J Ind Microbiol Biotechnol (2013) 40:393–397
DOI 10.1007/s10295-013-1241-2

SHORT COMMUNICATION

Construction of ploidy series of *Saccharomyces cerevisiae* by the plasmid YCplac33-GHK

Lihua Hou · Xiaoyang Li · Cong Wang · Xiaohong Cao · Haiyong Wang

Received: 17 December 2012 / Accepted: 2 February 2013 / Published online: 22 February 2013
© Society for Industrial Microbiology and Biotechnology 2013

Abstract An effective approach, using the plasmid YCplac33-GHK, is developed to construct a ploidy series of *Saccharomyces cerevisiae*. YCplac33-GHK harbors the *HO* gene under the control of galactose-inducible promoter and *KanMX4* as the selective marker. The simple method can solve the problem of industrial applications of strains with resistance genes.

Keywords *Saccharomyces cerevisiae* · Plac33-GHK · Polyploidy · Yeast

Introduction

Polyploidy has widely been observed in industrial fermentation of *Saccharomyces cerevisiae* (e.g., baking, brewing, distilling, and wine yeasts) [21]. Compared with diploid yeast, polyploidy yeast has more advantages in industrial fermentation [16]. Polyploidy can confer on yeast cells a greater level of protection against the occurrence of spontaneous lethal, changed gene dosages or detrimental recessive mutations, thus improving fermentation properties [19]. In recent years, growing attention has been devoted to the improvement of the desirable products, such as ethanol production and beer flavor by using polyploidy of *S. cerevisiae* [1, 6].

L. Hou (✉) · X. Li · C. Wang · X. Cao · H. Wang
Key Laboratory of Food Nutrition and Safety, Tianjin University of Science & Technology, Ministry of Education, Tianjin 300457, People's Republic of China
e-mail: lhhou@tju.edu.cn

Mutagenesis methods were used to construct ploidy series of *S. cerevisiae* in a previous study, which were laborious and time-consuming. Besides, polyploid series were constructed by repeating crosses between auxotrophic cells [22]. However, the method can be only applied for strains with specific genetic markers. It is thus impossible to work with industrial strains without any auxotrophy. In addition, the heat-induced endomitotic diploidization was used to generate polyploid cells of various wild-type strains of industrial yeast without specific selectable genetic markers [20]. However, this approach was low in efficiency to generate polyploid cells. Here, a simple and effective method of the plasmid YCplac33-GHK was developed to construct a polyploidy series.

Mating type of *S. cerevisiae* is determined by two different alleles of the mating-type (*MAT*) locus [15]. Like many other fungi, diploid cells can change into haploid cells through meiosis and haploid cells by mating of different mating-type cells. Moreover, *S. cerevisiae* has the capacity to alter some cells in a colony from one haploid mating type to another. *HO* gene encodes the site-specific endonuclease responsible for inducing mating-type gene switching, thus MAT a cells can change to MAT alpha cells or vice versa [9]. However, many mutations at the *HO* locus have been accumulated in the yeast strains used in industrial fermentations. In this study, the *HO* gene without any mutations was thus cloned into the single-copy plasmid YCplac33 [4] under the control of the inducible promoter of the yeast gene *GAL2*. Besides, *KanMX4* gene as the selective gene marker was also inserted into the vector. The plasmid that contains the *KanMX4* selectable marker, can be selected by resistance to G418 [8], thus resolving the problem of industrial strains without the selective marker.

Materials and methods

Yeast strains and growth conditions

The strain WT used in this study was derived from the diploid industrial strains TH-AADY of *S. cerevisiae* (Angel Yeast, China). The original (WT) strain was void of the *HO* gene. YPD was 20 g/l peptone, 10 g/l yeast extract and 20 g/l dextrose. YPDK was 20 g/l peptone, 10 g/l yeast extract, 20 g/l dextrose and 0.2 g/l G418 (Sigma, St Louis, MO, USA). YPGK was 20 g/l peptone, 10 g/l yeast extract, 20 g/l galactose and 0.2 g/l G418. 1 % (W/V) CH3COOK was used for the plates of CH3COOK. In addition, 1.5 % agarose were used in plates.

Plasmids construction

PCR using the previously described method [12] was carried out to clone the *HO* gene from the plasmid *HO* [11] with primers HO-R: 5′-gggcccgagctcatgctttctgaaaacacgac-3′ (*Sac*I) and HO-F: 5′-gggcccccggggttaagactgcattcatcac-3′ (*Sma*I). The resulting PCR product was digested and then inserted into the corresponding sites of the plasmid YCplac33, generating the plasmid pYCplac33-H. Subsequently, the promoter of *GAL2* was amplified using primers GAL-R: 5′-gggcccgaattccgccagatctgtttagcttg-3′ (*EcoR*I) and GAL-F: 5′-gggcccgagctcagtatcgaatcgacagcag-3′ (*Sac*I). The resulting PCR product was introduced into the plasmid pYCplac33-H, forming the plasmid pYCplac33-GH. Afterwards, the primers Kan-R: 5′-gggccctctagagcgaaggcacatctattac-3′(*Xba*I) and Kan-F: 5′-gggcccgcatgcgttctcctcaactgccatta-3′(*Sph*I) were used to amplify *KanMX4* from the plasmid pFA6-*KanMX4*. The resulting PCR product was cloned into the plasmid pYCplac33-GH, creating the plasmid pYCplac33-GHK.

Results and discussion

Ploidy series were constructed in several steps as follows. Firstly, the resulting plasmids were transformed into the strain WT by the lithium acetate method [18]. The resulting strains were cultivated on an YPDK plate at 30 °C for 2 days. A colony was screened from the above plate and streaked on an YPGK plate. After cultivation at 30 °C for 20 h, a colony selected from the YPGK plate was streaked on an YPDK plate. Subsequently, mating-type of 16 colonies were randomly selected from the YPDK plate. To analyze mating-type of yeast cells [13], colony PCR was carried out with primers MAT-F: 5′-agtcacatcaagatcgtttatgg-3′, MAT-a: 5′-gcacggaatatgggactacttcg-3′ and MAT-alpha: 5′-actccacttcaagtaagagtttg-3′. If the PCR product was 544 bp, the colony was MAT a cell. When the PCR product was 404 bp, it was MAT alpha cell. MATa/alpha cell was determined when there were two panels of 544 and 404 bp at one time. In this study, MAT a/a, MAT alpha/alpha and MATa/alpha cells were obtained. Sixteen random colonies from the YPDK plates were testing by PCR and the data is shown in Fig. 1. Lanes 3, 4, 8, and 14 were MAT a/a. Lanes 2, 5, 6, 7, 9, 10, 12, and 15 were MAT alpha/alpha. Lanes 1, 11, 13, and 16 were MAT a/alpha cells. MAT a/a cells and MAT alpha/alpha cells were then streaked on the YPD plate in order to lose the plasmid YCplac33-GHK by the previously described method [10]. Many colonies of MAT a/a cells on YPD plate were copied to YPDK plate. The colonies, which could grow on the YPD plate but not on YPDK, were the MAT a/a cells without the plasmid YCplac33-GHK, similar to MAT alpha/alpha cells. MAT a/a cells and MAT alpha/alpha cells without the plasmid were named H21 (*MAT a/a*) and H22 (*MAT alpha/alpha*), respectively. Figure 2 shows the PCR verification results of H21 and H22.

Secondly, H21 and H22 were mixed at 30 °C for 16 h. Some resulting cells were streaked on the YPD plate. Mating-type of the colonies selected from the above plate PCR was carried out to achieve tetraploid cells. The cells (*MAT a/a/alpha/alpha*) were selected and denominated H40 (Fig. 3). The strain H40 was streaked on the plates of CH_3COOK and cultivated at 28 °C for 3 days. Sporulation and ascus dissections, in which all four spore colonies did

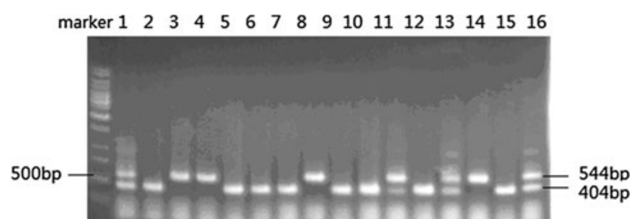

Fig. 1 Agarose gel electrophoresis of PCR product was used to test MAT a/a cells, MAT alpha/alpha cells, and MAT a/alpha cells. In this study, DNA marker included *14 lanes* of 250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000, 6,000, 8,000, and 10,000 bp

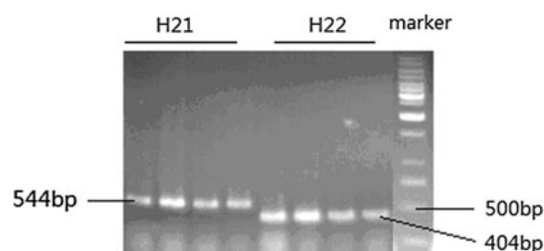

Fig. 2 Agarose gel electrophoresis of PCR product was used to test H21 and H22

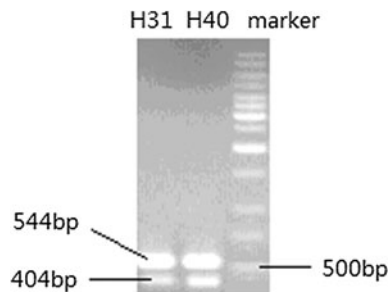

Fig. 3 Agarose gel electrophoresis of PCR product was used to test H31 and H40

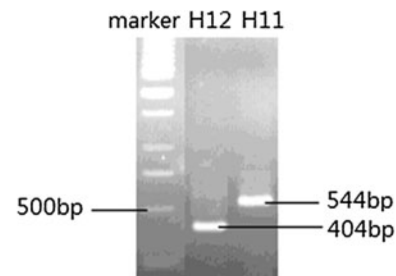

Fig. 4 Agarose gel electrophoresis of PCR product was used to test H11 and H12

not cross, were conducted to verify that H40 was tetraploid strain [17].

Finally, the diploid strain WT was streaked on KAC plates to form spores. The haploid cells (*MAT a*), H11, and haploid cells (*MAT alpha*), H12, were determined from the spores, respectively. H11 and H12 were tested by PCR (Fig. 4). H11 and H22 were mixed to obtain triploid cells H32 (*MAT a/alpha/alpha*). Meanwhile, H12 and H21 were crossed to construct triploid cells H31 (*MAT a/a/alpha*) (Fig. 3). It was found that spores from H31 to H32 were non-viable, which further indicated the strains H31 and H32 were triploid strain [17].

Cell morphology such as size and shape was observed using a microscope. Cell size increased with an increase of cell ploidy (data not shown). Meanwhile, no obvious differences of the shape between these cells were found. Our results agreed well with the data of other similar studies. DNA content, cell mass, and total protein of diploid strain WT, triploid strain H31, and tetraploid strain H40 are presented in Table 1. DNA was extracted from the mid-exponential phase cells with perchloric acid and determined using the diphenylamine assay [7]. The protein was examined by Bio-Rad method [3]. Dry cell mass was analyzed with weighing method [2]. The results further demonstrated that these strains were correctly constructed.

Genetic stabilization of the polyploid strains was analyzed by sub-cultivating for 12 generations. No distinctive difference of growth characteristic between the offspring and parent strains of tetraploidy was found (data not shown). FACS analysis [5] was carried out to further observe the stability of triploid strain and tetraploid strain. As shown in Fig. 5, the initial cells (top) and the cells for 12 generations (bottom) were stained with propidium iodide. The horizontal axis represents DNA content and the vertical axis indicates number of cells. The first and second peak in the histogram indicates prereplication and postreplication cells, respectively. These data demonstrated that the polyploid strains exhibited genetic stabilization.

Using the plasmid pYCplac33-GHK, another polyploid series [12] was constructed from the engineered strain

Table 1 DNA content, cell mass, and total protein of the triploid strain (H31), the tetraploid strain (H40) and the diploid strain (WT) per 10^9 cells is shown

Strains	DNA (μg)	Cell mass (mg)	Total protein (mg)
WT	49.18 ± 0.35	41.49 ± 0.62	30.53 ± 0.42
H31	62.41 ± 0.51	61.72 ± 0.69	48.42 ± 0.47
H40	86.50 ± 0.46	80.91 ± 0.73	59.38 ± 0.50

Data indicates the average and SD corresponding to at least three independent experiments

FTG2 (*MATa/alpha, fps1Δ::REPEAT, gpd2Δ::REPEAT, GLT1-PGK1*) obtained in previous work in our laboratory [14]. It was found that the tetraploid strain increased the ethanol yield by 4.67 % in the ethanol fermentation with the initial glucose of 20 %. Besides, aneuploid strains can be easily obtained using methyl benzimidazole-2-ylcarbamate on the base of the tetraploid strain. The tetraploid strain and the aneuploid strain can be used to improve the ethanol production in ethanol fermentation [12].

Conclusions

Here, we describe the method of plasmid YCplac33-GHK to construct a polyploidy series. The technique used the mate-type switching induced by the Ho protein encoded by the *HO* gene. The approach was a simple and effective way to achieve polyploid strains. Compared with the previous means, this method contained five advantages as follows. The genotype background of the polyploid strains constructed in this study was known, which benefited the further research on them. On the contrary, the genotype of the polyploid strains obtained by the previous means [11] was unknown because the mutations were uncertain. The second virtue was that the method can be used in both the yeast strains with selective maker (such as laboratory strain) and the industrial yeast strains without selective maker. However, many previously described methods [21] were only applied in the laboratory strain with specific

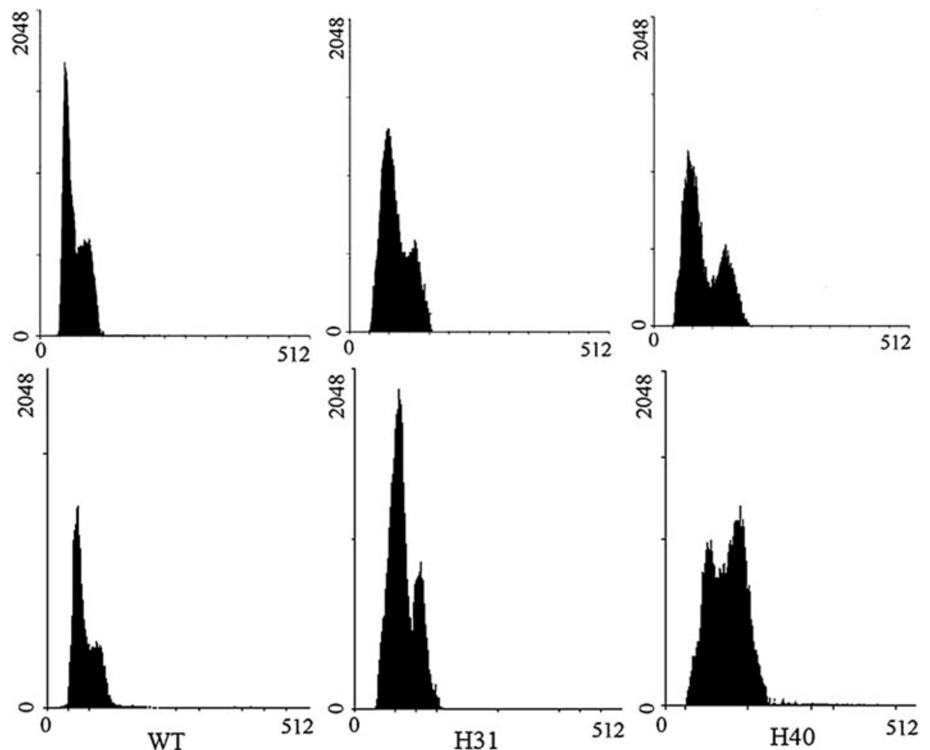

Fig. 5 FCAS of the initial cells (*top*) and the cells for 12 generations (*bottom*) of triploid strain H31, tetraploid strain H40, and the control strain WT

genetic markers. The third advantage was that the technique described here was simple and effective. Due to the low frequency of isolating polyploid cells, the previous methods [13] were laborious and time-consuming. As is known, the strains with resistance to drugs cannot be used in industry. The polyploid strains constructed in the present work overcome the difficulty of application in industry, which was the fourth merit of the method. In addition, the plasmid YCplac33-GHK can be finally lost in the host cells, thus avoiding the environmental pollution. It should be noted that the polyploid strains constructed by the plasmid YCplac33-GHK can provide enough yeast strains for the beer and ethanol fermentation industries.

Acknowledgments This work was supported by the following projects in China (2012AA022108, 2012GB2A100016, 31171731, 2013AA102106, IRT1166, 2012BAD33B04, 10ZCZDSY07000, and 31000768).

References

1. Bidenne C, Blondin B, Dequin S, Vezinhet F (1992) Analysis of the chromosomal DNA polymorphism of wine strains of *Saccharomyces cerevisiae*. Curr Genet 22:l–7
2. Borzani W (2001) A weighing method to identify the microbial growth phases in solid-state fermentation tests. World J Microbiol Biotechnol 16:601–605
3. Bradford MM (1976) A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
4. Caizares JV, Pallotti C, Saínz-Pardo I, Iranzo M, Mormeneo S (2002) The SRD2 gene is involved in *Saccharomyces cerevisiae* morphogenesis. Arch Microbiol 177(4):352–357
5. Carlson CR, Grallert B, Bernander R, Stokke T, Boye E (1997) Measurement of nuclear DNA content in fission yeast by flow cytometry. Yeast 13:1329–1335
6. Diiorio AA, Weathers PJ, Campbell DA (1987) Comparative enzyme and ethanol production in an isogenic yeast ploidy series. Curr Genet 12:9–14
7. Gercel-Taylor C (2005) Diphenylamine assay of DNA fragmentation for chemosensitivity testing. Methods Mol Med 111:79–82
8. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast 15:1541–1553
9. Haber JE (1998) Mating-type gene switching in *Saccharomyces cerevisiae*. Annu Rev Genet 32:561–599
10. Hegemann JH, Klein S, Heck S, Ldener U, Niedenthal RK, Fleig U (1999) A fast method to diagnose chromosome and plasmid loss in *Saccharomyces cerevisiae* strains. Yeast 15:1009–1019
11. Herskowitz I, Jensen RE (1991) Putting the *HO* gene to work: practical uses for mating-type switching. Methods Enzymol 194:132–146
12. Hou LH, Cao XH, Wang CL (2010) A novel approach for the improvement of ethanol fermentation by *Saccharomyces cerevisiae*. Can J Microbiol 56:1–6
13. Houston P, Simon PJ, Broach JR (2004) The *Saccharomyces cerevisiae* recombination enhancer biases recombination during interchromosomal mating-type switching but not in interchromosomal homologous recombination. Genetics 166:1187–1197
14. Kong QX, Gao JG, Cao LM, Zhang AL, Chen X, Zhao XM (2006) Improved production of ethanol by deleting *FPS1* and over-expressing *GLT1* in *Saccharomyces cerevisiae*. Biotechnol Lett 28:2033–2038
15. Kostriken R, Strathern JN, Klar AJ, Hicks JB, Heffron F (1983) A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. Cell 35:167–174

16. Querol A, Bond U (2009) The complex and dynamic genomes of industrial yeasts. FEMS Microbiol Lett 293(1):1–10
17. Salmon JM (1997) Enological fermentation kinetics of an isogenic ploidy series derived from an industrial *Saccharomyces cerevisiae* strain. J Ferment Bioeng 83:253–260
18. Schiestl RH, Gietz RD (1989) High-efficiency transformation of intact yeast cells using single stranded nucleic acids as carrier. Curr Genet 16:339–346
19. Takagi A, Harashima S, Oshima Y (1983) Construction and characterization of isogenic series of *Saccharomyces cerevisiae* polyploid strains. Appl Environ Microbiol 45:1034–1040
20. Tani Y, Tomohiro Y, Miyata A, Kurokui T, Hayashida S (1993) A novel method for breeding polyploid cells by heat-induced endomitotic diploidization in *Saccharomyces cerevisiae*. Biosci Biotech Biochem 57:2063–2066
21. Vezinhet F, Bloodin B, Hallet JN (1990) Chromosomal patterns and mitochondrial DNA polymorphism as tools for identification of enological strains of *Saccharomyces cerevisiae*. Appl Microbiol Biotech 32:568–571
22. Waghmare SK, Bruschi CV (2005) Differential chromosome control of ploidy in the yeast *Saccharomyces cerevisiae*. Yeast 22:625–639