Distinctly Different Characteristics of Flocculation in Yeast[1]

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ABSTRACT

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Flocculation of cells of *S. cerevisiae* NCYC 1109 and *S. cerevisiae* NCYC 234 was studied. Both cells flocculated 48 h after inoculation although the cells grown for only 20 h were non-flocculent. EDTA inhibited the flocculation of cells of *S. cerevisiae* NCYC 1109 and Ca^{2+} enhanced it. Protein-denaturants, D-mannose and D-glucose depressed the flocculation of cells of NCYC 1109. Treatment with proteolytic enzymes, photo-irradiation in the presence of methylene blue or periodate oxidation caused a loss of the floc-forming ability of the cells of NCYC 1109. On the contrary, the flocculation of cells of NCYC 234 was not affected by EDTA and Ca^{2+}. Protein-denaturants and monosaccharides did not inhibit the flocculent ability of cells of NCYC 234. Neither proteolytic enzyme treatments, photo-irradiation in the presence of methylene blue nor periodate oxidation, deprived the cells of NCYC 234 of the floc-forming ability. Cycloheximide repressed induction of the floc-forming ability of growing cells of NCYC 1109 significantly but not of growing cells of NCYC 234. These results suggest that the mechanisms of flocculation of NCYC 1109 and NCYC 234 are quite different.

Key words: Chemical modification, flocculation, intercellular interaction, *Saccharomyces cerevisiae*, yeast.

INTRODUCTION

Flocculation of brewer's yeast cells is important and interesting from both biochemical and industrial standpoints and is defined as the phenomenon wherein yeast cells adhere in clumps and sediment rapidly from the medium in which they are suspended[15].

Cells of *Saccharomyces cerevisiae* NCYC 1109 and NCYC 234 cultivated in YM medium for 20 h were non-flocculent, and co-flocculation occurred when non-flocculent cells of *S. cerevisiae* NCYC 1109 and non-flocculent cells of NCYC 234, both of which were cultivated in YM medium for 20 h, were mixed[10]. Effect of treatment with proteolytic enzymes and chemical modification of cell surface protein and carbohydrate components on the co-flocculation suggested strongly that the co-flocculation between cells of NCYC 1109 and cells of NCYC 234 resulted from interaction between surface protein components of cells of NCYC 1109 and surface carbohydrate components of cells of NCYC 234.

On the other hand, cells of both NCYC 1109 and NCYC 234 began to flocculate by themselves 48 h after inoculation. In this paper the self flocculation of both yeast strains was studied.

MATERIALS AND METHODS

Yeast strains

S. cerevisiae NCYC 1109 and NCYC 234 were used throughout. Both strains were ale yeast and obtained from the National Collection of Yeast Cultures.

Cultivation

The yeast cells, cultivated in a liquid medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% D-glucose (YM medium), were washed three times with sterilized deionized water and inoculated at a cell concentration of 1 μg/mL into fresh liquid medium of the same composition. Cultivation was carried out at 30°C with shaking on a reciprocal shaker (90 strokes/min, 7 cm). Yeast cells cultivated for the appropriate time were harvested and washed three times with deionized water.

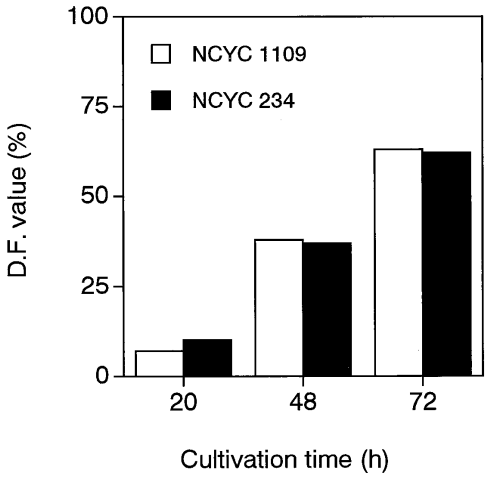

FIG. 1. Time course of flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234.

[1] A part of the contents of this paper was communicated as a poster presentation at the 10th International Yeast Symposium held in Arnhem, Netherlands in 2000.

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Estimation of flocculation

Unless stated otherwise, the **d**egree of **f**locculation of cells (D.F. value) was estimated by suspending cells in 5 mM $CaCl_2$ as described previously[11].

Effects of different concentrations of Ca^{2+}, protein-denaturants and monosaccharides on flocculation were studied by measuring D.F. values in various solutions in place of 5 mM $CaCl_2$.

Treatment of cells with proteolytic enzymes and chemical modification of cell surface protein and carbohydrate components

Trypsin. 10 mg of cells was incubated with 1 mg of trypsin (Sigma Chemical Co., Type III) at 30°C for 30 min in 2 mL of 0.05M Tris HCl buffer (pH 7.6).

Chymotrypsin. 10 mg of cells was incubated with 2 mg of chymotrypsin (Sigma Chemical Co., Type II) at 30°C for 30 min in 2 mL of 0.05M Tris HCl buffer (pH 7.8).

Photo-oxidation. 10 mg of cells was photo-irradiated in the presence of methylene blue and 8M urea at the room temperature, as described previously[12]. The [hν/MB] designation in the figures refers to photo-irradiated in the presence of methylene blue.

$NaIO_4$. 10 mg of cells was treated with 20 mM $NaIO_4$ at 0°C for 30 min in the dark.

After appropriate treatments described above, cells were washed three times with deionized water and then used in the flocculation experiments.

Addition of cycloheximide on growing cultures. After 1 μg/mL of cycloheximide (CHI), was added to the cell culture grown for 20 h, cells grown for 48 h and 72 h after inoculation were harvested and D.F. values were determined.

RESULTS

Time-course of flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234

Fig. 1 shows a time course of flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. Both cells of NCYC 1109 and cells of NCYC 234 cultivated for 20 h were non-flocculent. As shown before[10], co-flocculation occurred when non-flocculent cells of NCYC 234 and cells of NCYC 1109 grown for 20 h were mixed together (data not shown). Since they began to flocculate 48 h after inoculation, the self flocculation which occurred when

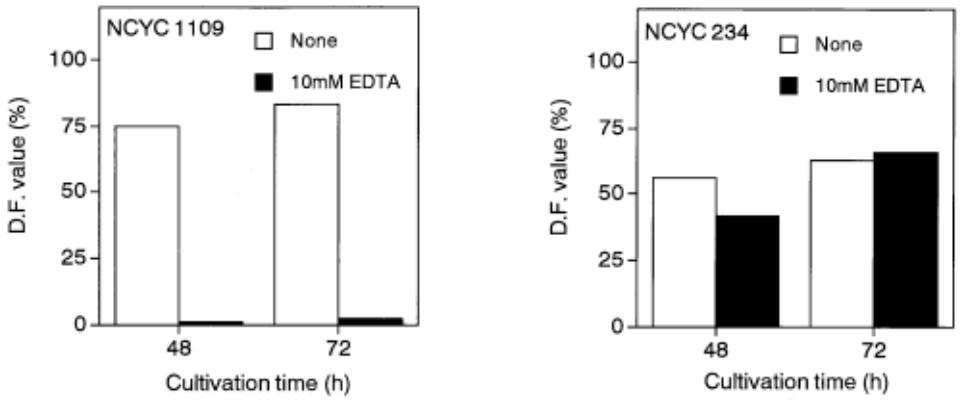

FIG. 2. Effect of addition of EDTA into a growing culture on the flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. 10 mM EDTA was added to the culture of cells of NCYC 1109 and cells of NCYC 234 cultivated for 48 h and 72 h and D.F. values were estimated.

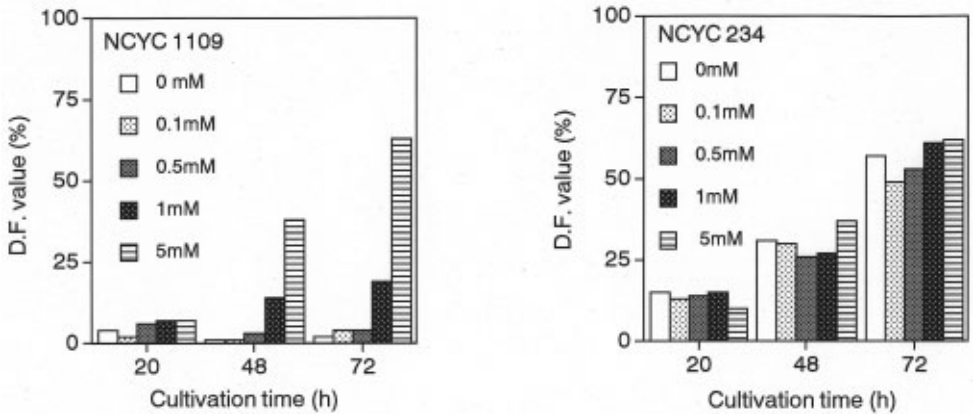

FIG. 3. Effect of Ca^{2+} on flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. After cells of NCYC 1109 and cells of NCYC 234 grown for 48 h and 72 h were harvested, washed three times with deionized water and suspended in different concentrations of $CaCl_2$ solutions, D.F. values were estimated.

cells of NCYC 1109 and cells of NCYC 234 were grown for 48 h and 72 h in YM medium, was studied as described below.

Calcium ion dependency of flocculation

Fig. 2 depicts the effect of addition of EDTA on flocculation of cells of NCYC 1109 and cells of NCYC 234 grown for 48 h and 72 h. Flocculent cells of NCYC 1109 grown for 48 h and 72 h failed to flocculate after addition of 10mM EDTA into the culture while EDTA addition did not affect the flocculation of cells of NCYC 234 significantly. Fig. 3 illustrates effect of Ca^{2+} on the flocculation of cells of 1109 and cells of NCYC 234 cultivated for 48 h and 72 h. Although flocculent cells of NCYC 1109 did not flocculate when they were washed and suspended in deionized water, the addition of Ca^{2+} promoted the flocculation. On the contrary, cells of NCYC 234 flocculated even in deionized water and Ca^{2+} did not affect the flocculation. These results suggest that Ca^{2+} is essential for the flocculation of cells of NCYC 1109 but not for cells of NCYC 234.

Effect of protein-denaturants on flocculation

The effect of protein-denaturants on flocculation is shown in Fig. 4. When 8 M urea or 5 M guanidine HCl was added to a cell suspension of NCYC 1109 in the presence of 5 mM Ca^{2+}, flocculation was significantly inhibited. On the contrary, these protein-denaturants failed to affect the flocculation of cells of NCYC 234.

Effect of treatment with proteolytic enzymes and chemical modification of cell surface protein and carbohydrate components on flocculation

Fig. 5 shows the effect of treatments with proteolytic enzymes on floc-forming ability of cells of NCYC 1109 and cells of NCYC 234. Treatment of cells of NCYC 1109 with trypsin or chymotrypsin caused an extensive loss of the floc-forming ability while flocculent ability of cells of NCYC 234 was not affected significantly by the treatment. Fig. 6 illustrates the effect of photo-irradiation in the presence of methylene blue on the floc-forming ability of cells of NCYC 1109 and cells of NCYC 234. It is

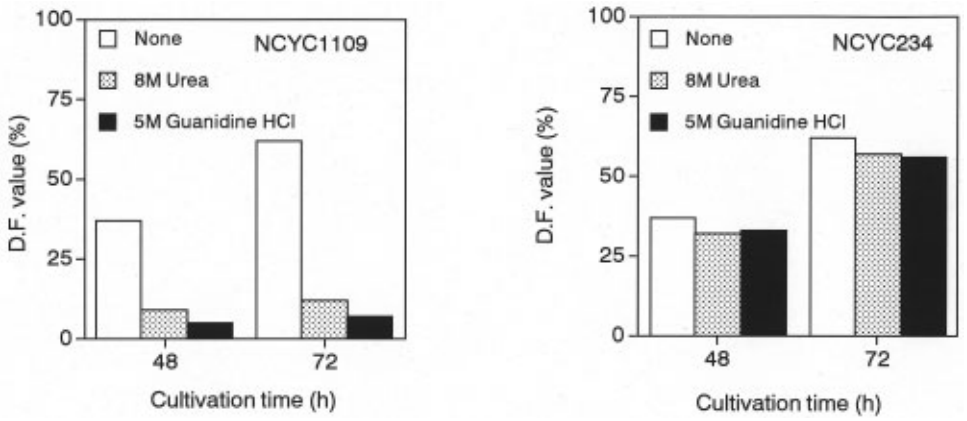

FIG. 4. Effect of protein-denaturants on flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. After cells of NCYC 1109 and cells of NCYC 234 grown for 48 h and 72 h were harvested, washed three times with deionized water and suspended in 8M urea or 5M guanidine HCl containing 5mM $CaCl_2$, D.F. values were estimated.

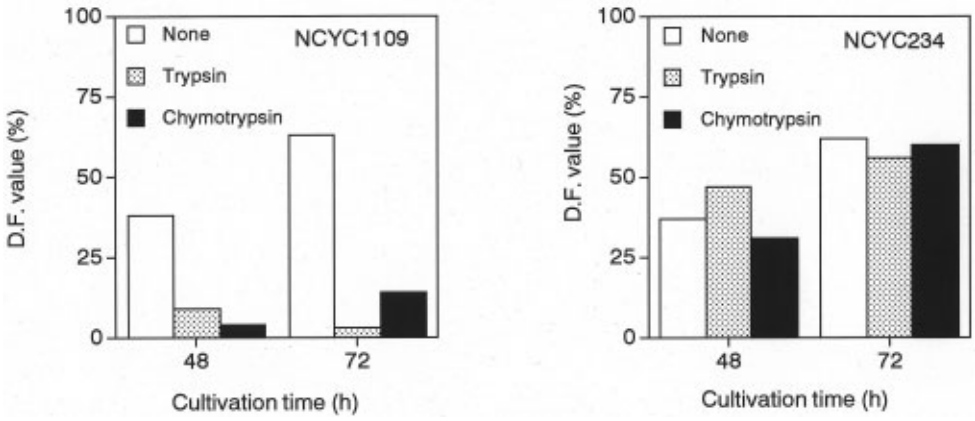

FIG. 5. Effect of treatment with proteolytic enzymes on floc-forming ability of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. After cells of NCYC 1109 and cells of NCYC 234 grown for 48 h and 72 h were harvested and washed three times with deionized water, they were treated with proteolytic enzymes. Then, they were washed three times with deionized water, suspended in 5mM $CaCl_2$ and D.F. values were estimated.

known that photo-irradiation in the presence of methylene blue preferentially brings about modification of imidazole groups of histidyl residues in proteins[3]. It has also been described that floc-forming ability of flocculent cells of beer yeast IFO 2018 is lost by photo-irradiation in the presence of a photo-sensitizer because of the destruction of the steric structure of a surface protein component essential for flocculation[11]. Photo-irradiation in the presence of methylene blue brought about a significant loss of the floc-forming ability of cells of NCYC 1109 while the flocculent ability of cells of NCYC 234 was not affected as much by the treatment. Treatment with periodate is known to result in the C-C bond cleavage of vicinal dihydroxy compounds including carbohydrates. As shown in Fig. 7, cells of NCYC 1109 oxidized with sodium periodate failed to flocculate. On the contrary, cells of NCYC 234 did not significantly lose the flocculent ability when they were treated with periodate.

Therefore, it is evident that both protein and carbohydrate components on the cell surface play essential roles in the flocculation of cells of NCYC 1109. On the other hand, it is probable that interaction of cell surface protein and carbohydrate components are not involved in the flocculation of cells of NCYC 234.

Effect of cycloheximide on induction of floc-forming ability

Fig. 8 shows the effect of cycloheximide on the induction of floc-forming ability of growing cells of NCYC 1109 and NCYC 234. Cycloheximide inhibited the induction of floc-forming ability of non-flocculent cells of NCYC 1109 significantly while the antibiotic failed to affect the induction of flocculent ability of cells of NCYC 234.

Effect of monosaccharides on flocculation

Fig. 9 depicts the effect of four monosaccharides on the flocculation of cells of NCYC 1109 and cells of NCYC 234 in the presence of 5 mM $CaCl_2$. The flocculation of cells of NCYC 1109 was strongly inhibited by D-mannose, D-glucose and D-fructose and partially by D-galactose, whereas the four monosaccharides tested failed to affect the flocculation of cells of NCYC 234.

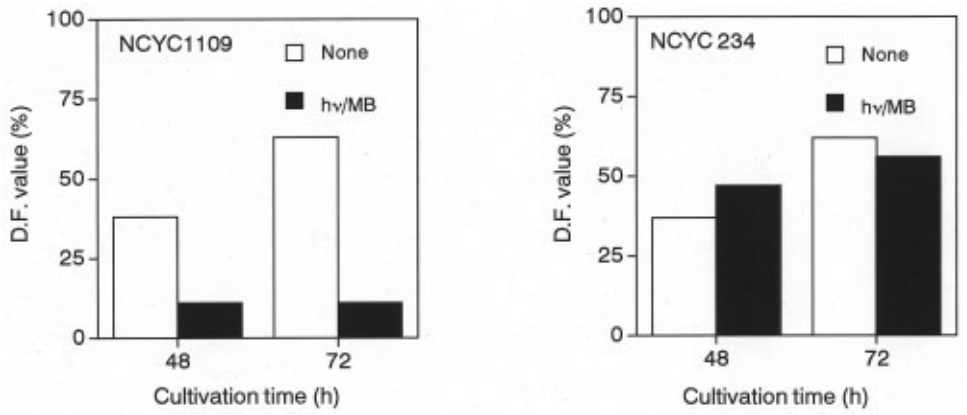

FIG. 6. Effect of photo-irradiation on floc-forming ability of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. After cells of NCYC 1109 and cells of NCYC 234 grown for 48 h and 72 h were harvested and washed three times with deionized water, they were photo-irradiated in the presence of methylene blue and 8M urea. Then, they were washed three times with deionized water, suspended in 5mM $CaCl_2$ and D.F. values were estimated.

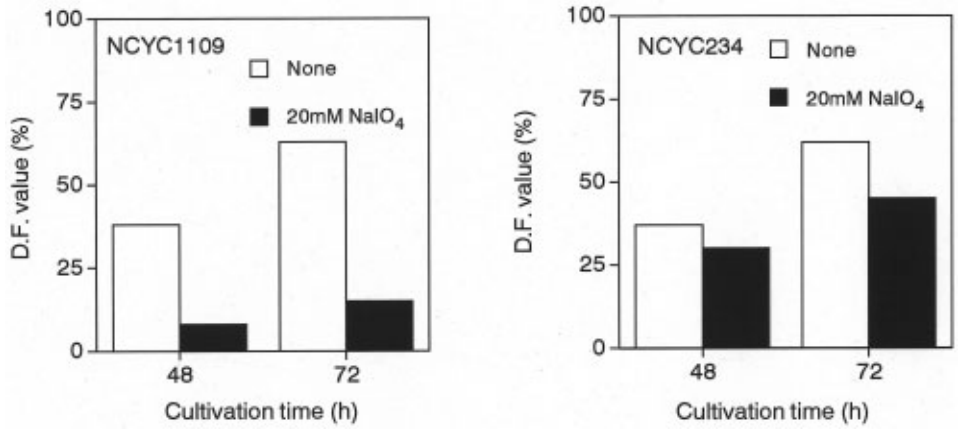

FIG. 7. Effect of periodate oxidation on floc-forming ability of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. After cells of NCYC 1109 and cells of NCYC 234 grown for 48 h and 72 h were harvested and washed three times with deionized water, they were treated with sodium periodate. Then, they were washed three times with deionized water, suspended in 5mM $CaCl_2$ and D.F. values were estimated. $NaIO_4$, treated with sodium periodate.

DISCUSSION

In 1982, Miki *et al.*[7,8] proposed the lectin-like theory of flocculation. A similar conclusion was reported through chemical modification of cell surface protein and carbohydrate of cells of beer yeast IFO 2018 from this laboratory[11]. Hitherto several papers have appeared which dealt with characteristics and function of the protein essential for yeast flocculation[1,2,4,6,14,18]. It was also reported that flocculation receptors were outer-chain mannan side-branches, two or three mannose residues in length[16].

As described in the previous paper[10], co-flocculation occurred between non-flocculent cells of NCYC 1109 and NCYC 234 grown for 20 h. It was described that a cell surface protein component of cells of NCYC 1109 and a cell surface mannan component of cells of NCYC 234 are absolutely necessary for the mutual recognition and intercellular interaction between the cells of both strains. Self flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234 which were grown for 48 h and 72 h was studied.

Flocculation of cells of NCYC 1109 was completely dependent upon Ca^{2+}, as was the case of flocculation of many other brewer's yeast strains[5,9,13,19,20]. Protein-denaturants inhibited the flocculation of cells of NCYC 1109. Not only proteolytic treatment and chemical modification of cell surface protein but also oxidative cleavage of cell surface carbohydrate deprived the cells of floc-forming ability. These results suggest that both protein and carbohydrate components on the cell surface are essential for the flocculation of cells of NCYC 1109. Non-flocculation of the cells of NCYC 1109 grown for 20 h might be caused by the lack of the mannan structure which is necessary for essential protein recognition. It is plausible that cycloheximide represses the enzyme which is involved in the synthesis of outer-chain mannan side-branches essential for flocculation. The flocculation of cells of NCYC 1109 was inhibited not only by D-mannnose, but also by D-glucose and D-fructose, indicating that the phenotype of flocculation of the cells of NCYC 1109 is the NewFlo one, according to Stratford and Assinder[17]. On the other hand, flocculation of cells of NCYC 234 was not affected by EDTA and Ca^{2+}. Protein-denaturants failed to inhibit the flocculation as well. Neither proteolytic treatment nor chemical modification of cell surface protein and carbohydrate components brought about significant deflocculation of cells of NCYC 234. None of the four monosaccharides tested affected the flocculation. These results suggest undoubtedly that the floc-formation of cells of NCYC 234 is not caused by an interaction between cell surface protein

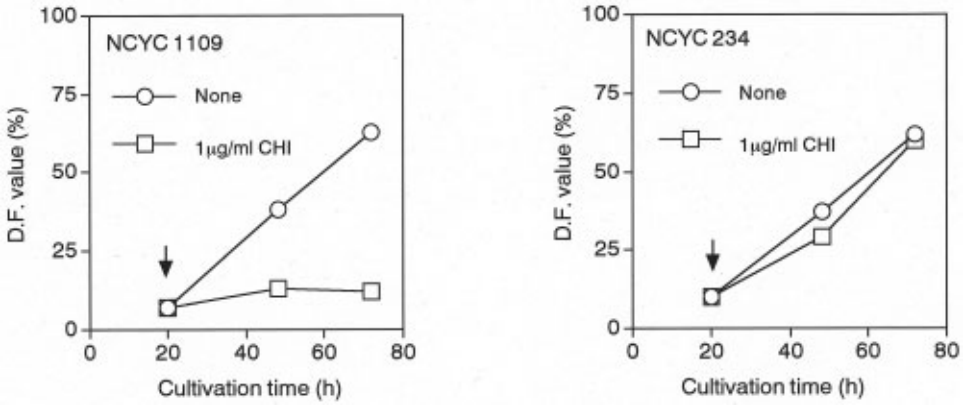

FIG. 8. Effect of cycloheximide on induction of the floc-forming ability of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. 1μg/mL of cycloheximide was added to a growing culture 20 h after inoculation. Cells of NCYC 1109 and NCYC 234 were harvested 48 h and 72 h after inoculation, washed three times with deionized water, suspended in 5mM $CaCl_2$, and D.F. values were estimated.

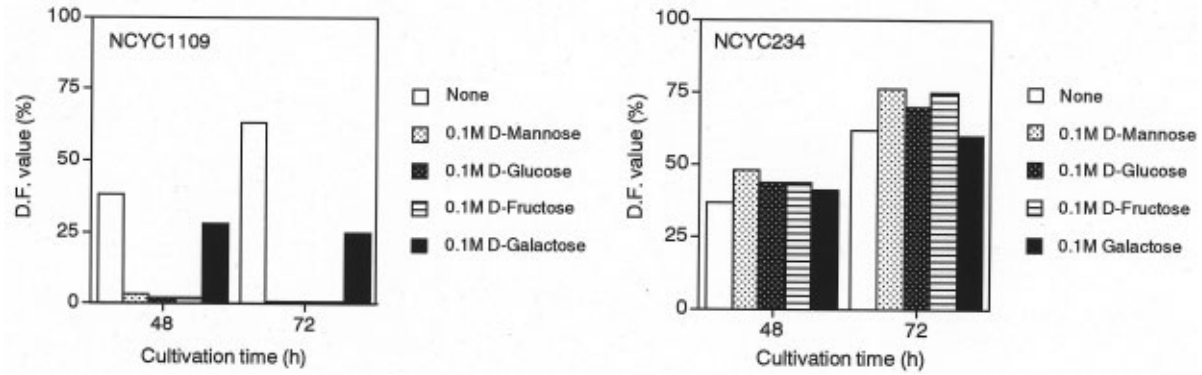

FIG. 9. Effect of monosaccharides on flocculation of cells of *S. cerevisiae* NCYC 1109 and NCYC 234. Cells of NCYC 1109 and NCYC 234 were grown for 48 h and 72 h, harvested, washed three times with deionized water and suspended in 0.1M D-mannose, 0.1M D-glucose, 0.1M D-fructose or 0.1M D-galactose containing 5mM $CaCl_2$, D.F. values were estimated.

and mannan components. It is probable that apparent aggregates are formed owing to the failure of cell division as cells are grown, though the exact mechanism at present is obscure.

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