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Why Do Flocculent Yeast Cells Not Flocculate in Early Growth Phase?

by

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Abstract

Cells of *Saccharomyces cerevisiae* IFO 2018 began to flocculate in the middle exponential phase, while they were dispersed in the early-growth phase. Cycloheximide strongly inhibited induction of floc-forming ability of the early-phase cells when the antibiotic was added to the growing culture. A definite co-flocculation occurred when the non-flocculent early-phase cells and flocculent cells were mixed. Photo-irradiation of early-phase cells in the presence of methylene blue did not weaken the co-flocculation with flocculent cells. After treatment with mercaptoethanol, early-phase cells co-flocculated with the complete cells much more strongly. The early-phase cells treated with periodate failed to co-flocculate with the complete cells. On the other hand, the complete cells deflocculated by treatment with mercaptoethanol or photo-irradiation did not co-flocculate with early-phase cells. Although flocculent cells deflocculated by periodate oxidation failed to co-flocculate with early-phase cells, mercaptoethanol treatment of the early-phase cells promoted the co-flocculation. It is suggested that early-phase cells are not able to produce surface protein component essential for self-flocculation.

Key Words: Yeast, *Saccharomyces cerevisiae*, flocculation, co-flocculation, early-phase cells, chemical modification

Abbreviations: D.F., degree of flocculation (see the text); hν/MB, photo-irradiated in the presence of methylene blue (see the text); CHI, cycloheximide.

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INTRODUCTION

In the preceding paper[1], it was described that co-flocculation occurs when flocculent cells of *Saccharomyces cerevisiae* IFO 2018 and cells of various genetically non-flocculent strains were mixed. It was indicated that both protein and carbohydrate components of yeast cell surface play essential parts in the interaction involved in flocculation and that cells of non-flocculent cells do not possess essential protein component although they have essential carbohydrate component.

S. cerevisiae IFO 2018 is a flocculent strain. Cells of *S. cerevisiae* IFO 2018 flocculate strongly in the late exponential phase, but remain dispersed in the early exponential phase.

In this paper, it is described that early-phase cells of *S. cerevisiae* IFO 2018 is non-flocculent because of lack of cell surface protein component essential for flocculation through means of chemical modification of cell surface components.

EXPERIMENTAL METHODS

Yeast strains

S. cerevisiae IFO 2018 was used throughout. The strain was lager and obtained from Institute for Fermentation, Osaka.

Cultivation

The yeast cells, cultivated in the semi-synthetic medium described in the previous paper[2], 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 28°C with shaking on a rotatory shaker. Yeast cells cultivated for appropriate time were harvested and washed three times with deionized water.

Estimation of flocculation

The degree of flocculation (D.F. value) of cells of a single strain and that of co-flocculation observed when flocculent cells and non-flocculent cells were mixed was estimated as described before[2].

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

Proteolytic enzemes

10 mg of cells was incubated with trypsin or chymotrypsin as described previously[3].

Mercaptoethanol-reduction

10mg of cells was treated with 0.1M mercaptoethanol in the presence of 8M urea, as described before[2]. [ME]

Photo-oxidation

10 mg of cells was photo-irradiated in the presence of methylene blue and 8M urea at the room temperature, as described before[3]. [hν/MB]

$NaIO_4$

10 mg of cells was treated with 20 mM $NaIO_4$ at 0℃ for 30 min in the dark, as described before[3].

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

Addition of cycloheximide on growing culture

After 1 μg/ml or 10 μg/ml of cycloheximide was added to cell culture grown for 16 h, cells grown for 20 h and 23 h after inoculation were harvested and D.F. values were determined.

RESULTS and DISCUSSION

Time-course of flocculation of cells of *S. cerevisiae* IFO 2018

Figure 1 shows a time course of flocculation of cells of *S. cerevisiae* IFO 2018. Cells cultivated for 16 h were non-flocculent and began to flocculate 19 h after inoculation. Therefore, cells cultivated for 16 h and 26 h were harvested and designated as non-flocculent early-phase cells and flocculent cells, respectively.

Effect of cycloheximide on induction of floc-forming ability

Figure 2 shows effect of cycloheximide on induction of floc-forming ability of

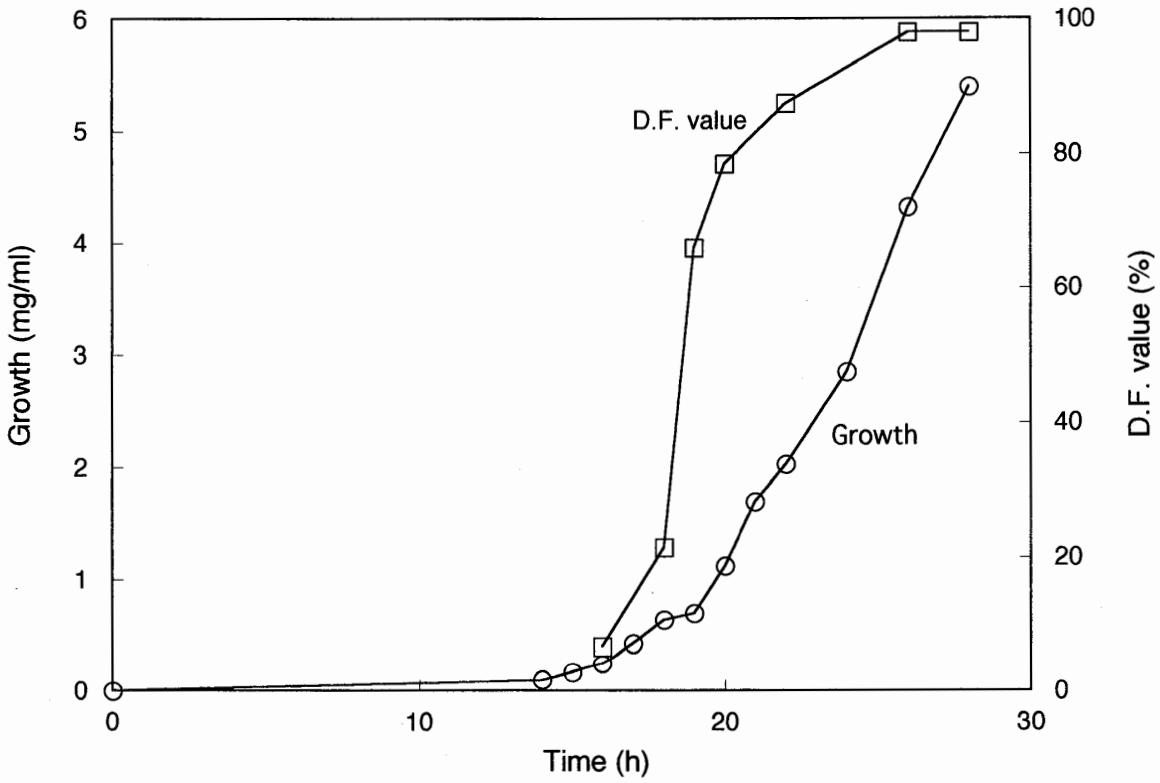

Figure 1. Time-course of Floc-Forming Ability of *S. cerevisiae* IFO 2018.

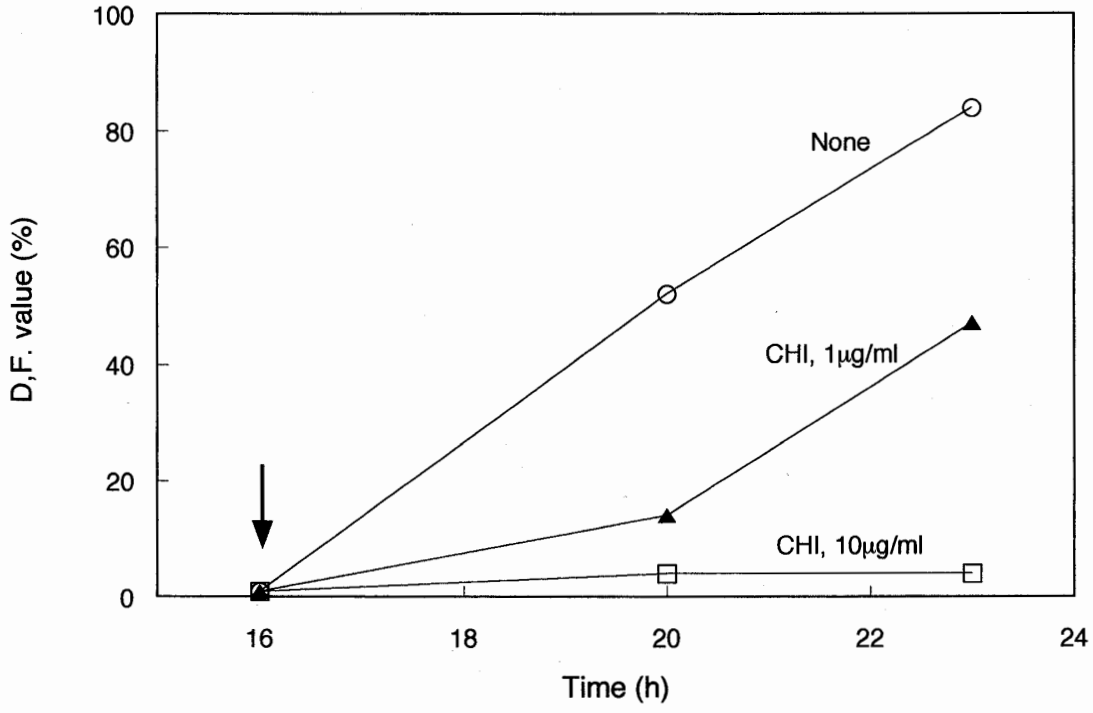

Figure 2. Effect of Cycloheximide on Induction of Floc-forming Ability of Non-flocculent Early-phase Cells.

growing non-flocculent cells of IFO 2018 which were cultivated for 16h (early-phase cells). Although cells began to flocculate strongly in the absence of cycloheximide 20h after inoculation, 10 μg/ml of cycloheximide inhibited the induction of floc-forming ability of non-flocculent early-phase cells completely.

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

As shown in Figure 3, a definite co-flocculation was observed when non-flocculent early-phase cells and flocculent cells were mixed. To study how the co-flocculation occurred, cell surface protein and carbohydrate components were treated with proteolytic enzymes or modified chemically.

Figure 3 shows also the effect of treatment with proteolytic enzymes of cell surface protein of non-flocculent early-phase cells on co-flocculation with flocculent cells. Treatment of early-phase cells with trypsin or chymotrypsin failed to affect the co-flocculation significantly.

Figure 4 illustrates effect of photo-irradiation of early-phase cells in the presence of methylene blue on the co-flocculation with flocculent cells.

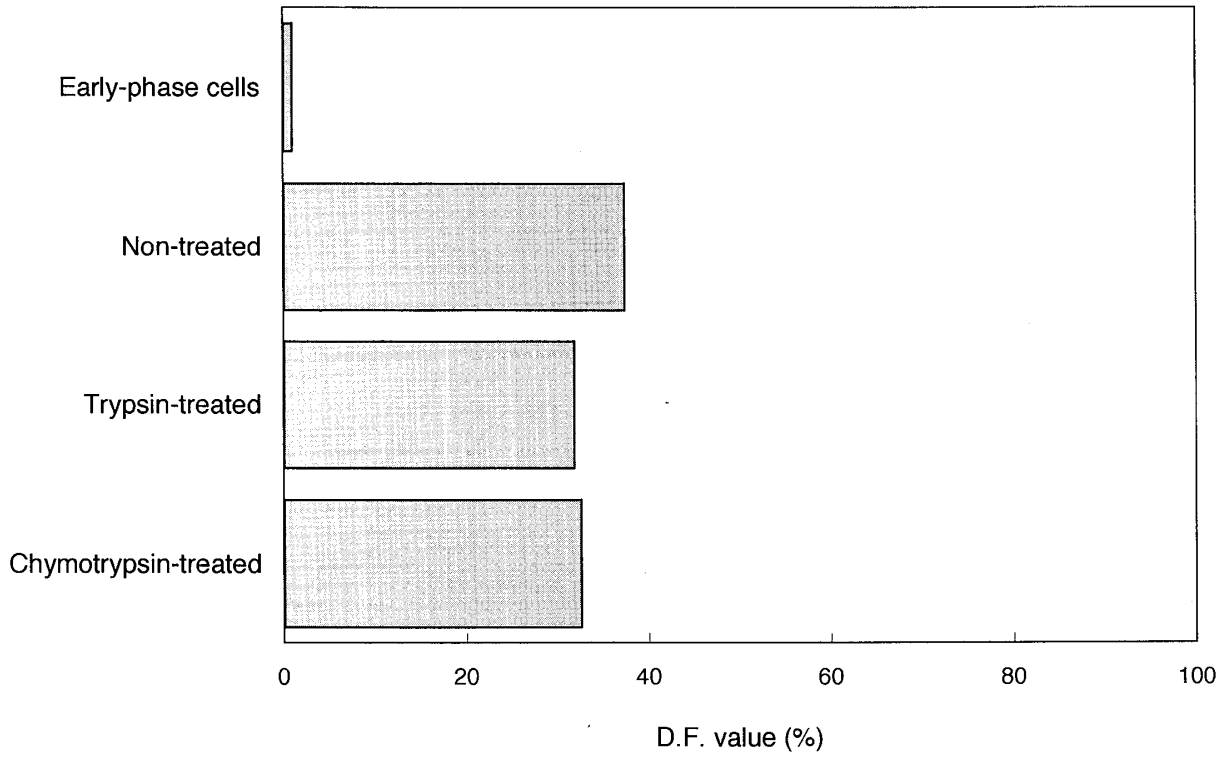

Figure 3. Effect of Treatment with Proteolytic Enzymes of Cell Surface Protein of Non-flocculent Early-phase Cells on Co-flocculation with Flocculent Cells.

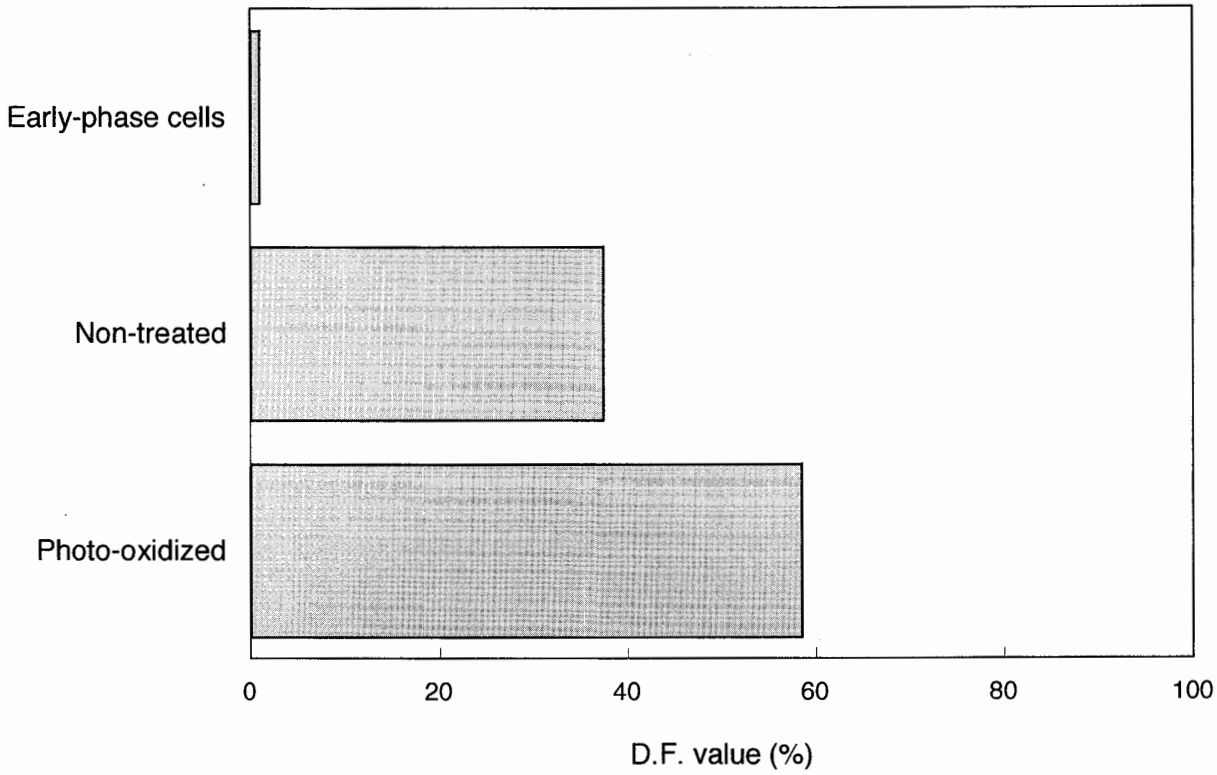

Figure 4. Effect of Phto-oxidation of Cell Surface Protein of Non-flocculent Early-phase Cells on Co-flocculation with Flocculent Cells.

It is known that photo-irradiation in the presence of methylene blue preferentially brings about modification of imidazole groups of histidyl residues in proteins[4]. It has also been described that floc-forming ability of flocculent cells of S. cerevisiae 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[5]. Photo-irradiation did not bring about a loss of the co-flocculation but somewhat enhanced the co-flocculation.

Figure 5 shows effect of reduction with mercaptoethanol of cell surface protein of non-flocculent early-phase cells on co-flocculation with flocculent cells. Mercaptoethanol-reduction of the early-phase cells, in particular, in the presence of 8M urea stimulated co-flocculation with flocculent cells significantly.

Treatment with periodate is known to result in the C-C bond cleavage of vicinal dihydroxy compounds including carbohydrates. As shown in Figure 6, periodate-oxidation of early-phase cells diminished co-flocculation with flocculent cells completely. These results suggest strongly that not surface protein component but surface carbohydrate component of early-phase cells is essential for co-flocculation with flocculent cells.

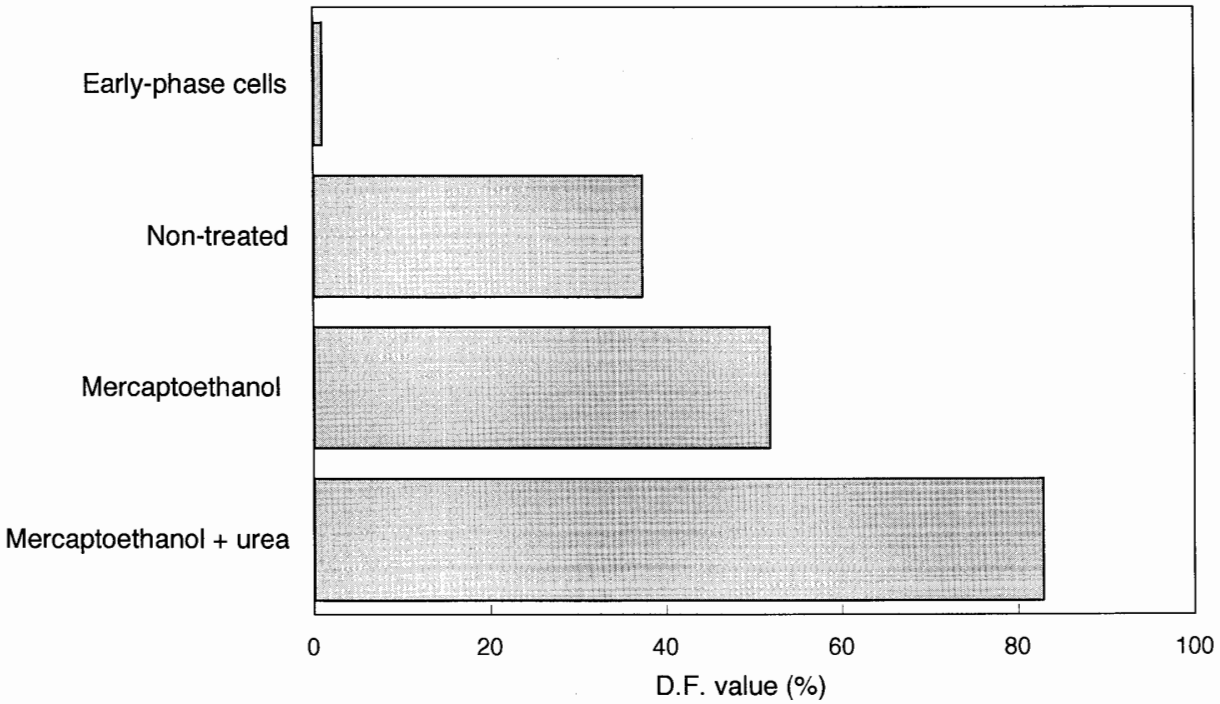

Figure 5. Effect of Reduction with Mercaptoethanol of Cell Surface Protein of Non-flocculent Early-phase Cells on Co-flocculation with Flocculent Cells.

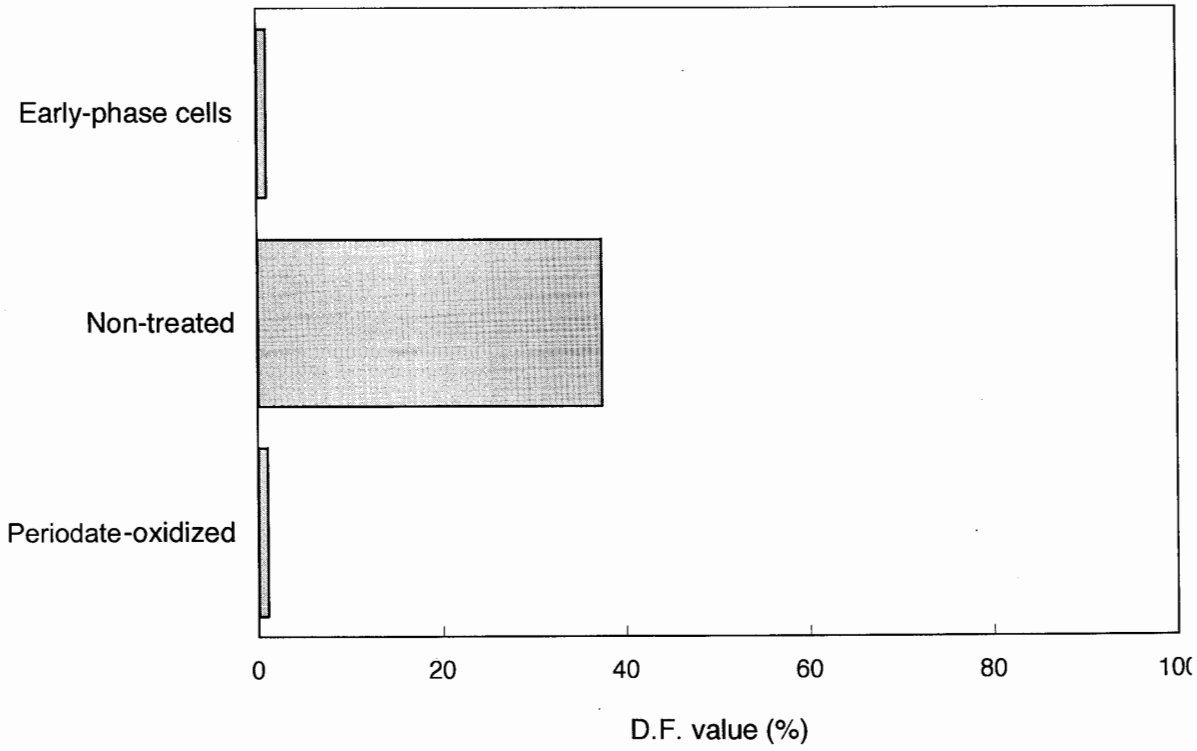

Figure 6. Effect of Periodate-oxidation of Non-flocculent Early-phase Cells on Co-flocculation with Flocculent Cells.

Next, effect of proteolytic treatment and chemical modification of flocculent cell surface protein and carbohydrate components of flocculent cells on co-flocculation with early-phase cells was investigated.

As shown in Figure 7, flocculent cells lost the floc-forming ability by treatment with protelytic enzymes. Cells deflocculated by the treatment with proteolytic enzymes failed to co-flocculate with non-flocculent early-phase cells. As shown in Figure 8 and Figure 9, flocculent cells lost the floc-forming ability by photo-oxidation and mercaptoethanol -reduction. Both cells deflocculated by photo-oxidation and mercaptoethanol-reduction did not co-flocculate with non-flocculent early-phase cells. These results suggest that surface protein components of flocculent cells are essential for self-flocculation of flocculent cells and co-flocculation with early-phase cells.

Figure 10 shows effect of periodate-oxidation of flocculent cells on self-flocculation of flocculent cells and co-flocculation with early-phase cells. Flocculent cells lost the floc-forming ability by periodate-oxidation, suggesting that mannan components on the flocculent cell surface also play an important part in the floc-forming ability of flocculent cells. Flocculent cells deflocculated by periodate-oxidation failed to co-flocculate with early-phase cells. Surprisingly, however, after treatment of early-phase cells with mercaptoethanol, flocculent cells defocculated by periodate-oxidation co-flocculated with early-phase cells, as shown in Figure 11. It is probable that periodate

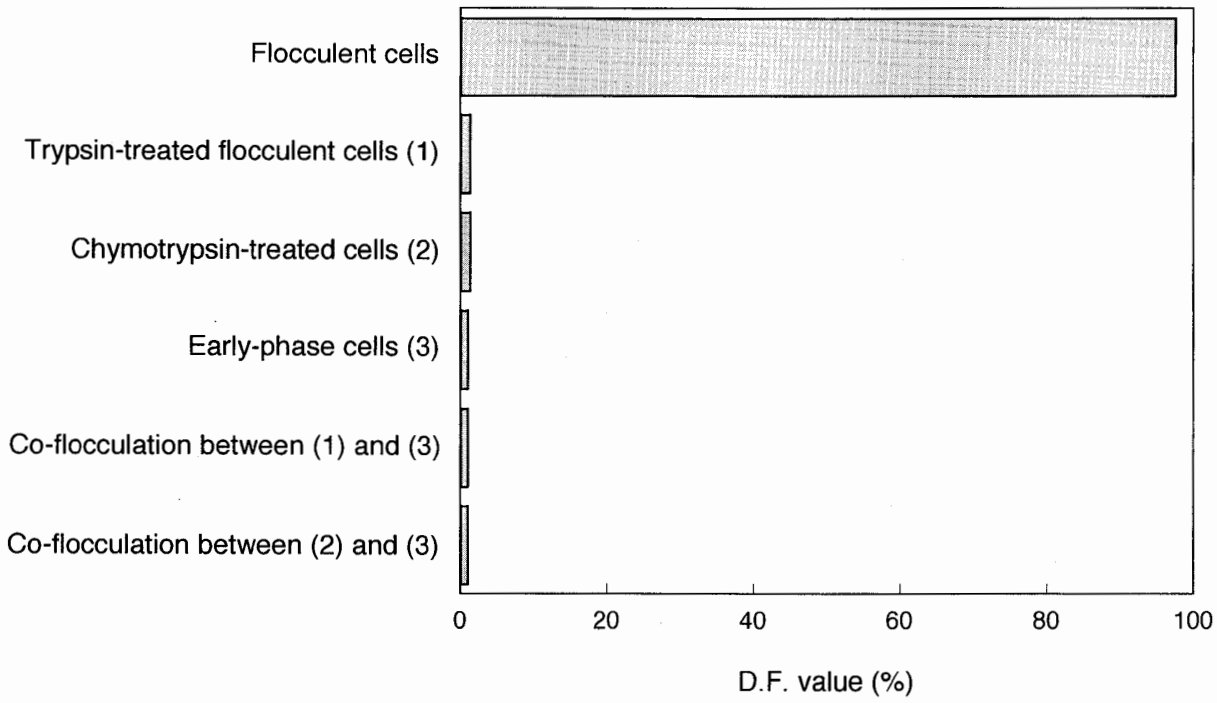

Figure 7. Effect of Treatment with Proteolytic Enzymes of Cell Surface Protein of Flocculent Cells on Co-flocculation with Early-phase Cells.

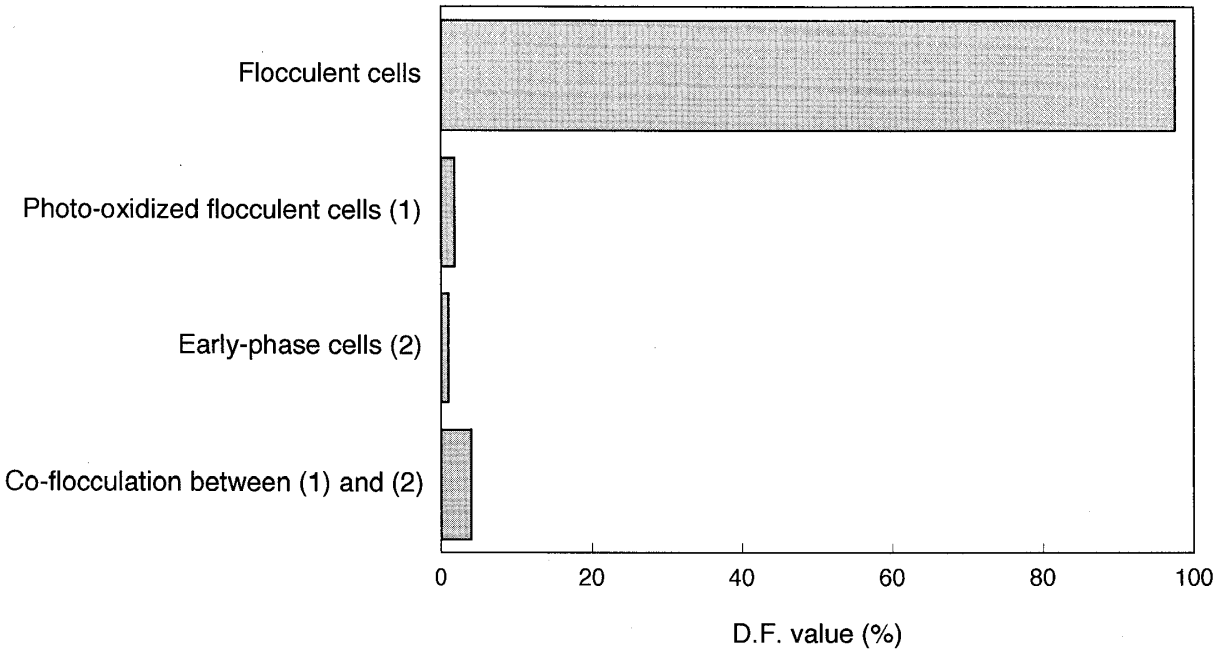

Figure 8. Effect of Photo-oxidation of Cell Surface Protein of Flocculent Cells on Co-flocculation with Early-phase Cells.

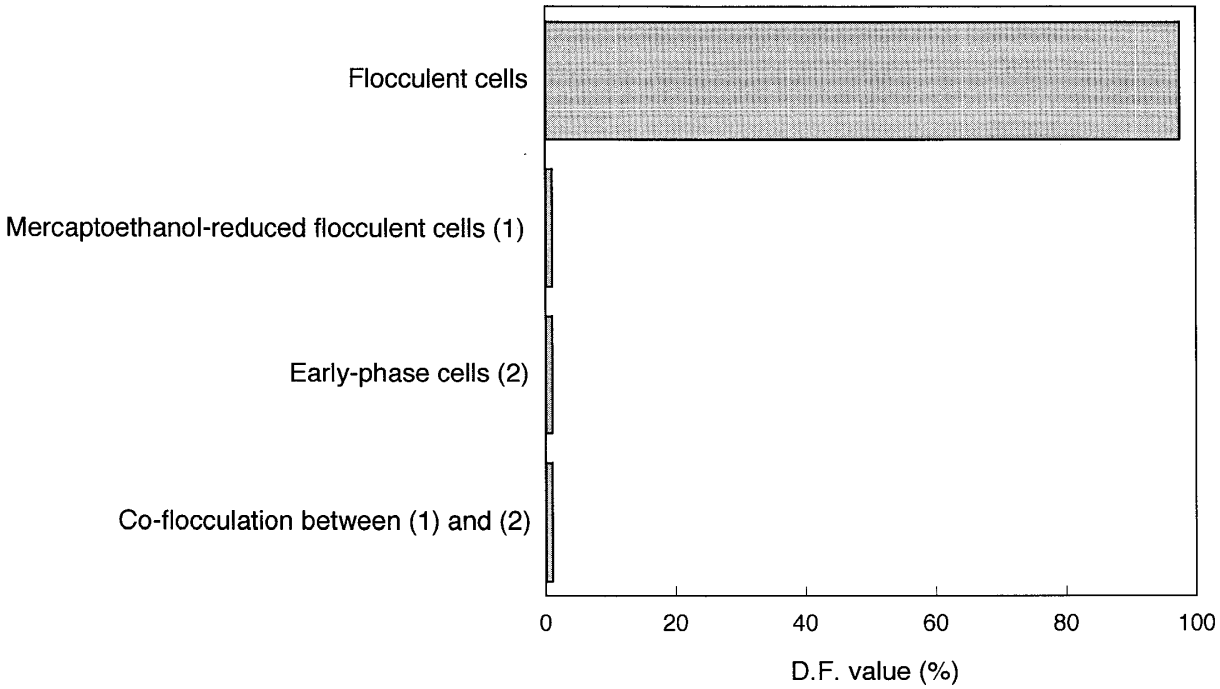

Figure 9. Effect of Reduction with Mercaptoethanol of Cell Surface Protein of Flocculent Cells on Co-flocculation with Early-phase Cells.

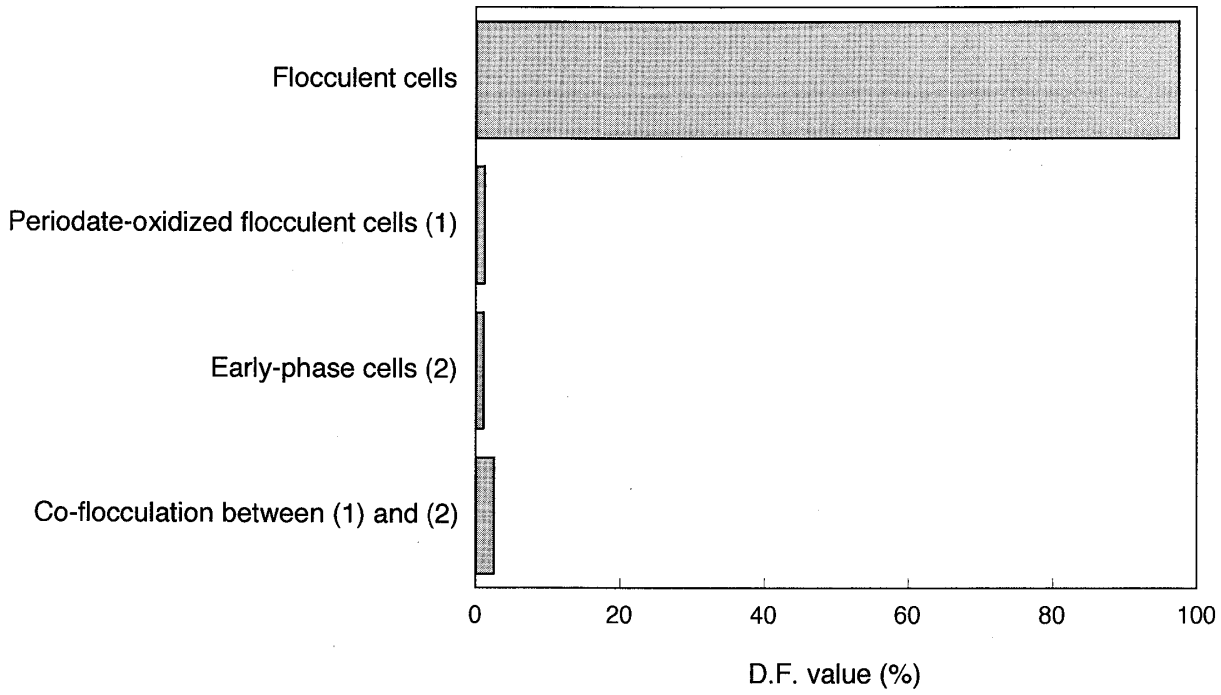

Figure 10. Effect of Periodate-oxidation of Cell Surface Protein of Flocculent Cells on Co-flocculation with Early-phase Cells.

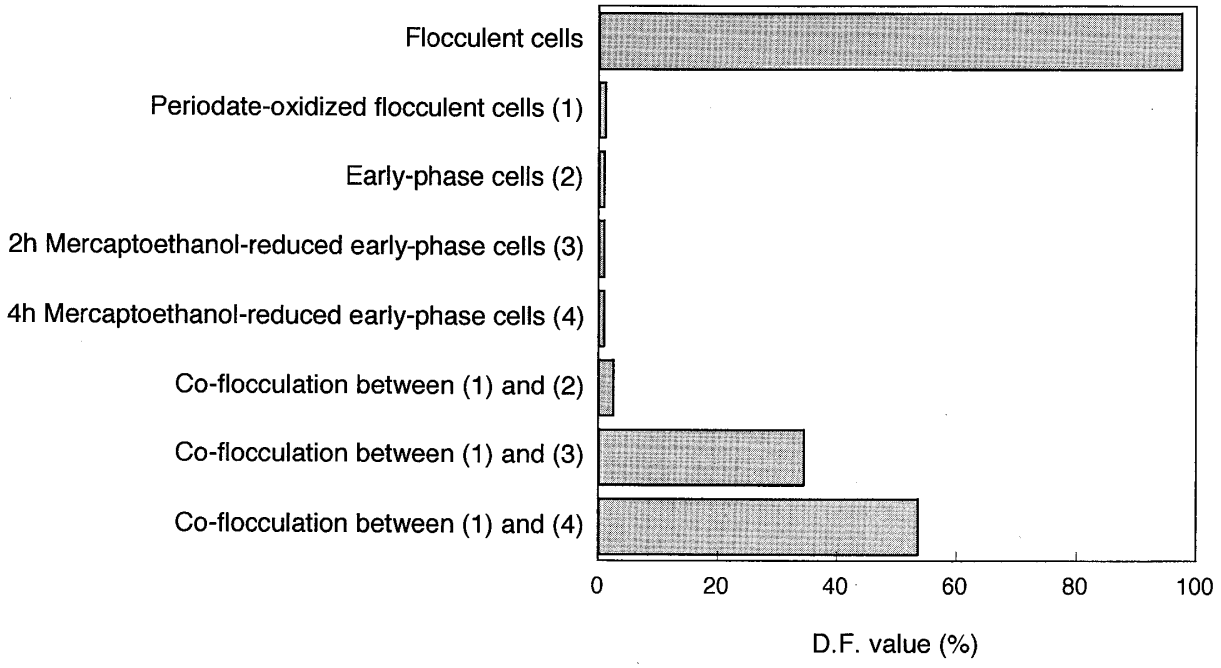

Figure 11. Effect of Reduction with Mercaptoethnol of Early-phase Cells on Co-flocculation with Periodate-oxidized Flocculent Cells.

treatment may affect a part of flocculent cell surface protein component essential for co-flocculation and deprive flocculent cells of co-flocculent ability with early-phase cells. In addition, it is possible that treatment with mercaptoethanol may unmask early-phase cell surface carbohydrate component essential for co-flocculation by reducing the S-S bonds on the cell surface.

These findings suggest that early-phase cells are not able to produce surface protein component essential for flocculation while they possess essential carbohydrate component.

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