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Short Communication

Lithium chloride-tolerant character of the heterobasidiomycetous yeast *Rhodotorula glutinis*

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The heterobasidiomycetous yeast *Rhodotorula glutinis* was able to grow in medium containing a high concentration of LiCl. This character of *R. glutinis* was presumed to be attributable to its ability to incorporate [^{14}C]-adenine and [^{14}C]-leucine into nucleic acids and proteins, respectively, in the presence of LiCl. Intracellular levels of Li^+ and Cl^- ions, production and accumulation of glycerol as an osmoregulator, and respiration in the LiCl-stressed condition were almost the same in the tolerant yeast *R. glutinis* and the sensitive yeast *Rhodosporidium sphaerocarpum*.

Key Words——heterobasidiomycetous yeast; LiCl-tolerance; *Rhodosporidium sphaerocarpum*; *Rhodotorula glutinis*.

Lithium chloride strongly inhibits the growth of most microorganisms. For example, *Saccharomyces cerevisiae* Meyen et Hansen barely grows in the presence of 0.5 M LiCl (Yagi, 1992a). Salt-tolerant yeasts *Zygosaccharomyces rouxii* (Boutroux) Yarrow (Yagi, 1988) and *Rhodotorula glutinis* (Fresenius) Harrison var. *salinaria* Hirosawa et Takada (currently *Rhodosporidium sphaerocarpum* Newell et Fell) (Hirosawa and Takada, 1969), which are able to grow in medium containing 3 M NaCl, are also unable to grow in media containing 0.2 M to 0.5 M LiCl. Thus, LiCl-hypertonicity inhibits the growth of most microorganisms. Until now, no report of naturally occurring microorganisms exhibiting tolerance to LiCl-hypertonicity exists to our knowledge, except for those on LiCl-resistant strains of *S. cerevisiae* and *Saccharomycopsis fibuligera* (Lindner) Klöcker, which were repeatedly adapted to high concentrations of LiCl (Laskowski, 1955, 1956; Yagi and Takada, 1973; Yagi, 1992a, 1993). Recently, we found that the heterobasidiomycetous yeast *Rhodotorula glutinis* (Fresenius) Harrison was strongly tolerant to LiCl-hypertonicity as well as to NaCl-hypertonicity. In this paper, we report the mechanism(s) of LiCl-tolerance of *R. glutinis*, in comparison with *R. sphaerocarpum*, which exhibits sensitivity to LiCl.

Rhodotorula glutinis (IFO 1125) and *R. sphaerocarpum* (IFO 1938) were used and abbreviated as Rg and Rs strains, respectively. Both strains were precultured in YPD-30 medium consisting of yeast extract 5 g, peptone 5 g, KH_2PO_4 5 g, $MgSO_4 \cdot 7H_2O$ 2 g, glucose 30 g and distilled water 1 L. The precultured cells were inoculated at a density of 5×10^5 cells/ml in 80 ml of YPD-30 medium with various concentrations of NaCl or LiCl and cultured at 26°C on a reciprocating shaker at 120 strokes/min. To determine the intracellular levels of glycerol and inorganic ions during the short treatment of salt stress, a larger inoculum of 10^8 cells/ml medium was used. Growth was monitored by measuring the absorbancy at 660 nm of cultures diluted with salt solutions isotonic to the respective growth media. Glycerol in cells and culture medium was determined enzymatically by the glycerokinase method (F-kit glycerol, Boehringer-Mannheim, Mannheim, Germany) (Yagi, 1988, 1992b). The total production of glycerol was estimated from the sum of the amounts of glycerol in cells and culture medium. Intracellular Li^+, K^+ and Cl^- ions were extracted twice with hot 0.5 N HNO_3 from the cells. The Li^+ and K^+ ions were measured by flame photometry in an atomic absorption spectrophotometer (model 208; Hitachi Ltd., Tokyo, Japan) and the Cl^- ions were colorimetrically quantitated (Iwasaki et al., 1952; Yagi, 1992b). Respiratory activities of cells in the medium containing various concentrations of LiCl were measured with a Warburg manometer at 26°C. To investigate incorporation of radioactive precursors, the cells grown in salt-free medium or cultured for a further 24 h in the medium with 0.5 M LiCl or NaCl were used. To incorporate radioactive leucine, the concentrations of yeast extracts and polypeptone in YPD-30 medium were reduced to 0.1 g/L. [8-^{14}C]-Adenine hydrochloride or [U-^{14}C]-leucine was added to the medium with or without salts at final concentration of 3.7 kBq/ml and incubated for 60 min or 3 h. At appropriate intervals, a 500-μl sample was taken into cooled tubes containing 500 μl of 10% trichloroacetic acid (TCA). The resultant TCA-insoluble materials were collected on a Whatman GF/C glass fiber disk and washed three times with 1 ml of 5% TCA. The radioactivity on the disks was counted in vials containing 5 ml of Scintisol

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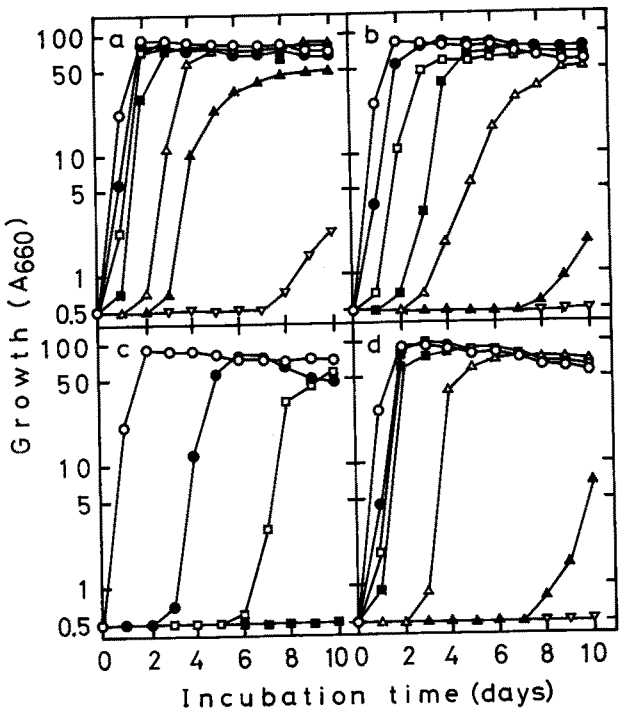

Fig. 1. Time course of growth of *R. sphaerocarpum* (a and c) and *R. glutinis* (b and d) in media containing various concentrations of NaCl (a and b) and LiCl (c and d). Symbols in a and b indicate growth in medium containing the following concentrations of NaCl: ○, no addition; ●, 1 M; □, 1.5 M; ■, 2 M; △, 2.5 M; ▲, 3 M; ▽, 3.5 M. Symbols in c and d indicate growth in medium containing the following concentrations of LiCl: ○, no addition; ●, 0.1 M; □, 0.3 M; ■, 0.5 M; △, 1.0 M; ▲, 1.5 M; ▽, 2.0 M.

EX-H (Dojindo Lab., Kumamoto, Japan) in a Packard TriCarb liquid scintillation counter. The radioactivities in the TCA-insoluble fraction were estimated as the amounts of incorporation into nucleic acids or proteins.

The growth of the Rs and Rg strains was examined in YPD-30 medium containing various concentrations of NaCl and LiCl (Fig. 1). The Rs strain grew well in the presence of up to 3 M NaCl, though a slight prolongation of the lag phase was observed. It could grow in the presence of 0.3 M LiCl with a longer lag phase, but not at all in the presence of 0.5 M LiCl (Fig. 1, a and c). On the other hand, the Rg strain was able to grow even in the presence of 1 M LiCl as well as in the presence of 2.5 M NaCl (Fig. 1, b and d). Both strains also grew well in the presence of 2 M or 3 M KCl or 1.4 M $MgCl_2$ (osmotic pressure equivalent to 2 M NaCl) (data not shown). Thus, the two strains exhibited similar tolerance to hypertonicity due to NaCl, KCl or $MgCl_2$, but quite different tolerance to hypertonicity due to LiCl.

To examine whether Li^+ and Cl^- ions penetrated into the cells, the intracellular levels of these ions were measured in cells of both strains which had been incubated for 6 h in media with 0.5 M or 1.0 M LiCl (Fig. 2). The intracellular levels of Li^+ ions of both strains increased in proportion to the concentration of LiCl in the medium, and reached a maximum in medium with 1.0 M LiCl after

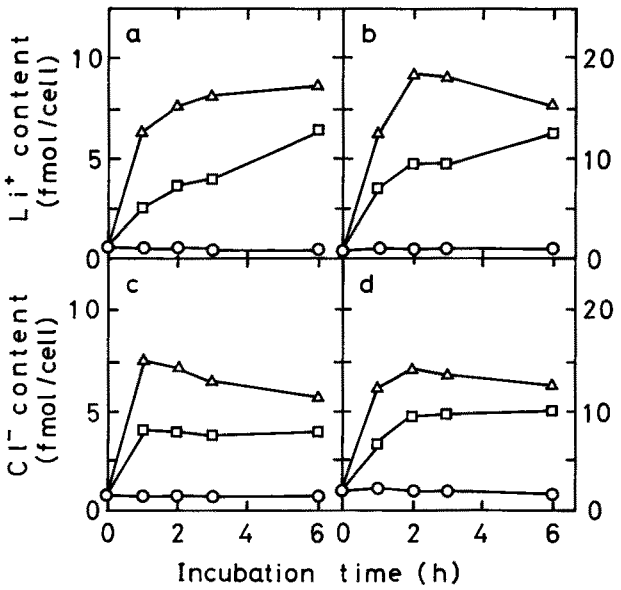

Fig. 2. Changes in levels of Li^+ (a and b) and Cl^- (c and d) ions in *R. sphaerocarpum* (a and c) and *R. glutinis* (b and d) incubated for 6 h in medium containing various concentrations of LiCl. Symbols: ○, salt-free; □, 0.5 M LiCl; △, 1.0 M LiCl.

2 to 4 h of incubation. The intracellular levels of Cl^- in both strains also increased to a constant level after 1 or 2 h of incubation. We determined cell volume of both strains by measuring the packed cell volume (Yagi, 1992b). The cell volumes of the Rg and Rs strains were $68.9 \pm 10.5\ \mu m^3$ and $25.9 \pm 5.8\ \mu m^3$, respectively, that of the Rg strain being about 2.6 times larger than that of

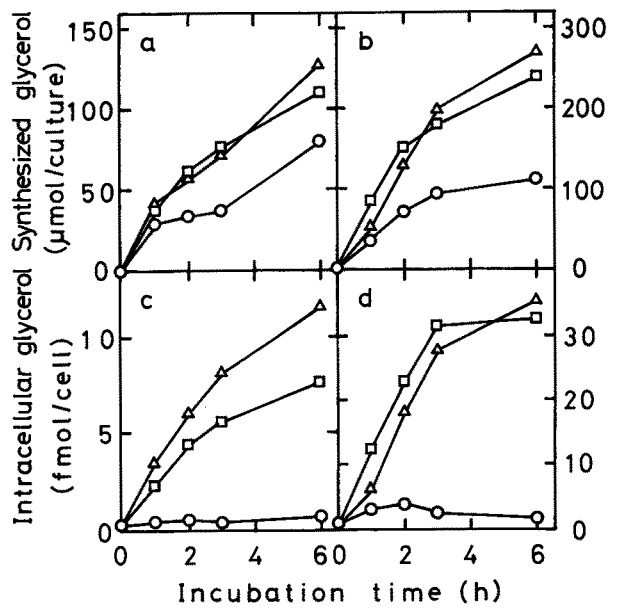

Fig. 3. Changes in levels of synthesized glycerol (a and b) and intracellular glycerol (c and d) in *R. sphaerocarpum* (a and c) and *R. glutinis* (b and d) incubated for 6 h in medium containing various concentrations of LiCl. Symbols: ○, salt-free; □, 0.5 M LiCl; △, 1.0 M LiCl.

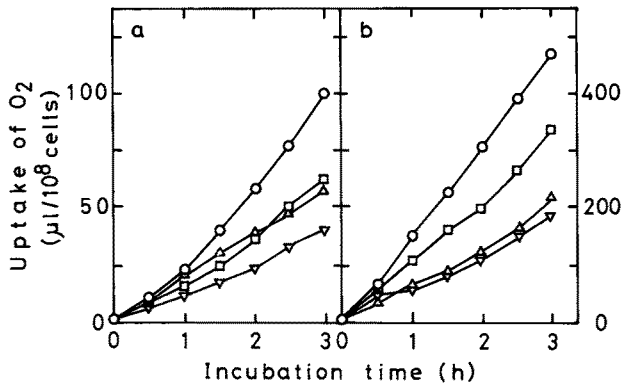

Fig. 4. Changes in uptake of O_2 in *R. sphaerocarpum* (a) and *R. glutinis* (b) incubated for 3 h in medium containing various concentrations of LiCl. Symbols: ○, salt-free; □, 0.5 M LiCl; △, 1.0 M LiCl, ▽, 1.5 M LiCl.

the Rs strain. The differences in the intracellular levels of the ions between the two strains were considered to be due to the difference in their cell volumes. These results indicate that a considerable amount of Li^+ and Cl^- ions penetrated into cells of both strains, and that the tolerance of the Rg strain to LiCl-hypertonicity is not caused by impermeability of the cells to LiCl.

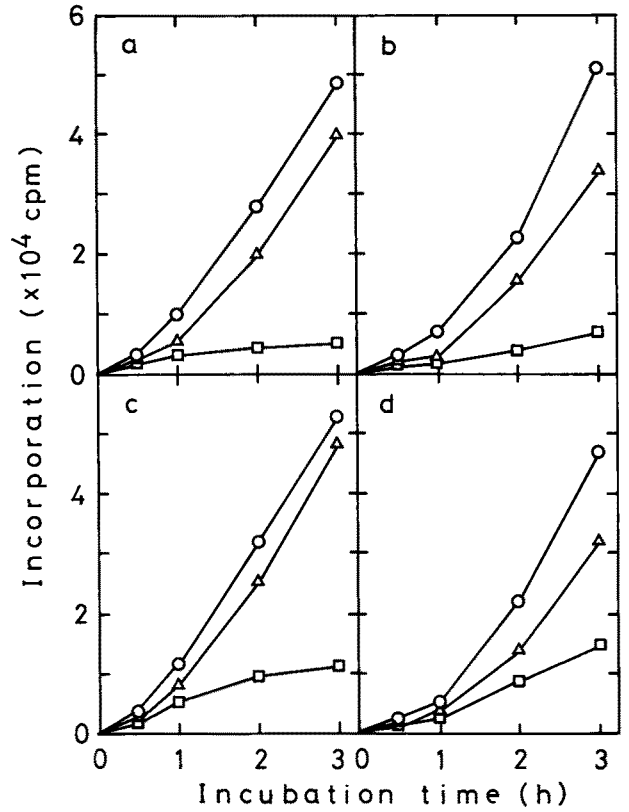

Fig. 5. Incorporation of [^{14}C]-adenine (a and b) and [^{14}C]-leucine (c and d) into TCA-insoluble fraction in cells of *R. sphaerocarpum* (a and c) and *R. glutinis* (b and d) in the presence of 0.5 M LiCl or 0.5 M NaCl. Symbols: ○, salt-free; □, 0.5 M LiCl; △, 0.5 M NaCl.

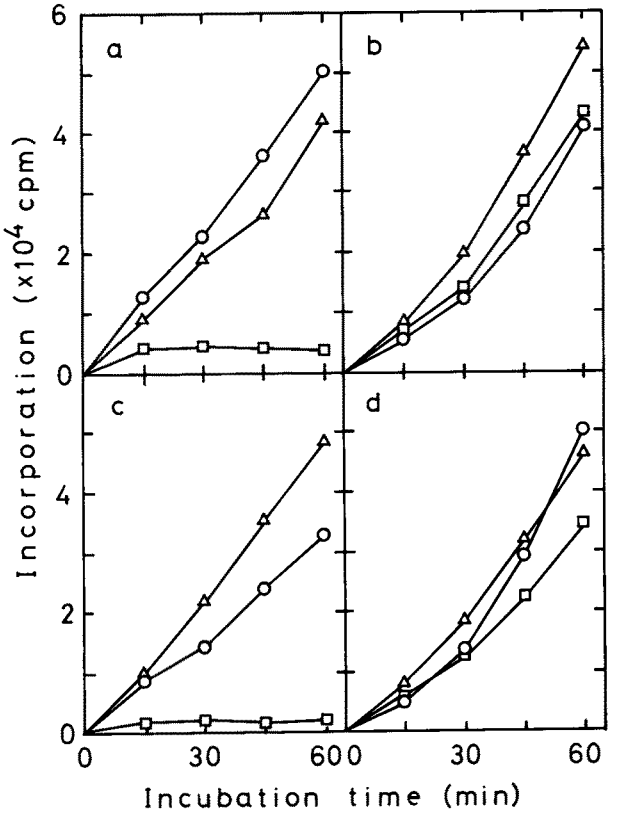

Fig. 6. Incorporation of [^{14}C]-adenine (a and b) and [^{14}C]-leucine (c and d) into TCA-insoluble fraction in cells of *R. sphaerocarpum* (a and c) and *R. glutinis* (b and d) incubated under identical conditions for 24 h in media with 0.5 M LiCl or 0.5 M NaCl. Symbols: ○, salt-free; □, 0.5 M LiCl; △, 0.5 M NaCl.

The Rs strain is known to accumulate glycerol as a major osmoregulator in the NaCl-hypertonic condition (Yagi and Ashibe, 1994). The effects of LiCl on the synthesis and intracellular accumulation of glycerol were then examined (Fig. 3). In both strains, synthesis of glycerol was accelerated in the presence of 0.5 M LiCl, although no further acceleration was found in the presence of 1 M LiCl (Fig. 3, a and b). Intracellular accumulation of glycerol was not inhibited in either strain by LiCl-hypertonicity (Fig. 3, c and d). Thus there was no difference between the strains in the synthesis and intracellular accumulation of glycerol in the LiCl-hypertonic condition. The effects of LiCl on respiration in both strains were then examined in media containing various concentrations of LiCl. The oxygen uptake was reduced by almost the same extent in the two strains in the presence of high concentrations of LiCl (Fig. 4). A similar effect of LiCl-hypertonicity on liberation of carbon dioxide was observed in both strains (data not shown).

The effects of LiCl-hypertonicity on incorporation of [^{14}C]-adenine and [^{14}C]-leucine into nucleic acids and proteins were then examined in cells immediately after the exposure to LiCl-hypertonicity or after incubation for a further 24 h in the LiCl-stressed condition. The incorpo-

ration of both substances into nucleic acids and proteins was inhibited immediately after exposure to LiCl-hypertonicity in both Rg and Rs strains, but not inhibited in the case of NaCl-hypertonicity (Fig. 5). However, the Rg cells that had been incubated for a further 24 h in medium with 0.5 M LiCl rapidly incorporated [^{14}C]-adenine and [^{14}C]-leucine in the presence of 0.5 M LiCl to the same extent as those in the salt-free condition (Fig. 6, b and d). The Rs cells treated in the same manner were unable to incorporate adenine and leucine when incubated in the presence of 0.5 M LiCl for 60 min (Fig. 6, a and c) or 3 h (data not shown). These results indicated that the Rg cells are able to synthesize nucleic acids and proteins in the LiCl-stressed condition, although time is required to induce this character. This character is considered to enable *R. glutinis* to grow in medium containing a high concentration of LiCl.

Literature cited

Hirosawa, N. and Takada, H. 1969. Salt susceptibility of *Rhodotorula glutinis* var. *salinaria* isolated from salt farm in Japan. Trans. Mycol. Soc. Japan **10**: 35–39.

Iwasaki, I., Utsumi, S. and Ozawa, T. 1952. New colorimetric determination of chloride using mercuric thiocyanate and ferric ion. Bull. Chem. Soc. Japan **25**: 226.

Laskowski, W. 1955. Resistance of *Saccharomyces* to high concentrations of lithium chloride. Science **121**: 299–300.

Laskowski, W. 1956. The genetics of resistance of *Saccharomyces* to lithium chloride. Genetics **41**: 98–106.

Yagi, T. and Takada, H. 1973. Growth characteristics of a yeast, *Endomycopsis fibuligera*, adapted to hypertonicity with sodium chloride. Trans. Mycol. Soc. Japan **14**: 211–220.

Yagi, T. 1988. Intracellular levels of glycerol necessary for initiation of growth under salt-stressed condition in a salt-tolerant yeast, *Zygosaccharomyces rouxii*. FEMS Microbiol. Lett. **49**: 25–30.

Yagi, T. 1992a. Isolation and some properties of lithium chloride-resistant strains of *Saccharomyces cerevisiae*. Trans. Mycol. Soc. Japan **33**: 193–201.

Yagi, T. 1992b. Regulation of intracellular osmotic pressure during the initial stages of salt stress in a salt-tolerant yeast, *Zygosaccharomyces rouxii*. Microbios **70**: 93–102.

Yagi, T. 1993. Promotive effect of a reduction in the level of the carbon source on growth of a LiCl-resistant strain of *Saccharomyces cerevisiae* in the presence of LiCl. Trans. Mycol. Soc. Japan **34**: 153–157.

Yagi, T. and Ashibe, E. 1994. Accumulation of glycerol by the heterobasidiomycetous yeast *Rhodosporidium sphaerocarpum* in response to external hypertonicity due to NaCl. FEMS Microbiol. Lett. **115**: 213–218.