from spores in the same ascus. After 24 hr incubation in VHG-BII medium, cells were transferred to sporulation medium. Figs. 7, 8, 9 and 10; strains derived from C2213, C2235a, C2235b and C2235b in Table 1, respectively.

posed of homothallic (D/D) and heterothallic (a/α) diploid strains originating from a single ascus. Heterothallic diploid strains were obtained by the cross of a and α haploid segregants. They were cultured in VHG-BII medium for 24 hrs and transferred to the sporulation medium. Spore forming cells were counted daily. As shown in Figs. 7-10, homothallic strains sporulated in significantly higher percentage than heterothallic strain in each of the groups tested. The homothallic character and higher sporulation ability always accompanied together, indicating that the D gene affects the sporulation ability.

DISCUSSION

In *Saccharomyces* yeast heterozygote for mating type alleles (a/α) sporulates, but homozygotes (a/a or α/α) do not. In the latter, even the initiation of DNA synthesis

necessary for spore formation does not occur in the sporulation medium (Roth and Lusnak 1970). It has also been known that yeast cells produce a special new type of RNA at an early stage of sporulation, and this RNA is detectable even in a/α disomic strains which do not produce mature asci (Kadowaki and Halvorson 1971). Thus, heterozygosity of mating type alleles, a/α seems necessary for cells to sporulate. And the effect of *D* gene on sporulation seems to be explained in terms of its mutator action, which brings about a/α heterozygosity in homothallic diploid strains. In this paper, however, we have demonstrated that *D* gene itself causes higher sporulation ability, and not through its mutator action.

Imada (1971) has shown a possibility that *HMx* homothallism-controlling genes (Takahashi 1958) show the same type of effect on sporulation as that of *D* gene shown in the present paper. He also observed the same effect of the homothallism-controlling genes on sporulation ability even when an acetate medium was used for presporulation culture.

Thus, we may conclude that homothallism-controlling genes have an effect of promoting sporulation.

SUMMARY

Effect of a homothallism-controlling gene on sporulation was studied, using isogenic yeast strains carrying and not carrying *D* gene. In all cases tested, *D* gene showed distinct promotive effect on sporulation, irrespective of the period of incubation in presporulation or sporulation medium. However, *D* gene has no significant effect on vegetative growth in presporulation culture.

ACKNOWLEDGMENTS

We would like to express our hearty thanks to Prof. J. Ashida of Ehime University and Prof. Y. Masuda and Mr. C. Shimoda of Osaka City University for their invaluable discussions.

LITERATURE CITED

Croes, A. F., 1967 Induction of meiosis in yeast. II Metabolic factors leading to meiosis. Planta **76**: 227-237.

Hawthorne, D. C., 1963 Direct mutation of the mating type alleles as an explanation of homothallism in yeast. Proc. 11th Intern. Cong. Genetics **1**: 34-35.

Imada, T., 1971 Sporulation and sex controlling gene in *Saccharomyces cerevisiae*. Master Thesis, Osaka City University.

Kadowaki, K., and H. O. Halvorson, 1971 Appearance of a new species of ribonucleic acid during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **105**: 826-830.

Roth, R., and K. Lusnak, 1970 DNA synthesis during yeast sporulation. Genetic control of an early developmental event. Science **168**: 493-494.

Takahashi, T., 1958 Complementary genes controlling homothallism in *Saccharomyces*. Genetics **43**: 705-714.

Takahashi, T., 1970 Ascospore formation and sex controlling genes in *Saccharomyces*. Bull. Brew. Sci. **16**: 31-40.

Yanagishima, N., C. Shimoda, T. Takahashi, and N. Takao, 1970 Responsiveness of yeast cells to auxin, animal sex hormones and yeast sexual hormones in relating to sex controlling genes. Develop. Growth and Differ. **11**: 277-286.