Cloning of a new FLO gene from the flocculating Saccharomyces cerevisiae IM1-8b strain

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Abstract

A flocculation conferring gene was cloned from a genomic library of the flocculating strain Saccharomyces cerevisiae IM1-8b as a 5 kb DNA fragment. The shortest DNA fragment (XbaI-XbaI) able to confer the flocculating phenotype was 3.1 kb. Southern analysis revealed that this gene was not homologous to the already reported FLO1 gene since strong hybridization signals were obtained when chromosomes IV and XII were probed with a digoxigenin-labelled fragment and no signal at all was detected for chromosome I. Partial sequencing data unequivocally ascribed the cloned fragment to chromosome XII. The gene was detected in a variety of S. cerevisiae strains regardless of their being phenotypically flocculating. This gene which, we propose as FLO2, is able to complement the flo1 mutation and is suppressed by suppressors (fsu3) that do not affect other FLO genes.

Keywords: Saccharomyces cerevisiae; Flocculation; FLO2; Cloning

1. Introduction

Flocculation in Saccharomyces cerevisiae describes a reversible process of cell aggregation (Ca²⁺-dependent) by which yeast cells adhere, forming clumps that spontaneously sediment in the medium. Because it leads to efficient separation of the yeast cells from the fermenting medium, this characteristic is a suitable property for yeast strains involved in certain industrial fermentations such brewing, wine making and the biological production of ethanol [1] (for a review see [2]).

Flocculation in S. cerevisiae involves protein-sugar interactions between specific lectin-like surface proteins and the cell wall mannan [3]. This hypothesis is supported by the fact that protein denaturation causes an irreversible loss of flocculation capacity but does not affect receptor sites. Moreover, flocculation can also be reversibly inhibited by the presence of sugars [4,5].

Flocculation in S. cerevisiae is under genetic control. Several genes affecting flocculation have been reported and mapped using classical genetic methods. Two genes are dominant: FLO1, allelic with FLO8 [6], located at 37 cM from the ade1 marker [7] and 4.7 cM from the PHO11 gene [8] on chromosome I; FLO5, mapped on chromosome VIII at 34...
Table 1

Saccharomyces cerevisiae strains used in this study

<table>
<thead>
<tr>
<th>Yeast strains</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM1-8b</td>
<td>MATa leu2-3, 112 his4 sta6 sta10 FLO1</td>
<td>A. Jiménez*</td>
</tr>
<tr>
<td>164S</td>
<td>MATa leu2-3, 112 ura3-53 trp1</td>
<td>C.E.C.T.</td>
</tr>
<tr>
<td>DBY746</td>
<td>MATa his3-1 leu2-3, 112 ura3-52 trp1-289</td>
<td>C.E.C.T.</td>
</tr>
<tr>
<td>NR8</td>
<td>MATa leu2-3, 112 ura3-52 flo1</td>
<td>N.M. Reboredo [22]</td>
</tr>
</tbody>
</table>

*Centro de Biología Molecular, Madrid, Spain; C.E.C.T., Spanish Type Culture Collection.

cM from the PET3 [6] and 30.5 cM from FURI [9], and the recessive genes flo3, flo6 and flo7 [10]. These genes behave in a semi-dominant manner and are considered to be possible alleles of FLO genes that are highly sensitive to suppression [8]. The sequences of chromosomes I and XI reveal a further two possible flocculation genes in S. cerevisiae. One of them is located near the end of the left arm on chromosome I (FLO9) and the other lies on chromosome XI (FLO10). In addition, three other pseudogenes have been detected on chromosomes I and VIII; mutations in several genes including the regulatory genes TUP1, SSN6, SFL1 and PHO2, that result in flocculation and other pleiotropic effects, have also been reported. Moreover, flocculation identical to that of FLO1-containing strains has been reported when heterologous genes, such as HTLV1-1 Tax transactivator, are expressed in yeast [2].

FLO1 has been cloned [11,12], sequenced [13,14] and the Flo1 protein localized in the cell wall with its amino-terminus exposed to the medium [15], suggesting a direct role of this protein in the flocculation process. According to this, FLO genes must be structural flocculation genes and could be regulated by TUP1 and SSN6, which would form part of a regulatory cascade.

In this paper we report on the cloning and partial characterization of a new gene coding for flocculation in S. cerevisiae. We show evidence of its mapping on chromosome XII and propose a designation of FLO2.

2. Materials and methods

2.1. Strains and plasmids

The yeast strains of S. cerevisiae used in this study together with their genotypes and origins are listed in Table 1. The Escherichia coli strain employed in the cloning procedures was NM522: Δ (lac proAB), thi, hsd Δ 5, supE, [F, proAB, lacIq, ZΔM15] λ−. The yeast-E. coli vector pRS316 was obtained from Stratagene and has been described previously [16].

2.2. Culture media, transformation and general genetic techniques

S. cerevisiae was grown in YEPD medium or minimal medium SD prepared according to Sherman et al. [17]. Uracil, adenine and amino acids were added to SD medium when necessary as indicated in [17]. Yeast cells were transformed with circular or linear DNA using the lithium acetate procedure [16]. Crosses between yeast strains, sporulation and dissection of tetrads were performed as described in [17].

E. coli was grown in LB medium [18] supplemented with ampicillin (50–100 μg/ml) when required for selection. Media were solidified with 2% bacto agar (Difco). E. coli transformation was performed using the CaCl2/RbCl2 method or electroporation [18].

2.3. Flocculation measurements

Colonies of yeast cells were grown for 3 days in tubes containing 5 ml of YEPD or selective media. To test flocculation, tubes were shaken vigorously on a vortex machine and the flocc-forming ability was estimated ‘de visu’ by subjective grading.

2.4. Nucleic acid manipulation

Chromosomal and plasmid DNA from yeast was obtained as described previously [16]. Plasmid DNA from E. coli was isolated by the alkaline lysis method.
Fig. 1. (A) Flocculation brought about by plasmid pSV1 in strain 1645. Untransformed S. cerevisiae strain 1645 (1) and transformed with pSV1 (2). (B) Phase-contrast observations of the same cultures.
[18] followed by further purification with a CsCl gradient. Small-scale preparations were carried out by the alkaline lysis method [18].

Chromosome block preparations and pulsed field gel electrophoreses (PFGE) were carried out in a Pharmacia Pulsaphor System, as described by the manufacturer. Electrophoresis conditions were: 1% agarose gels in 0.5×TBE buffer (50 mM Tris base, 50 mM boric acid, 1 mM EDTA; pH 8.0) run for a total of 27 h at 200 V. Pulse time was stepped from 70 s after 15 h to 120 s for 12 h.

2.5. Southern analysis

PFGE-separated chromosomes or total yeast genomic DNA digested with restriction endonucleases and separated by electrophoresis (5 μg/ lane) were denatured [18] and then transferred to nitrocellulose membranes (Schleicher and Schuell) using the Pharmacia 2016 VacuGene vacuum blotting system. Probes were labelled with digoxigenin using the digoxigenin-labelling kit from Boehringer Mannheim following the directions of the manufacturer. Hybridizations were carried out under standard high stringency conditions [18]. After hybridization with the probe, the filter was incubated with anti-DIG antibody conjugated to alkaline phosphatase and treated as recommended by the manufacturer.

2.6. Sequencing strategy and sequence analysis

The yeast insert in pSV5 was partially sequenced by the dideoxy-nucleotide chain-termination method [19] with sequenase 2.0 and [α-32P]dATP (Amersham). The DNA sequence was analysed by using the programs from the GCG (Software package of the Wisconsin University Biotechnology Center). Search for homologies was carried out with FASTA [20] or BLAST [21] programs.

3. Results and discussion

Flocculation in strain IM1-8b is governed by the FLO1 gene [8]. The phenotype, however, is suppressed by a single gene (fsu3) present in a larger number of non-flocculating strains. However, this suppressor is unable to suppress the FLO1 genes of other flocculating strains [8]. Accordingly, we decided to study the genetic determinant governing flocculation in this strain and also to attempt the cloning of the FLO1 gene.

3.1. Preparation of a suppressor-free strain

Laboratory strains frequently harbor flocculation suppressor genes, causing erratic and non-monogenic
with pSV1 and all of the retransformants showed a calcium-dependent [3] stable flocculation phenotype. The 5 kb insert was digested with different restriction enzymes and subcloned into pRS316 which gave rise to plasmids pSV4, pSV5 and pSV6 (Fig. 2). The results of these experiments allowed the identification of a 3.1 kb XbaI-XbaI insert as the smallest fragment able to confer the flocculating phenotype. It was also observed that those plasmids whose insert had been digested with EcoRI or SpeI did not induce flocculation.

3.3. Effect of flocculation suppressors

As indicated above, the flocculating character of strain IM1-8b is specifically suppressed by fsu3 which, however, has no effect on other flocculent strains [8]. Strain DBY746 was characterized as harboring such a suppressor because of the observed non-monogenic segregation when crossed with IM1-8b; when DBY746 was transformed with pSV1 no flocculation was observed.

3.4. Chromosomal assignment

Mapping of the cloned gene was performed by hybridization of the XbaI-XbaI probe (digoxigenin-labeled) with the chromosomes separated by PFGE. As shown in Fig. 3, two hybridization signals corresponding to chromosomes IV and XII of strain IM1-8b were detected. The probe also gave similar results with other S. cerevisiae strains, regardless of the flocculation phenotype. From these results alone, it could be concluded that this flocculation-conferring new gene existed in two copies per haploid genome. However, sequencing of a fragment (300 bp, Fig. 2) of the coding insert confirmed that the gene was unequivocally on the right arm of chromosome XII (segments L445 and L455, coordinates 452000-455000 and 459000-462000 respectively) and no homology was found with chromosome IV. According to database analysis part of L445 segment was repeated in L455. These results demonstrate that the cloned gene does not share homology with FLOI, which is present in strain IM1-8b, as shown previously [8] and also that it is different from the rest of the reported flocculation genes so far assigned to chromosomes I, VIII and XI. Because the new gene

Fig. 3. Southern analysis. (A) Ethidium bromide-stained gel of fractionated chromosomes by PFGE. (B) Southern hybridization using the XbaI-XbaI fragment shown in Fig. 2 as a probe. Lanes 1,3, strain IM1-8b; lanes 2,4, strain 1645.

3.2. Isolation of a new flocculation gene

A genomic library of strain IM1-8b was prepared in the centromeric shuttle plasmid pRS316 (URA3) and used to transform the strain 1645 (ura3). Three out of $10^4$ Ura$^+$ transformants were able to flocculate in liquid medium (Fig. 1A,B). The three plasmids were isolated and turned out to be identical. Further studies were carried out with pSV1 (insert size of 5 kb, Fig. 2). Strain 1645 was retransformed
does confer a strong flocculation phenotype, comparable in all aspects to that induced by FLO1, we propose a designation of FLO2.

3.5. Complementation of flo1 mutations with FLO2

The FLO1 gene has been cloned and sequenced by several groups and it now seems quite clear that FLO genes codify for cell wall glycoproteins having the carboxy-terminal domain embedded in the plasma membrane and the amino-terminal domain exposed to the external surface [2,15].

Two hypotheses can be proposed for a possible role of FLO2. (i) It could be a structural gene, similar in function (but not in sequence) to FLO1, that would normally be silent in all *S. cerevisiae* strains but otherwise active when cloned in the shuttle plasmid pRS316 and (ii) it would be a regulator gene with the ability to activate a silent flocculation gene in strain 1645 (Southern analysis and sequencing data have shown that many non-flocculating strains have silent genes); FLO2 would thus be inactive in strain 1645 but readily expressed when cloned in plasmid pRS316, then activating a flocculation gene.

To gain insight into these possibilities, we next attempted to complement the *flo1* mutation with FLO2. The NR8 flo1 mutant [22] was transformed with pSV1. In all cases the transformants showed a remarkable flocculation phenotype, despite their not having a functional FLO1 gene. These results would support the first of the two hypotheses, although further work is necessary to clarify the function of FLO2 since Southern analysis and data from the *Saccharomyces* genome database have shown that non-flocculating strains have silent FLO genes (other than FLO1) that could in turn be activated by FLO2.

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References


