Technical Papers

Resistance of Saccharomyces to High Concentrations of Lithium Chloride

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Yeasts frequently occur in mediums containing high concentrations of salts, that is, in food brines (*1*) or in sea water (*2*). Mrak and Bonar noted that the ability of several species of *Debaryomyces* to grow in high salt concentrations was reduced when the cultures were grown for a time on wort agar without additional salt. Takada and Nagai (*3*) investigated the plasmolytic behavior and the content of amino acids of *Saccharomyces ellipsoideus* adapted to high concentrations of NaCl. Growth and variability of this yeast on NaCl medium was studied by Yanagishima (*3*).

In experiments on the genetics of resistance of *Saccharomyces* (*4*) to relatively high concentrations of a variety of salts, several strains were obtained that can grow in a synthetic medium containing lithium chloride in concentrations up to 1.0*N* and do not lose their resistance after transfer in normal medium. The resistant strains were obtained by exposing unadapted stock cultures to various concentrations of the salt, which was added to DIFCO yeast nitrogen base medium, with 1-percent glucose as the carbon source. When liquid medium was used, 5000 to 20,000 cells were inoculated in 5 ml of the medium plus salt concentrations of 0.5*N* to 1.0*N*. Growth was observed in some tubes after 8 to 10 days; the cultures that grew were transferred to fresh salt medium every second or third day. The strains thus obtained remained resistant after 12 transfers in normal medium. Resistant mutants were also obtained on solid medium containing LiCl in this same range of concentrations. Ten to 20 days after plating, a comparison with the control plates (without added LiCl) revealed that only a fraction of the cells had produced colonies on the salt medium. Some of the largest colonies were selected, and these remained resistant after several transfers in normal medium.

Twenty-seven independent haploid and diploid strains were thus obtained, all of which can grow on 0.5*N* and some on 1.0*N* LiCl medium. In order to analyze the genetic basis of the resistant strains, more than 60 crosses of various combinations were made. The diploids resulting from crosses between haploid LiCl-resistant strains and nonresistant strains grew, in all cases, on LiCl medium. Therefore, resistance to LiCl is dominant to nonresistance. Evidence of the genetic basis of resistance to LiCl was obtained by dissecting asci from several crosses and testing the spore cultures. Sixteen crosses were made and four nonallelic dominant genes (L_1, L_2, L_3, and L_4) have thus far been identified (*5*). Two of these genes (L_1 and L_2) were already present in two strains at the beginning of the experiments, whereas the other two (L_3 and L_4) arose after exposure of the strains to medium containing LiCl (as described earlier). It is not yet known whether LiCl is mutagenic in its effect or simply serves as a selective agent for the accumulation of mutants of spontaneous origin.

The response of four haploid strains carrying each of the dominant genes for LiCl resistance to increasing concentrations of LiCl is given in Table 1. For comparison, the response of a nonresistant haploid strain of the recessive genotype $l_1\ l_2\ l_3\ l_4$ is also shown. It will be noted that strains carrying gene L_1 exhibit fair growth on 0.3*N* LiCl medium within 6 days of incubation. Strains carrying gene L_2 exhibit good growth within 4 days on 0.5*N* LiCl medium, and strains with L_3 or L_4 need only 2 days to show good growth on the same medium. On concentrations of 0.75 to 1.0*N* LiCl medium, only strains containing the genes L_2, L_3, or L_4 will grow. The last produces the most vigorous growth.

A spectrophotometric analysis of resistant cultures grown on LiCl medium has shown a considerable decrease of the potassium content of the cells as compared with the controls of the same cultures grown

Table 1. Response of haploid strains carrying different genes for LiCl resistance to increasing concentrations of LiCl.

Genotypes for resistance to LiCl	Normality of LiCl concentration in the medium*							
	0	0.1	0.2	0.3	0.4	0.5	0.75	1.0
$l_1\ l_2\ l_3\ l_4$	+ (2)†	+ (4)	± (6)	–	–	–	–	–
$L_1\ l_2\ l_3\ l_4$	+ (2)	+ (3)	± (4)	± (6)	–	–	–	–
$l_1\ L_2\ l_3\ l_4$	+ (2)	+ (2)	+ (2)	+ (2)	+ (3)	+ (4)	± (5)	± (7)
$l_1\ l_2\ L_3\ l_4$	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (5)	± (5)
$l_1\ l_2\ l_3\ L_4$	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)

* Growth on solid mediums of different concentrations of LiCl is roughly compared with the following symbols: (i) + is good growth, equal or nearly equal to control culture growing on LiCl-deficient medium; and (ii) ± is fair growth, less efficient than growth of control.

† The numbers in parentheses give the average number of days necessary for the indicated state of growth.

on normal medium. Only 20 to 30 percent of the original potassium content could be found when the cultures were grown in 1.0*N* LiCl medium or were exposed to a 1.0*N* LiCl solution for 24 to 48 hr. The sodium content of the cells did not show a significant change in any case. In cultures grown on LiCl medium, lithium could be demonstrated only in the cells if the cultures were not washed more than twice. Nonresistant strains, grown on normal medium, were also exposed to LiCl solutions for 24 to 48 hr. They showed the same decrease in the potassium content as the resistant strains grown on the salt medium. The sodium content of the nonresistant strains exposed to LiCl solutions was also unchanged in comparison with control cultures.

The decrease of the potassium content after treatment with LiCl is of special interest since it is known that the K^+ ion plays an important role during fermentation in the yeast cell (*6–8*). It has also been pointed out that Li^+ interferes with the carbohydrate metabolism in yeast, sea urchin larvae (*9, 10*), and bacteria (*11*). An antagonistic effect of Li^+ and K^+ in yeast with regard to glucose fermentation was reported by Lindahl (*10*). Whether there is a direct exchange in the yeast cell of K^+ by Li^+ or whether Li^+ acts as a glycolytic inhibitor like iodoacetic acid or sodium fluoride which causes the loss of K^+ (*7*) remains to be determined. The fact that no Li^+ could be found in the yeast cells after several washes does not support the first possibility.

On the basis of these facts, it may be stated that the effect of LiCl on the decrease of the potassium content is the same in nonresistant and resistant strains. The ability of the latter to grow on LiCl medium is under gene control. If potassium is normally essential to the cell (*6–8*), the mutation enables the cell to grow in the presence of LiCl with a considerably decreased amount of potassium. Therefore, it can be assumed that a general difference exists between mutant and normal cells in regard to their requirement for a minimal amount of potassium.

This assumption could be supported by the results of fermentation tests in a glucose medium lacking LiCl and deficient in potassium (*12*). Several LiCl-resistant and nonresistant strains were inoculated into test tubes containing 8 ml of the medium, part of which filled a small inverted test tube (Durham tube). After 1 wk of incubation it appeared that only the LiCl-resistant strains had filled the inverted tubes with gas, whereas the nonresistant strains had produced no gas or only a small amount of it. In addition, it should be mentioned that strains containing the genes L_2, L_3, or L_4 filled the inverted tubes with gas in a shorter period of time than strains with the gene L_1. Further experiments to determine the significance of potassium for normal and mutant strains are in progress (*13*).

References and Notes

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1. K. Kroemer and G. Krumbholz, *Arch. Mikrobiol.* **3**, 384 (1932) ; J. Lodder, *Zentr. Bakteriol. Parasitenk. Abt. II* **86**, 227 (1932) ; F. M. Mrak and L. Bonar, *ibid.* **100**, 289 (1939).
2. C. E. ZoBell, *Marine Microbiology* (Chronica Botanica, Waltham, Mass., 1946).
3. H. Takada, *J. Inst. Polytech., Osaka City Univ. Ser. D* **4**, 17 (1953) ; S. Nagai, *ibid.* **4**, 35 (1953) ; N. Yanagishima, *ibid.* **5**, 29 (1954).
4. The yeast strains used in these experiments are genetic strains that are morphologically like *S. cerevisiae* and were largely derived from this species.
5. A detailed description of the crosses will be published elsewhere.
6. J. M. Muntz, *J. Biol. Chem.* **171**, 653 (1947).
7. G. T. Scott, M. A. Jacobson, and M. E. Rice, *Arch. Biochem.* **30**, 282 (1950).
8. O. Meyerhof and A. Kaplan, *Arch. Biochem. and Biophys.* **33**, 282 (1951).
9. P. E. Lindahl, *Arch. Entwicklungsmech. Organ.* **128**, 661 (1933).
10. ———, *Naturwiss.* **22**, 105 (1934) ; ———, *Acta Zoologica* **17**, 179 (1936).
11. W. Braun, *Bacteriol. Revs.* **11**, 75 (1947).
12. The medium used was a modification of DIFCO yeast nitrogen base medium (with glucose as carbon source) in which the potassium salts were replaced by sodium salts. The possibility remains that traces of K^+ were still present as impurities of other compounds. For the present purpose, however, it was considered as sufficient if the K^+ content was largely decreased in comparison to the standard medium.
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Hemocyanin and Radioactive Copper

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In view of the recent revival of interest in the binding of copper to the hemocyanins (*1–4*), we wish to report some observations made several years ago in these laboratories (*5*), that emphasize the extremely low degree of dissociability of the copper-protein bond. During the preliminary phase of a series of investigations in which radioactive copper has been used to study the exchangeability of copper in the copper enzymes, ascorbic acid oxidase (*5–7*), and tyrosinase (*8*), we had occasion to carry out an exploratory study of copper exchange in the hemocyanin of *Busycon canaliculatum*.

Filtered whole *Busycon* serum containing 0.22 to 0.26 percent copper based on the dry weight of the serum was employed. The procedures including the methods of radioactivity assay for the hemocyanin-radiocopper exchange experiments were similar to those previously published describing the exchange studies on ascorbic acid oxidase (*6, 9*). Unbuffered columns of Amberlite IR-100 (Na^+), 25 by 0.8 cm, were used for separating from the protein solution the unbound radiocopper remaining in solution after the hemocyanin had been exposed to the radiocopper for the desired length of time. All experiments were carried out at *p*H 6.5 to 7.0, approximating the natural *p*H of *Busycon* serum.