TITLE

The Role of the Heat Shock Proteins in the Thermotolerance of Distillery Yeast Strains.

SUMMARY

1. Stable haploid derivatives of a diploid homothallic distillery yeast strain were constructed by targeted disruption of the *HO* gene.

2. Additional haploid strains isogenic to the distillery yeast, but having the *ho* and *ura3-52* mutations of a laboratory strain were also constructed by genetic crosses, for use in gene disruption and overexpression studies.

3. The *HSP104* gene of the distillery yeast was disrupted to study its role in stress tolerance. The disruptant was found to be more sensitive to heat shock as expected. Its survival was also poor under high temperature fermentation conditions, indicating that *HSP104* provides stress tolerance to yeast cells under these conditions.

4. *HSP104* coding sequence was cloned under the control of a strong constitutive GPD promoter. Either 5'-region of the coding sequence or the entire coding sequence was cloned with or without 200 bp upstream region of *HSP104.* These constructs were linearized within *HSP104* and targeted to the *HSP104* locus of the yeast genome. In the constructs with only 5'-region of *HSP104,* after integration, the *HSP104* fully comes under the control of GPD promoter. In the constructs with entire coding sequence of *HSP104,* the gene gets duplicated with one gene under the control of native heat shock promoter and the other under the control of GPD promoter.

5. Pattern of overexpression of *HSP104* in the recombinant yeast strains was checked by western blot analysis. The constructs with the GPD promoter about 200 bp away from the coding sequence produced negligible amount of Hsp104 both at 25°C and 37°C. The constructs with the coding sequence next to the GPD promoter produced a large amount of

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10. To directly identify mutants impaired in thermotolerance, there is as yet no reliable plate screen. Parallel screening of a large number of such mutants in liquid broth is also difficult with existing methods. For simultaneous monitoring of individual mutants in mixed populations we have developed a novel method named as quantitative target display. Here mutants are obtained by transposon mutagenesis and DNA fragments corresponding to the mutated genes are amplified and used to distinguish the mutants from each other.

11. Conditions were optimized to selectively amplify only the DNA flanking transposon insertions (ie. target sequences). Selective amplification was confirmed by amplifying the targets from known mutants.

12. Amplification of targets is quantitative, that is, the intensity of the amplified DNA is proportional to the abundance of the corresponding mutant.

13. DNA fragments corresponding to over 1000 mutants can be simultaneously amplified and displayed by quntitative target display. Primers with selective bases at their 3'-termini were used in 16 different combinations to display a limited number of fragments per lane.

14. A mixed population of mutants mutated in various HSP genes were tested for their heat shock tolerance by quantitative target display. The *HSP104* and *TPS2* mutants disappeared on heat shock, as expected. Even a small change in the abndance of a mutant could be monitored. These results confirm that this method can be used to quantitatively follow the abundance of mixed population of mutants during selection.

Hsp104 constitutively. The amount of Hsp104 seen in this construct at 25°C was about five-fold higher than that seen in wild type strain at 37°C.

6. In spite of the high level of Hsp104 in the recombinant yeast strains, their heat shock tolerance or survival during high temperature fermentation was not any better than wild type strain. Since normal level of heat shock tolerance was seen, the protein is functional.

7. The lack of improvement in heat shock tolerance in the distillery strain on *HSP104* overexpression is perhaps due to difference(s) in the genetic make up of this strain from the laboratory strains. When the same overexpressing plasmid was integrated in the genomes of two different laboratory strains, a substantial improvement in heat shock tolerance of cells grown at 25°C was seen, though it was not as much as that of wild type cells subjected to 37°C preincubation. This indicated that overexpression of *HSP104* alone is not sufficient for heat shock tolerance.

8. To check if simultaneous overproduction of Hsp104 and Ssa1 would improve heat shock tolerance, such yeast strains were constructed and checked. Overexpression of *SSA1* in a laboratory strain already overexpressing *HSP104* further improved its thermotolerance, though overexpression of *SSA1* alone did not help. Simultaneous overexpression of *HSP82* in strain already overexpressing *HSP104* did not improve the thermotolerance.

9. To directly identify genes which on overexpression alone or in combination with *HSP104* increase thermotolerance, a yeast genomic overexpression library (under the control of a constitutive promoter) was transformed into yeast and transformants with increased thermotolerance were enriched by repeated heat shock. Five plasmids conferring increased thermotolerance to cells already overproducing Hsp104 were identified, and are being characterized.