

Effect of Salt Stress on the Growth of Yeast *Saccharomycopsis fibuligera*

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ABSTRACT

The effect of salt condition on the growth of cells was examined in both shaking and standing culture conditions. The cells were able to grow in a shaking culture after adaptation to the condition of 1 M NaCl, but the growth rate was slower than that of the condition without NaCl. The cells grew in the shaking culture containing 1 M KCl and the growth rate was higher than that of the culture with 1 M NaCl but lower than that of the culture without salt. The cells adapted to the condition of 1 M NaCl were able to grow in a standing culture and produced mycelial-form cells and their forms were slimmer than those of mycelial cells formed in the culture containing no NaCl. The growth of cells in the standing culture was stimulated by plant hormones such as indoleacetic acid (0.05 µg/ml), naphthaleneacetic acid (0.5 µg/ml) and 2,4-dichlorophenoxyacetic acid (0.05 µg/ml). The adapted cells grew under the condition of 1 M NaCl in standing culture, although the growth rate was lower than that of normal standing culture conditions and growth was somewhat stimulated by those plant hormones in the mycelial form cells. The cells were also able to grow in the presence of 2 M NaCl after adaptation to the condition of standing culture, although their growth rate was lower and their mycelial forms were slimmer than those grown in the presence of 1 M NaCl. Furthermore some bubble-shaped hollow cells were observed among them. It is suggested that the high concentration of Na⁺ affects the components of the cell structure.

INTRODUCTION

Dimorphic changes are observed under some environmental conditions in *Saccharomycopsis fibuligera* (Takada et al., 1963; Yagi, 1977). Dimorphism in fungi has been considered as a good model of primitive morphogenesis. Cells of *S. fibuligera* grow in a yeast-like form under shaking culture conditions and they change their form from yeast-like to mycelial form under the standing culture conditions. We previously reported that the cells harvested from the shaking culture at a log phase exhibited a very low ATPase activity, whereas the cells harvested at an early stationary phase exhibited a high ATPase activity in both *Saccharomyces cerevisiae* (Ota, 1982, 1983, 1985 and 1986) and *S. fibuligera* (Ota and Morishita, 1986). The mycelial form cells of *S. fibuligera* harvested from the standing culture at a log phase exhibited a very high ATPase activity (Ota and Morishita, 1983, 1987 and 1988a,b). For amylase activity in the cells of *S. fibuligera*, mycelial form cells were higher than

yeast-like form cells (Ota and Morishita, 1989a). The cells of *S. fibuligera* were able to grow in a shaking culture after adaptation to the condition of 1 M NaCl. The adapted cells harvested at a log phase exhibited a considerable amount of ATPase activity, although the cells before adaptation exhibited a low ATPase activity (Ota and Morishita, 1983).

This communication describes the effect of salt and plant hormones on the growth in the cells of *S. fibuligera*.

METHODS

Yeast strain

Saccharomyces fibuligera (Linder) Dekker, IFO 0103, was used throughout this study.

Growth and conditions

Cells of *S. fibuligera* were grown at 30°C on YM medium containing 5 g polypeptone, 3 g malt extract and 20 g glucose per litre. Yeast-like form cells were grown under shaking culture conditions of about 95

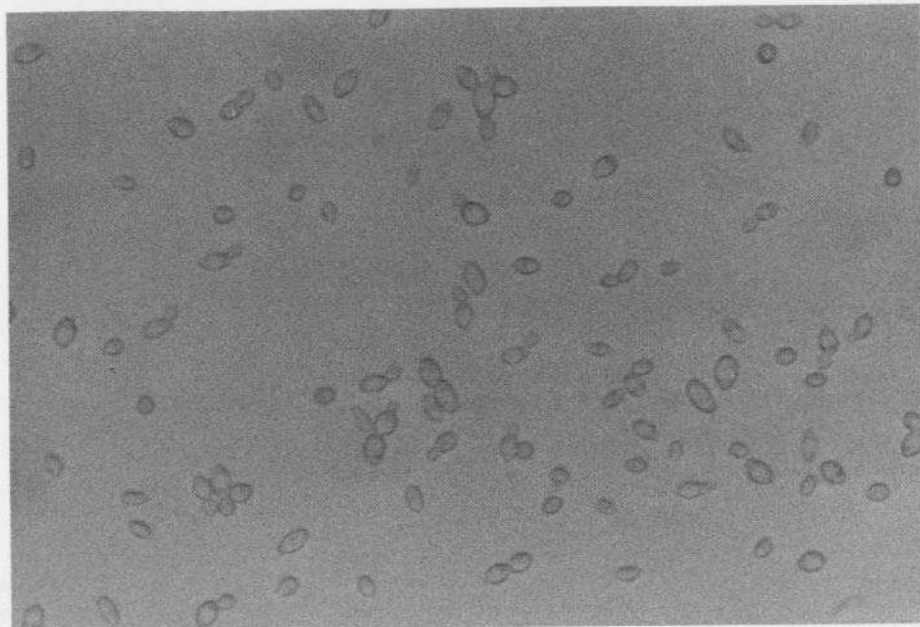


Fig. 1. Yeast-like form cells at a log phase incubated in a shaking culture at 30°C for 1 day.

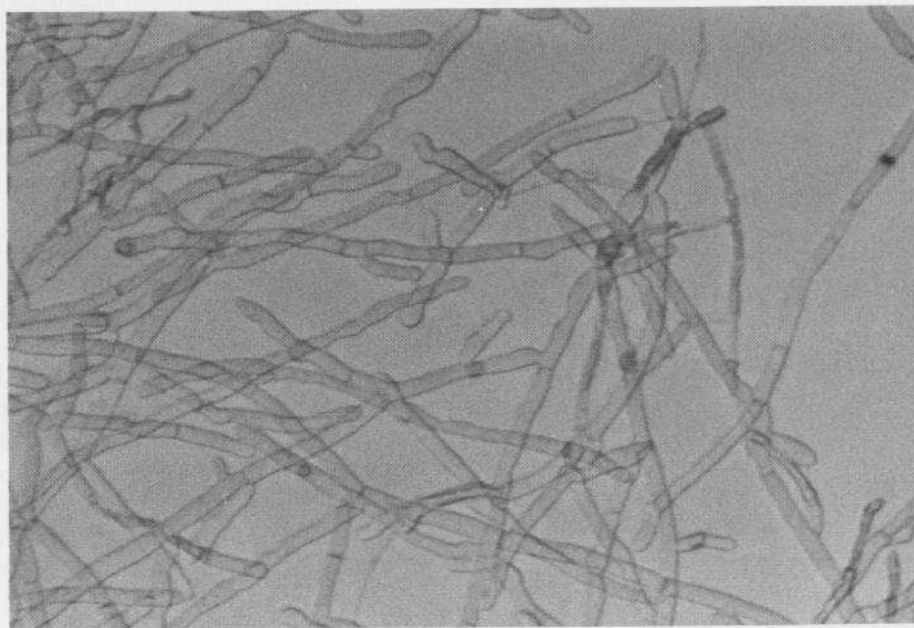


Fig. 2. Mycelial-form cells incubated in a standing culture at 30°C for 3 days.

strokes/min and mycelial-form cells were grown under standing culture conditions. For the measurement of salt effect on the growth, the final concentration of 1 M or 2 M NaCl, 1 M KCl or MgCl₂ was added to the medium. For the effect of plant hormones, the final concentration of 0.05 µg/ml of indoleacetic acid, or 2,4-dichlorophenoxyacetic acid, 0.5 µg/ml of naphthaleneacetic acid was added to the medium.

RESULTS

Figure 1 shows the yeast-like form cells of *S. fibuligera* incubated for 1 day under shaking culture conditions. Many budding cells were observed among the cells at a log phase. Figure 2 shows the mycelial-form cells of *S. fibuligera* incubated for 3 days under standing culture conditions. A consider-

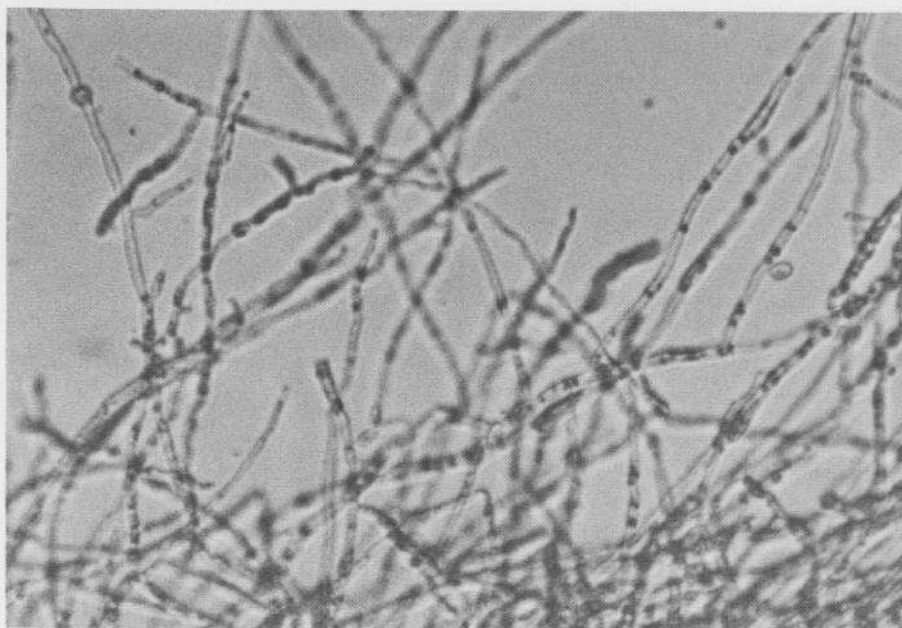


Fig. 3. Mycelial-form cells incubated in a standing culture containing 1 M NaCl at 30°C for 15 days.

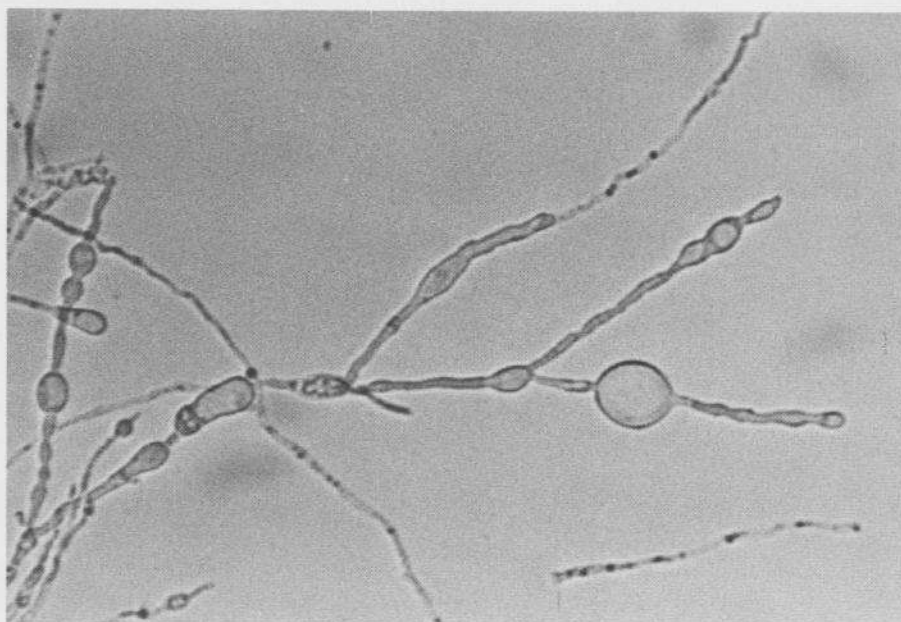


Fig. 4. Mycelial-form cells incubated in a standing culture containing 2 M NaCl at 30°C for 24 days.

able amount of the growth of mycelial form cells was observed. The mycelial-form cells under standing culture conditions manifested their slow growth and the growth curve of mycelial-form cells rose gradually during the incubation and reached its peak in 10–15 days (Ota and Morishita 1988a, b). Figure 3 shows mycelial-form cells of *S. fibuligera* incubated for 15 days under standing culture in the

presence of 1 M NaCl. The cells under standing culture containing 1 M NaCl grew more slowly after the adaptation than those containing no NaCl. The yeast-like form cells of *S. fibuligera* under shaking culture containing 1 M KCl grew faster than those containing 1 M NaCl, but slower than those containing no NaCl. Figure 4 shows the mycelial-form cells of *S. fibuligera* incubated for 24 days under standing

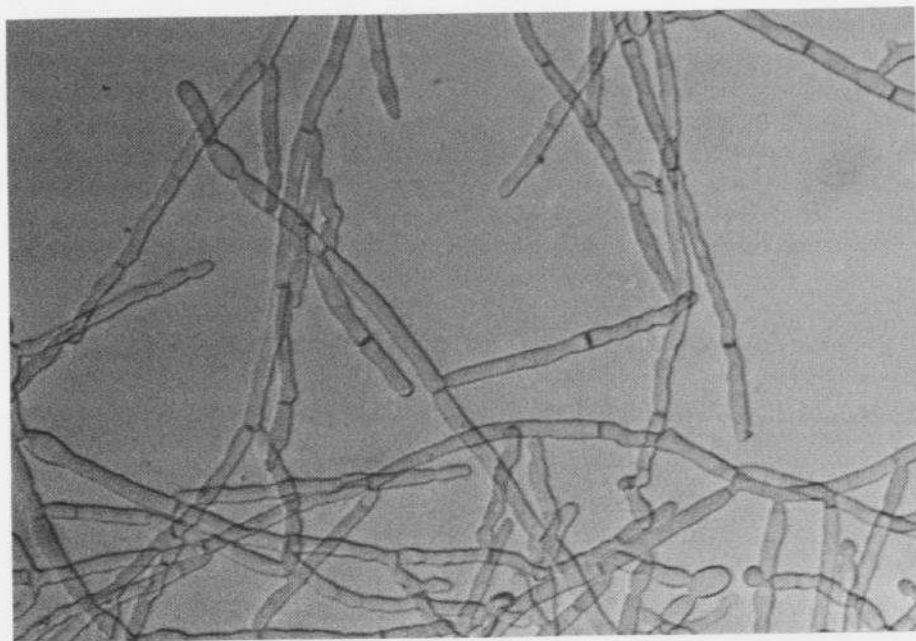


Fig. 5. Mycelial-form cells incubated in a standing culture containing indoleacetic acid (0.05 µg/ml) at 30°C for 3 days.

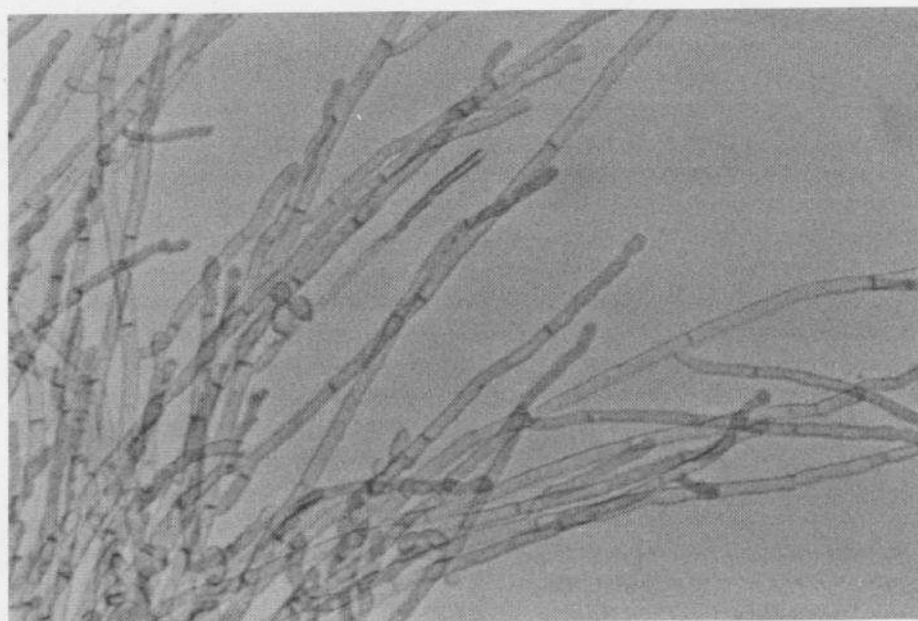


Fig. 6. Mycelial-form cells incubated in a standing culture containing naphthaleneacetic acid (0.05 µg/ml) at 30°C for 3 days.

culture containing 2 M NaCl. The cells under the standing culture containing 2 M NaCl grew more slowly after the adaptation than those containing 1 M NaCl and their form was more slimmer. Some bubble-shaped hollow cells were observed among them. Figure 5 shows the growth of the cells of *S. fibuligera* under standing culture conditions containing indoleacetic acid. The growth of the cells was

somewhat stimulated by a plant hormone, indoleacetic acid, at the concentration of 0.05 µg/ml. Higher concentration of the hormone inhibited the growth. Figure 6 shows the growth of the *S. fibuligera* under standing culture conditions containing naphthaleneacetic acid. The growth of the cells was slightly stimulated by a plant hormone, naphthaleneacetic acid, at the concentration of 0.5 µg/ml.

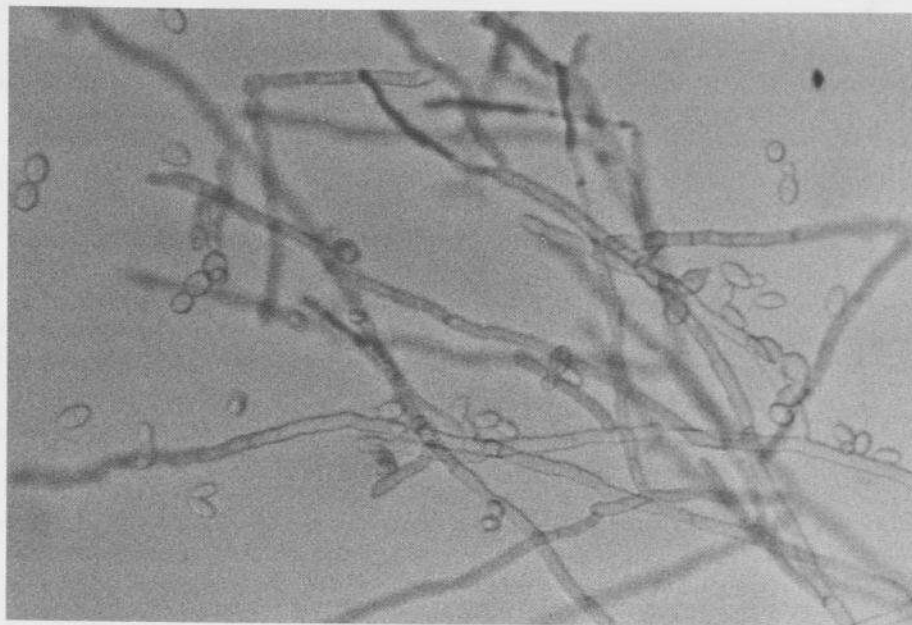


Fig. 7. Mycelial-form cells incubated in a standing culture containing 2,4-dichlorophenoxyacetic acid (0.05 $\mu\text{g/ml}$) at 30°C for 3 days.

Figure 7 shows the growth of the cells of *S. fibuligera* under standing culture conditions containing 2,4-dichlorophenoxyacetic acid. The growth was only slightly stimulated by a plant hormone, 2,4-dichlorophenoxyacetic acid at the concentration of 0.05 $\mu\text{g/ml}$. Higher concentration of the hormone inhibited the growth. These plant hormones affected the growth of the cells under the standing culture conditions containing 0.5 M NaCl as described above, but could not get rid of the depression resulting from the 0.5 M NaCl.

CONCLUSIONS

The cells of *S. fibuligera* were able to grow in a shaking culture after adaptation to the condition of 1 M NaCl, but at a rate of growth lower than that of the cells grown in the absence of NaCl. On the other hand the cells were able to grow in a shaking culture containing KCl and the growth rate was higher than that of the cells grown in the presence of 1 M NaCl. These results suggest that 1 M NaCl had an effect on the cell growth, not only osmotically but also structurally. We previously reported that a large number of the cells of *S. fibuligera* which had been incubated under shaking culture conditions for 7 days looked hollow and concluded that the cells might have been broken and consequently their contents had leaked out. On the contrary, a large number of the cells grown in the presence of 1.6 M sorbitol were intact and the cells differed greatly from those grown in the presence of 1.6 M sucrose in their shape and mor-

phology; consequently it is suggested that 1.6 M sucrose not only induced osmotic stress on the cells but also affected their structural conformation (Ota, A. and Morishita, H., 1989b,c). The cells were able to grow in a standing culture with 2 M NaCl after adaptation to the condition, but their growth rate was lower and their mycelial forms were slimmer than those of the cells containing 1 M NaCl and some bubble-shaped hollow cells were observed among them. Morphology of the cells grown in the presence of 2 M NaCl is very different from that of the cells grown in the presence of 1.6 M sucrose. It is concluded that the high concentration of Na^+ had an effect on the cell structure that was different from 1.6 M sucrose.

Plant hormones, such as indoleacetic acid and naphthaleneacetic acid, had some stimulation effect on the cell growth both in the standing culture without NaCl and with 0.5 M NaCl. It seems likely that these plant hormones affect only the extension of mycelial form cells.

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