INFLUENCE OF AERATION AND GLUCOSE CONCENTRATION IN THE FLOCCULATION OF *Saccharomyces cerevisiae*

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SUMMARY

The influence of glucose concentration and aeration rate on the flocculation ability of *Saccharomyces cerevisiae* was studied. A low aeration rate exerted a positive effect on flocculation whereas a high aeration inhibited this phenomenon. A positive effect was achieved with a high glucose concentration. By controlling both of these factors it was possible to modulate the flocculation expression of this strain.

INTRODUCTION

Flocculation of yeasts is usually defined as the ability of the cells to aggregate spontaneously and form flocs which sediment rapidly in culture medium (Stewart et al., 1976). This phenomenon has traditionally been exploited in brewing industries (Stewart, 1975).

The aggregation of *S. cerevisiae* is under genetic control (Stewart et al., 1976; Miki et al., 1982a; Yamashita and Fukui, 1983; Stewart and Russell, 1986; Esser et al., 1987). In addition to the involvement of chromosomal genes, there is evidence that mitochondrial DNA influences the flocculation expression (Holmberg and Kiellandt-Brandt, 1978; Hinrichs et al., 1988).

In the cell walls of yeast, the mannans are generally associated with proteins (mannoproteins) which are exposed on the external surface (Cabib et al., 1982). Flocculation would appear to involve the surface of the cells. Chemical and enzymatical treatments inhibit flocculence ability irreversibly, which indicates the involvement of proteins or glycoproteins located on the yeast surface (Nishihara et al., 1977, 1982; Miki et al., 1982a). The α-mannan also appears to be implicated because mannose, various mannosyl derivates and ConcanavalinA competitively inhibit the cell-cell interaction (Taylor and Orton, 1978; Miki et al., 1982a; Kihm et al., 1988).

Unfortunately, there is no consensus of opinion about physico-chemical mechanisms involved in yeast flocculation. Several mechanisms have been proposed: a calcium bridge between anionic groups on the surface of adjoining
cells (Mill, 1964; Beavan et al., 1979); the "lectin-like" theory which implicates a specific lectin, bound to α-mannan of the adjoining cells (Miki et al., 1982a), using Ca$^{2+}$ to ensure the correct conformation of the lectin [see Rose (1984) and Calleja (1987) for reviews of flocculation].

Environmental factors also influence the flocculation ability of yeast cells. The cultural conditions which induce or repress flocculation are one of the most controversial subjects. For example, the wort components have been described as stimulators by some authors and as inhibitory or innocuous by others. Lindquist (1953) reports that fermentable sugars such as glucose, mannose, maltose and sucrose repress the flocculation. Otherwise, Nishihara (Nishihara et al., 1976a) verified that cells growing in a medium with glucose, fructose, mannose, galactose, sucrose and maltose flocculated strongly. Others claim that no single nutritional element (carbohydrates, aminoacids and proteins) can give a complete explanation of the flocculation process (Amri et al., 1979; Bonaly et al., 1981). Patel and Ingledew (1975a,b) claim that differences in sugar’s metabolism can explain the flocculation mechanism.

The influence of aeration involves similar contradictions. Some authors think that aeration stimulates flocculation, while others consider that it has an inhibitory effect (Calleja, 1987). Miki (Miki et al., 1982b) observed that in aerobic cultures the flocculence was not significantly affected; it was repressed in anaerobic growth but recovered in stationary phase or upon aeration.

Mitochondrial function appear to be necessary for flocculation expression. In the presence of glycolytic and respiratory inhibitors and uncouplers, the flocculation induction is repressed (Nishihara et al., 1976b). Moreover, respiratory-deficient mutants exhibit a change in flocculation capacity when compared to parental strains (Stewart et al., 1976); this influence seems to be strain specific (Holmberg and Kielland-Brandt, 1978; Hinrichs et al., 1988). The induction of flocculence is repressed by cycloheximide, but not by chloramphenicol. This suggests that the synthesis of cytoplasmatic proteins but not mitochondrial is necessary (Nishihara et al., 1976b).

Some of the studies indicated above report results on the influence of several fermentable sugars. Other authors, like Miki (Miki et al., 1982b), performed experiments on the combined effects of glucose and aeration. However even in this case, the influence of low glucose concentration was not investigated, and the tested range of aeration rate was rather narrow.

To our knowledge, this is the first work which intends to exploit simultaneously the effect of a combined wide range of both aeration and glucose concentration on flocculence.