Effect of Growth Media and Strains on Structural Stability in Small Chromosomes (Chromosome I, VI and III) of Bottom-Fermenting Yeast

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ABSTRACT

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The PFGE analysis results of 50 clones, each isolated from cultures of two lager strains (P and Q), after passage of 700 generations in wort or YEPD, showed that small chromosomal DNAs (I, VI and III) are susceptible to changes during successive cultivation. These changes may be strain-dependent (P > Q) and the media may also affect the instability in the chromosomal DNAs (YEPD > wort).

Key words: Bottom-fermenting yeast, genetic instability, PFGE.

INTRODUCTION

It has been reported that the length of the chromosomes of *S. cerevisiae* changed during successive cultivation. Southern analysis using the DNA probe *URA3* showed that recombination between homologous chromosomes in chromosome V of *S. carlsbergensis* happened during cell division[4]. Also in wine yeast, changes in the length of chromosome I and VI were observed during the process of vegetative propagation[3]. We have previously reported that during the course of studying the karyotypes of brewing yeasts with Pulsed Field Gel Electrophoresis (PFGE), differences in PFGE patterns among some strains from the same origins (lager strains P and Q) were found in small chromosomal DNAs such as chromosome I, VI and III[5]. The results of Southern analyses using *FLO1, ACT1* and *HIS4* probes strongly supported our finding about instability of these chromosomal DNAs[5]. Further analysis of small chromosomes of Q-derived strains showed differences in banding patterns near chromosome VI[11]. This finding enabled us to classify them into five types.

In this article the structural stability in small chromosomes (I, VI and III) was analysed with PFGE, after repeated cultivation of two lager yeasts (P and Q).

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EXPERIMENTAL

Yeast strains

Lager strain P (SBC0001) and Q (SBC0002) were the parental strains used in this study. *S. cerevisiae* YNN295 (Bio-Rad) was used as the size standard. P and Q strains were shown as strains A and B in our previous paper[5].

Media and cell growth in serial cultivation

Single-colony isolates from strain P and Q with the same PFGE patterns as those of their parental strains were used in this trial. Each yeast was inoculated into a 100 mL flask containing wort (11°P) or YEPD (10 g yeast extract/L, 20 g peptone/L, 20 g glucose/L) and cultivated with shaking (100 rpm) at 20°C for 2 days. A portion of the culture was re-inoculated into the next flask and cultivated under the same conditions. The yeast was pitched at 10^5 cells/mL and grew to 10^8 cells/mL, i.e. approximately 10 generations. This procedure was repeated 70 times, corresponding to the passage of approximately 700 generations. Finally, 50 clones were isolated from each culture and analysed with PFGE.

Pulsed-field gel electrophoresis conditions

The electrophoresis and sample preparation were performed according to the methods described in our previous papers[5,8].

RESULTS AND DISCUSSION

We investigated instability in the small chromosomes (I, VI and III) of derivatives obtained from P and Q lager strains after serial cultivations of 700 generations in wort or YEPD. Table I shows that the frequency of the structural modification was larger in the P strain than in the Q strain. The three small chromosomes of the two lager strains were not equally susceptible to structural modification. Chromosome I in strain P and chromosome VI in strain Q were comparatively unstable. The frequency of the structural modification was dependent on the media. In the case of YEPD, the frequency was higher than in the case of wort. The results suggested that the susceptibility in chromosomal DNAs during successive cultivations might be strain-dependent and that the growth media also might affect instability in the chromosomal DNAs.

Fig. 1 shows the PFGE patterns of chromosome I regions in P-derived strains after successive cultivation. In

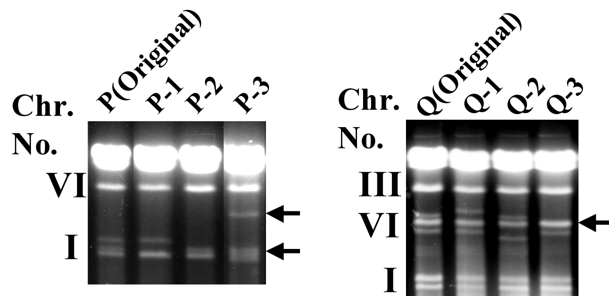

FIG. 1. The differences in PFGE patterns of derivative strains obtained from P and Q strains after passage of 700 generations.

strain P, chromosome I was the most unstable (Table I). In 21 of the 25 clones showing some alterations in small chromosomes, changes were observed in this region. These strains were classified into three types (P-1, -2 and -3). In the original strain P, three bands were observed in the region of chromosome I. In type P-1 (one clone), the middle band in this region was not detected, whereas in type P-2 (19 clones), the highest band was not detected. In type-3 (one clone), a band was observed in the region between chromosome VI and chromosome I. This may be the same type as strain A35-3 reported in a previous paper[5].

In strain Q, chromosome VI was the most unstable (Table I). In five of the six clones, showing some alterations in small chromosomes, changes were observed. These strains were also classified into three types (Q-1, -2 and -3). In original strain Q, three bands were observed in the region of chromosome VI. We have previously reported differences in the banding patterns near chromosome VI of Q-derived strains and this enabled us to classify them into five types[11]. In type Q-1 (one clone), the highest band in this region was observed in a higher region than that of the original strain Q. This type was not found in the previous study. In type Q-2 (one clone), the length of the lowest band was shortened, whereas in type Q-3 (three clones) the highest band was not detected. These types correspond to type-3 and type-4 respectively in the previous study[11]. In chromosome VI and III of P-derived strains and in chromosome III of Q-derived strain, minor differences were observed.

The genetic alterations described in this study might affect the brewing performance of these strains. Therefore, many of the physiological and genetic properties of brewing yeasts used in our breweries were studied in our laboratory for several years. The physiological properties included extract consumption curves, flocculation, extracellular proteinase A activity, production of sulphite, ester profiles, etc[11]. However, until now, there was no evidence showing a clear relationship between brewing performance and such genetic changes. From the results of our long term study of the many brewing properties of yeast, flocculation was found to be the most variable[6]. It is generally believed that the flocculation characteristic of bottom-fermenting yeast is unstable[1,2,10]. We have previously shown that a decrease in the flocculation of bottom-fermenting yeast occurred when the ratio of the non-flocculent cells to the flocculent cells increased in the yeast cell population. Proposed models for the conversion of flocculent to non-flocculent cultures from the results of Southern and Northern analyses revealed that genetic changes in the Lg-*FLO1* gene were responsible for the loss of flocculation[6,7].

Table I. Instability in small chromosomes (I, VI and III) of derivatives from P and Q strains after passage of 700 generations.

Yeast	Medium	Chr. I	Chr. VI	Chr. III	Total
P	Wort	5	1	0	6/50 (12%)
	YEPD	16	1	2	19/50 (38%)
Q	Wort	0	1	0	1/50 (2%)
	YEPD	0	4	1	5/50 (10%)

The numbers express the changes in banding patterns of derivatives to the corresponding chromosome region.

Taking into consideration the genetic instability observed in the chromosomal DNA banding patterns, Restriction Fragment Length Polymorphism (RFLP) of the mt-DNA and the flocculation gene Lg-*FLO1,* bottom-fermenting yeast are thought to be genetically very unstable in nature. It is generally accepted that the brewing yeast is a polyploid consisting of chromosomes from *S. cerevisiae* and *S. bayanus.* The chromosomes of the bottom-fermenting yeast were analysed using various DNA probes, and it was shown that the chromosomes of *S. bayanus* existed in the bottom-fermenting yeast, in addition to the chromosomes of *S. cerevisiae*[9,13]. Because of complexity in its generation process, it was thought that the genetic rearrangement such as the mutual translocation between various chromosomes, etc. occurred[12]. This may be related to the instability in the yeast's genome.

To investigate whether the genetic alteration observed in this study affects their brewing performance, further analyses for the alteration points in chromosomes of derivative strains obtained from strain P and Q will be undertaken.

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