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GENETIC CONTROL OF HYDROGEN SULFIDE RETENTION IN SACCHAROMYCES CEREVISIAE

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Summary: The ability to retain hydrogen sulfide (H_2S) in Saccharomyces cerevisiae is under nuclear gene control. Mutants with the ability to retain greater amounts of H_2S than the parent have been isolated and characterised.

Introduction:

It is well known that fermenting yeasts reduce sulfur, sulfites and sulfates to sulfides. In dilute acidic media such as wine and beer, sulfide has a low threshold value and thus causes an objectionable aroma. Although much is known about its production (Rankine, 1963; Wainwright, 1970; Schutz and Kunkee, 1977; Eschenbruch, 1974; Rupela and Tauro, 1979) the problems of controlling its release into the growth medium is yet unsolved. Selection of yeasts that release less of H_2S into the growth medium has been one of the approaches used to reduce the H_2S content of these beverages. Using bismuth sulfite agar we have earlier shown that it is possible to isolate spontaneous mutants from both a wine yeast and a haploid yeast which release less H_2S into the growth medium (Rupela and Tauro, 1984). In this paper we report that the ability to retain a greater amount of H_2S within the yeast cells can be altered by nuclear gene mutation.

Materials and Methods:

The haploid yeast strain S. cerevisiae S-288Cα was from Professor R.K. Mortimer, Donner Laboratory, University of California, Berkeley, USA, and strain S-288Ca was derived from the former. These cultures were maintained on YEPD agar

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slopes (Yeast extract, 0.2%, Peptone 0.5%, Dextrose 2% and Agar 2%). Spontaneous mutants that retain greater amounts of H_2S (H_2S(ret) were isolated from each of the two strains independantly by the method described earlier (Rupela and Tauro, 1984). On the YEPD-bis agar medium, the parental cultures have a brown phenotype while the H_2S retainers have a black phenotype.

To establish the genetic control of this phenomenon, preliminary genetic analysis was carried out using two H_2S(ret) mutants of the opposite mating types as described by Sherman et al (1972). From each cross, at least 10 four spored asci were dissected and the segregation ratios were determined using asci from which all the four spores were viable.

The amount of H_2S within the cells and in the growth medium was estimated as described by Acree et al (1971) and by Rupela and Tauro (1984).

Results and Discussion:

Earlier, we had reported the isolation of two mutant types namely H_2S(ret) and H_2S(ex) which differed in the amount of H_2S retained or excreted, respectively (Rupela and Tauro, 1984). We had also reported that the ability to retain this chemical within the cells is an energy dependant phenomenon and that inducing respiratory deficiency would allow greater excretion of this chemical into the growth medium without making the strains dependant on sulfur amino acids. To examine the nature of genetic control of the H_2S(ret) phenomenon, two H_2S(ret) mutants of the opposite mating type selected randomly were crossed between themselves as well as with the parental culture. The diploids were isolated by micromanipulation and analysed further (Table 1). On YEPD-bis agar the H_2S(ret) diploid was black while the diplid from the back cross was brown. This suggested that the two mutants used were noncomplementing. Further, on sporulation and tetrad analysis, it was found that the H_2S(ret) phenotype segregated like a normal nuclear gene segregation, indicating that the ability to retain greater amounts of H_2S within the cells can be altered by nuclear gene mutation.

To further establish that the character segregates quantitively, all four spores from one ascus from the back cross were cultured in YEPD-bis broth and the amount of H_2S in culture broth and in the cells was determined. It was found that the amount of H_2S retained within the cells is greater in the spores with the H_2S(ret) character and the pattern is consistent with nuclear gene segregation.

Table 1. Genetic analysis of H_2S(ret) mutation*

Yeast strain	Phenotype on YEPD-bis agar	Spore segregation		H2S content	
		Brown	Black	Broth (ppb)	Cells (ug/g)
S-288C α, haploid	brown	-	-	-	-
S-288C a, haploid	brown	-	-	676	122
H2S(ret), haploid	black	-	-	520	207
H2S(ret) a x H2S(ret)α diploid	black	0	4	-	-
H2S(ret) a x S-288Cα, diploid	brown	2	2	-	-
Spore 1	black	-	-	510	281
2	brown	-	-	678	165
3	brown	-	-	780	165
4	black	-	-	570	274

*Cells were grown in 200 ml of YEPD broth containing 0.8% bismuth sulfite for 24 hr, after which the cells and supernatent were separated by centrifugation at 3,000 rpm for 15 minutes. The H2S content of the pellets and the supernatent was determined as described by Acree et al (1971); Rupela and Tauro, 1984.

These results confirm our earlier finding that yeast strains which can retain more of H_2S within the cells can be isolated by genetic alteration. In this paper we show that this character is under nuclear gene control. The number of loci that determine this character is at present unknown. Our intention in doing the preliminary genetic analysis was only to verify if this phenomenon is under nuclear or cytoplasmic gene control. Unlike the excretion phenomenon, the ability to retain H_2S is not a pleiotrophic effect of respiratory deficiency. The two mutants used in this study were derived independantly from two parents and their inability to complement is only incedental. Detailed complementation studies are required to establish the number of loci that determine the H_2S(ret) phenomenon in yeast.

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