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Effect of Rare Sugars on Physiology of Yeast

by

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Abstract

Effect of rare sugars on physiology of yeast *Candida tropicalis* pK233 was studied. Of rare sugars tested, L-sorbose exerted a significant effect. L-Sorbose inhibited growth strongly and brought about pseudhyphal growth in the absence of D-glucose. This effect was not shown in the presence of D-glucose. L-Sorbose caused a decrease of respiratory activity. Cell lysis, cell death and leakage of intracellular components, such as nucleotides and potassium ion, were brought about by L-sorbose.

Key word ; rare sugar, L-sorbose, yeast, *Candida tropicalis* pK233

INTRODUCTION

Rare sugars are monosaccharides and their derivatives which exist rarely in nature. Because of their rarity and expensiveness, the research on rare sugars has not being done yet. New enzymes capable of changing abundant natural sugars to rare sugars were discovered by Izumori and mass production of rare sugars from cheap D-glucose and D-fructose became possible now[1),2)]. Physicochemical property and physiological activity of rare sugars are studied at present. Application of rare sugars to medical supplies, functional foods and cosmetics are being developed[3),4)]. In this study, we describe effect of rare sugars on the physiology of *C. tropicalis* pK233, a dimorphic yeast.

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MATERIALS AND METHODS

Yeast strain

Candida tropicalis pK233 was used throughout.

Cultivation

The yeast cells, cultivated in YP medium including 1% yeast extract and 2% peptone, were washed three times with sterilized deionized water and inoculated at a cell concentration of 1 μg/ml into fresh medium of the same composition or fresh medium including 2% each rare sugar described below. Cultivation was carried out at 30℃ with shaking on a rotatory shaker.

Rare sugars used

D-Allose, L-sorbose, D-sorbose, L-psicose, D-psicose, L-fructose, L-tagatose, D-tagatose were used. Allitol was also used as a derivative of rare sugar.

Effect of rare sugars on physiology of resting cells

Respiratory activity

Respiratory activity was determined through change of dissolved oxygen using DO meter.

Measurement of cell viability

Cell viability was measured by methylene blue staining.

Measurement of leakage of intracellular components

Culture was harvested by centrifugation and washed with distilled water repeatedly until the absorbance of the supernatant at 260nm was negligible. The cells were suspended at the concentration of 1mg/ml in distilled water or 2% L-sorbose and incubated at 30℃. The suspension was then sampled at appropriate time. After low-speed centrifugation, the supernatant was sampled. Nucleotides and potassium ion in the supernatant was measured according to method of Mizoguchi *et al.*[5)-7)] and ICP emission spectral analysis, respectively.

RESULTS AND DISCUSSION

Effect of rare sugars on growth of *C. tropicalis* pK233

Fig. 1 shows time course of cell concentration of *C. tropicalis* pK233 grown in YP media including 2% each rare sugar. L-Sorbose lengthened lag phase and caused a significant delay of beginning of the cell growth. L-Tagatose also lengthened lag phase and allitol repressed the growth rate. In contrast, L-fructose caused some increase of the cell concentration at stationary phase. It is possible that L-fructose was dissimilated by the cells. Other rare sugars tested failed to affect the cell growth（data not shown）.

Next, effect of L-sorbose, which inhibited cell growth significantly, on the morphology of the cells was studied.

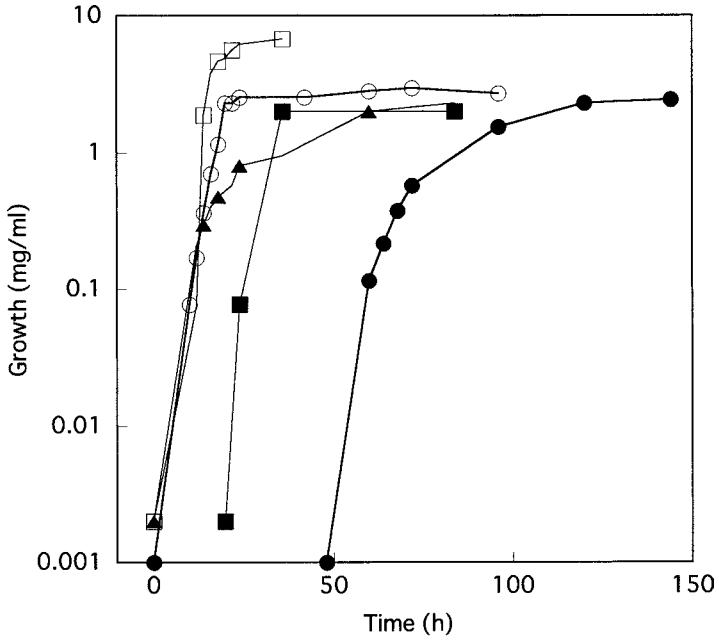
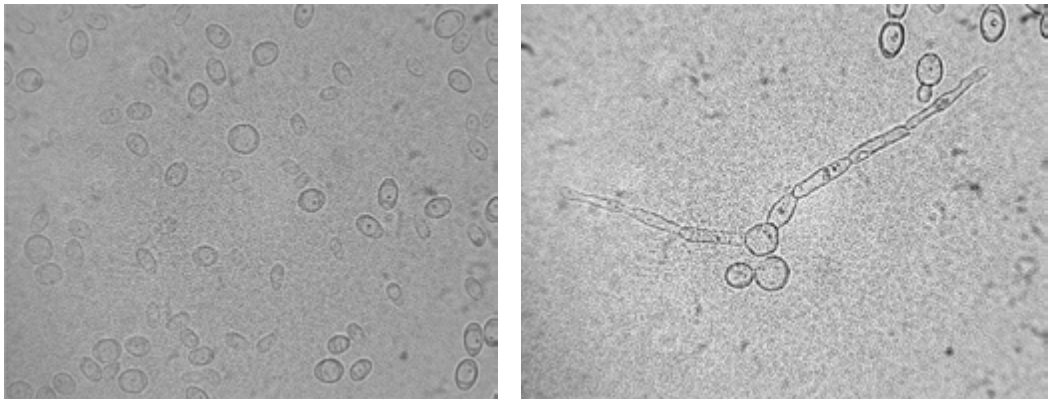


Fig. 1 Effect of rare sugars on growth of *C. tropicalis* pK233
○; none, ●; L-sorbose, ■; L-tagatose, ▲; allitol, □; L-fructose

Effect of L-sorbose on morphology of *C. tropicalis* pK233

While the cells were grown in yeast form in YP medium, L-sorbose caused pseudohyphal development in *C. tropicalis* pK 233 as shown in Fig. 2.



L-Sorbose(−), 72h　　2% L-Sorbose, 72h

Fig. 2　Microphotographs of *C. tropicalis* pK233

Effect of D-sorbose on growth of *C. tropicalis* pK233

Effect of D-sorbose, the enantiomer of L-sorbose which affected growth and morphology of *C. tropicalis* pK233 significantly, on cell growth was investigated. As shown in Fig. 3, D-sorbose did not inhibit the cell growth at all.

Among rare sugars tested, L-sorbose, L-tagatose and allitol affected cell growth. Effect of these rare sugars on the cell growth was studied in the presence of D-glucose.

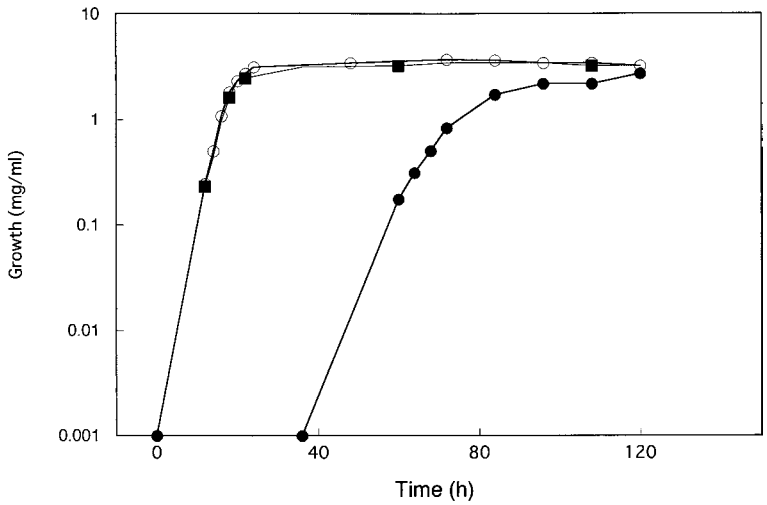


Fig. 3　Effect of D-sorbose on growth of *C. tropicalis* pK233
○; none, ●; L-sorbose, ■; D-sorbose

Effects of rare sugars on growth of *C. tropicalis* pK233 in the presence of D-glucose

Fig. 4 shows effect of 2% L-sorbose, L-tagatose and allitol on cell growth in YP medium including 2% D-glucose. In principle the presence of D-glucose enhanced growth rate. Any rare sugar, including L-sorbose, failed to inhibit the cell growth at all.

Next, physiological effect of L-sorbose, which significantly inhibited cell growth of resting cells in the absence of D-glucose, was studied.

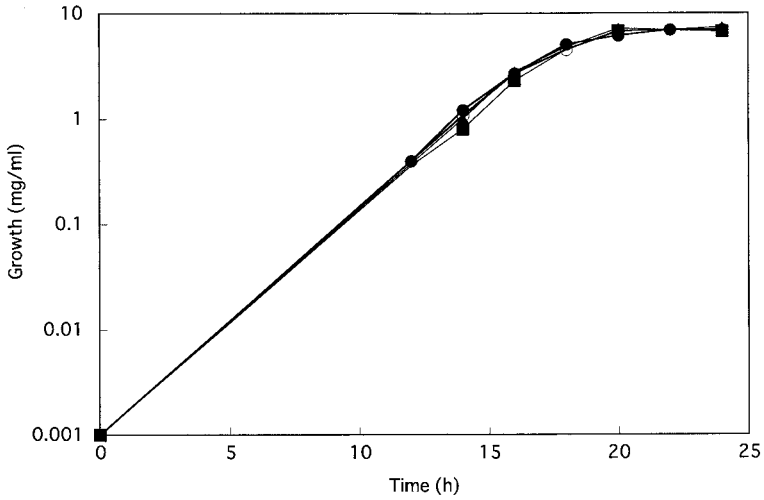


Fig. 4 Effect of rare sugars on growth of *C. tropicalis* pK233 in the presence of D-glucose
○; none, ●; L-sorbose, ■; L-tagatose, ▲; allitol

Effect of L-sorbose on respiratory ability of resting cells of *C. tropicalis* pK233

Fig. 5 shows respiratory ability of cells which were grown in YP medium including no L-sorbose for 24h, washed with deionized water and suspended in deionized water or 2% L-sorbose and incubated at 30℃ for appropriate time. L-Sorbose caused a significant decrease of respiratory activity and the respiratory activity of cells incubated in 2% L-sorbose for 24h decreased to 20% of the original activity.

Effect of L-sorbose on cell concentration and viability of resting cells of *C. tropicalis* pK233

Time-course of cell concentration and viability of cells grown in YP medium including no L-sorbose for 24h, washed with deionized water, suspended in deionized water or 2% L-sorbose and incubated at 30℃ was shown in Fig. 6. L-Sorbose brought about decrease of cell concentration. Cell viability was also decreased by L-sorbose.

L-Sorbose caused cell death and cell lysis. Therefore effect of L-sorbose on

the leakage of intracellular components such as nucleotides and potassium ion was investigated under the same condition described above.

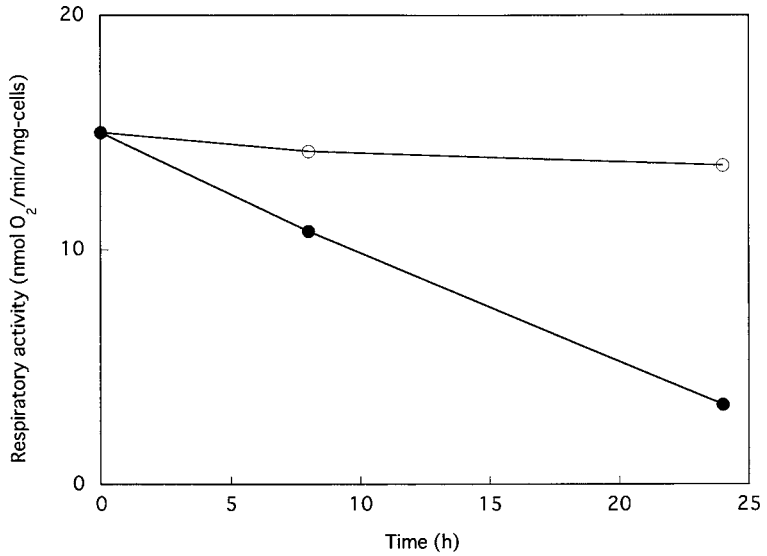


Fig. 5 Effect of L-sorbose on respiratory activity of resting cells of *C. tropicalis* pK233
○; none, ●; L-sorbose

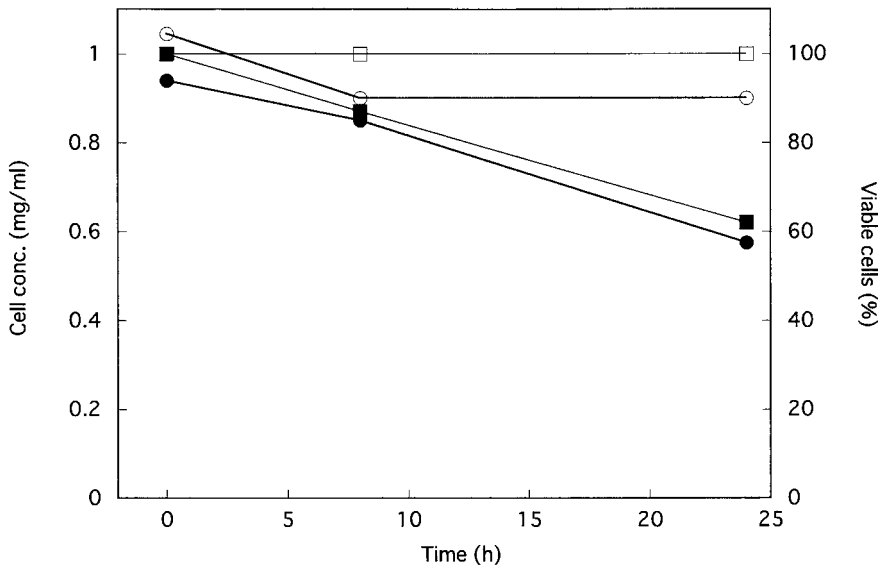


Fig. 6 Effect of L-sorbose on cell concentration and viability of resting cell of *C. tropicalis* pK233
○; cell nonc.(none), ●; cell conc.(L-sorbose),
□; viability(none), ■; viability(L-sorbose)

Effect of L-sorbose on leakege of intracellular components of resting cells of *C. tropicalis* pK233

Nucleotides

Fig. 7 shows time-course of leakage of intracellular nucleotide of cells which were grown in YP medium including no L-sorbose for 24h, washed with deionized water and suspended in deionized water or 2% L-sorbose and incubated at 30℃ for appropriate time. L-Sorbose promoted a significant leakage of intracellular nucleotides while no leakage of intracellular nucleotides occurred when the resting cells were incubated in water for 24h.

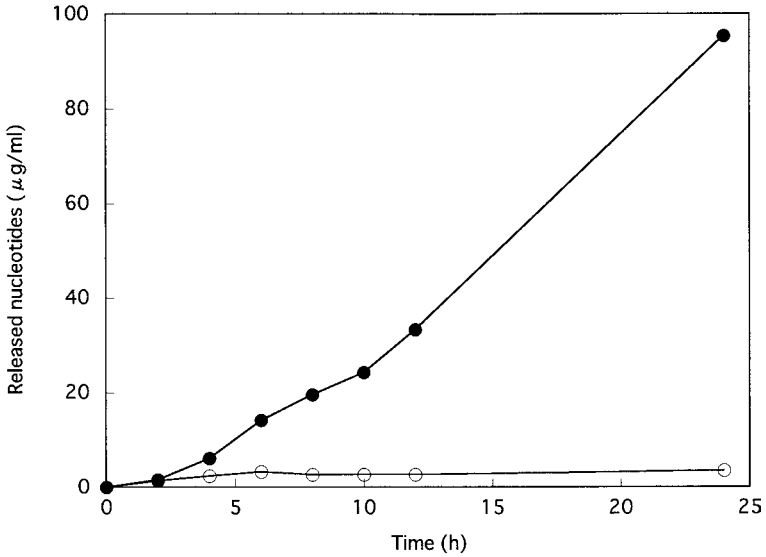


Fig. 7 Effect of L-sorbose on leakage of intracellular nucleotides of reating cells of *C. tropicalis* pK233
○; none, ●; L-sorbose

Potassium ion

Fig. 8 shows time-course of leakage of intracellular potassium ion of cells which were grown in YP medium including no L-sorbose for 24h, washed with deionized water and suspended in deionized water or 2% L-sorbose and incubated at 30℃ for appropriate time. L-Sorbose caused a significant leakage of intracellular potassium ion as well while no leakage of intracellular potassium ion occurred when the resting cells were incubated in water for 24h.

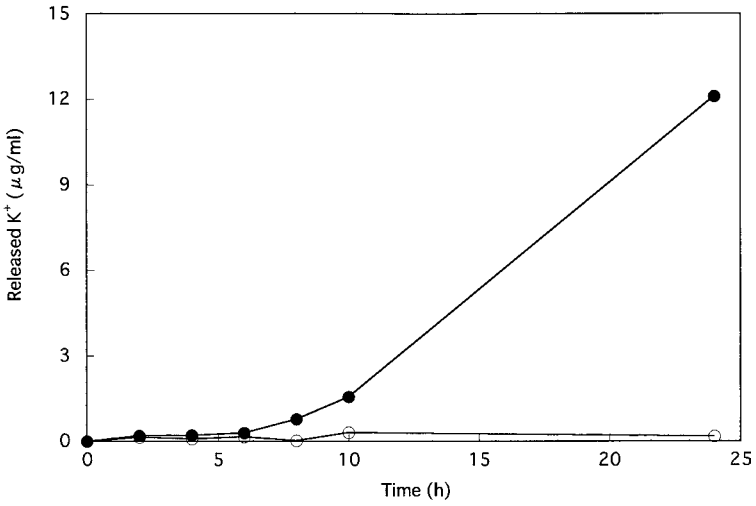


Fig. 8 Effect of L-sorbose on leakage of intracellular potassium ion of reating cells of *C. tropicalis* pK233
○; none, ●; L-sorbose

While the cells of *C. tropicalis* pK233 were grown in yeast form in the medium including D-glucose, they were grown in pseudohyphal form in the medium including n-alkane as the carbon source[8]. Ethanol caused pseudohyphal development and leakage of various intracellular components even in the medium including D-glucose[9)-11)]. Inositol antagonized ethanol and repressed the pseudohyphal development. It was claimed that antagonistic action of ethanol and inositol on the dimorphism of *C. tropicalis* pK233 might be related to cytoplasmic membrane transduction[12), 13)]. The effect of rare sugars on the physiology of dimorphilic cells of *C. tropicalis* pK233 was investigated. Among rare sugars tested, L-sorbose affected the physiology of the cells in the absence of D-glucose significantly. L-Sorbose caused a significant delay of the beginning of growth, pseudohyphal development, cell death, cell lysis, decrease of respiratory activity and leakage of intracellular components such as nucleotides and potassium ion. It is probable that L-sorbose affects cytoplasmic membrane, brings about cell lysis and death, and inhibits the cell growth, though the exact mechanism is obscure at present.

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