THE GENETICS OF RESISTANCE OF SACCHAROMYCES TO LITHIUM CHLORIDE

WOLFGANG LASKOWSKI

Department of Botany, University of Washington, Seattle, Washington

Received July 18, 1955

Yeast strains have been reported to occur in media containing high salt or sugar concentrations (Lodder 1932; Kroemer and Krumbholz 1932; Mraik and Bonar 1932; Gray 1945; Scarr 1951). Strains resistant to relatively high salt concentrations have also been produced experimentally by several investigators. The adaptation of Saccharomyces ellipsoideus to copper sulfate and to sodium chloride has been studied by Yanagishima (1952, 1954), Nagai (1953), and Takada (1953). Perlman and O'Brien (1954) have reported a strain of S. cerevisiae resistant to cobalt nitrate, and Sussman and Bradley (1953) have obtained strains of Saccharomyces which tolerate sodium arsenate.

The results reported below arose out of an investigation of the means of achieving and maintaining resistance to relatively high salt concentrations. The questions posed at the outset of the investigation were as follows: Is the effect transitory and if so, is the change which has occurred a cytoplasmic adaptation or an unstable nuclear modification; or, if the effect is lasting, what is the nature of the heritable change responsible for resistance, and what is the pattern of inheritance? Yeast is well-suited to a study of these problems since it will grow in a synthetic medium to which various salts in known concentration may be added. Also, hybrids of known parentage are easily produced and the genetics of resistance may be interpreted from the segregations in asci obtained from the hybrids.

Several genetic strains of yeast (Saccharomyces) have therefore been exposed to media containing deleterious concentrations of potassium chloride, sodium chloride, magnesium chloride, lithium chloride, or manganese chloride, and cultures have been obtained which are resistant to one or another of these salts. In the trials made thus far, it has turned out that only those strains adapted to LiCl or MnCl₂ were stable, retaining their resistance after 10 to 20 transfers in normal medium. The first genetic analyses were undertaken with the mutants resistant to LiCl and the results of these are dealt with in this paper.

MATERIALS AND METHODS

The yeast strains for these experiments were provided through the courtesy of Mr. D. C. Hawthorne. They were heterothallic and morphologically like Saccharomyces cerevisiae. Each strain carried a number of genetic markers which were useful as indicators of successful hybridization in the crosses described below.

1 Fulbright research scholar. Present address: Institut für Genetik, Freie Universität Berlin, Berlin-Dahlem, Germany.
2 This investigation was supported in part by funds from grant E-328, National Institutes of Health, Public Health Service, and from the Biological and Medical Fund of the State of Washington.
The LiCl-free medium in which the cultures were grown consisted of Difco yeast nitrogen base (6.7 g/l), supplemented with adenine and uracil (10 mg/l of each) and glucose (10 g/l). This will be referred to as YNB medium. For a LiCl-containing medium, LiCl was added to the above before autoclaving. Bacto-Agar (2%) was added for solid medium.

The cultures were tested for LiCl resistance as follows: A sample of a culture was inoculated into 5 ml YNB medium and incubated for 24 hrs. The culture was then diluted in sterile tap water and a loopful (ca. 10⁴ cells) of the suspension was streaked on plates of solid YNB + LiCl medium. The plates were incubated at 30°C and were examined daily for growth. In describing the experimental results, the term "rapid grower" is applied to those cultures which exhibited after two days as much growth on YNB + LiCl medium as on YNB medium alone; the term "slow grower" describes a culture which made imperceptible or scant growth after two days on the LiCl medium and which exhibited moderate to good growth on the sixth day; the "nongrower" did not show detectable growth during the six-day period of observation.

The cultures were crossed by the mass-mating technique of LINDEGREN and LINDEGREN (1943). A heavy inoculum from each of two cultures of opposite mating type was placed in 2 ml of YNB medium. After zygotes were formed, the cells were transferred to fresh medium at 24 hr intervals for three or four days to enrich the content of diploid cells. Single diploid cells were isolated with a micromanipulator and the cultures derived from these were sporulated on Fowell's medium (FOWELL 1952).

Only asci containing four spores were dissected. All segregants were tested for mating type and for at least four other characteristics, namely, their requirement for adenine, histidine, uracil, and methionine. Only the asci which gave the expected 2:2 segregation ratio for each of the markers are considered in this paper. In the five asci which exhibited unexpected segregation, four were segregating 3:1 for uracil independence:dependence and one was segregating 1:3 for histidine independence:dependence. The reasons for these discrepancies have not been determined.

EXPERIMENTAL RESULTS

**Origin of the LiCl-resistant strains**

The response of haploid and diploid strains to various concentrations of LiCl in solid YNB medium is indicated by the examples given in table 1. Growth was markedly affected in some strains at a concentration as low as 0.2N; at 0.4N and above, there was no visible growth on the plate two days after plating although there was reasonably good growth of some of these strains after 6 days, at concentrations up to 0.75N. At 1.0N, none of the strains exhibited perceptible growth even after three or four weeks.

In those cases of retarded but definite growth on LiCl medium, the number of colonies which arose on the plate was approximately the same as the number on the control plate; the colonies were simply smaller on LiCl medium. However, individual colonies of distinctly larger size also occurred on LiCl medium. These were especially obvious on plates in which the retardation of general growth was most pronounced.
TABLE 1

Growth of haploid and diploid cultures on YNB medium containing various concentrations of LiCl. The resistant mutants 1L.5, 1011L.5, and 1080L.5 arose on plates of YNB + 0.5N LiCl; 1011L1 arose on a plate of YNB + 1.0N LiCl. The amount of growth exhibited after two and six days is expressed as: + = normal or nearly normal; ± = distinctly less than normal; − = imperceptible

<table>
<thead>
<tr>
<th>Concentration of LiCl</th>
<th>Cultures not previously exposed to LiCl</th>
<th>Mutants selected after exposure to LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haploids</td>
<td>Diploid</td>
</tr>
<tr>
<td></td>
<td>101</td>
<td>1073</td>
</tr>
<tr>
<td>0.0N</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>0.1</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>0.2</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.3</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.4</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.5</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.75</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1.0</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

and they occurred also on plates in which there was no general growth. Samples of such colonies on 0.5N and 1.0N LiCl medium were transferred into YNB medium. After 24 hours these samples were tested for growth on LiCl medium and were found to be resistant to the concentration to which each had been exposed. After 10 to 20 transfers, at 24 hour intervals, in YNB medium, the cultures were again tested on LiCl medium and nearly all were found to have retained their resistance.

Twenty-seven haploid and eight diploid resistant strains were thus obtained. Those isolated from plates containing 0.5N LiCl made as good growth at this concentration after two days as on YNB medium and grew progressively less well on 0.75N and 1.0N LiCl medium (table 1). Those obtained from 1.0N LiCl plates exhibited a normal amount of growth after two days on 1.0N medium.

Inheritance of resistance to 0.5N LiCl

The mutant strain 1L.5 was obtained by plating strain 1 on 0.5N LiCl medium, as described above. Strain 1 itself did not grow at this concentration and grew only poorly, in fact, when exposed to concentrations above 0.2N (table 1). A cross between 1L.5 and 1073, an unadapted strain which exhibited slow growth on 0.5N, yielded the hybrid H23. The hybrid grew as well on 0.5N medium as its resistant parent, 1L.5. Twelve asci were obtained from H23 and the segregations in these asci are given in table 2. Two days after plating, two of the segregants of each ascus had made good growth on 0.5N medium. In three of the asci, the remaining two segregants were slow growers on this medium, exhibiting perceptible growth after four to six days of incubation. In eight of the asci, one of the segregants grew slowly and one did not make perceptible growth in six days. In one ascus, both of the remaining segregants were nongrowers. The presence of two rapid growers in each ascus indicated segregation for a single gene for resistance to 0.5N LiCl and the evidence that the hybrid was also a rapid grower indicated that this gene is dominant. The segregation
TABLE 2
Segregations for resistance to 0.5N LiCl, in asci from hybrid H23

<table>
<thead>
<tr>
<th>Number of asci</th>
<th>Phenotype of segregants</th>
<th>Rapid growth</th>
<th>Slow growth</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3
Segregations for resistance to 0.3N and 0.5N LiCl, in asci from hybrid H60. RG = rapid growth; SG = slow growth; NG = no growth

<table>
<thead>
<tr>
<th>Number of asci</th>
<th>Segregation for growth on 0.5N LiCl</th>
<th>Segregation for growth on 0.3N LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RG</td>
<td>SG</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

for slow growth suggested that the hybrid was heterozygous at at least one other locus concerned with resistance to LiCl, the gene or genes for resistance having been contributed by parent 1073, itself a slow grower on 0.5N LiCl medium.

Further evidence that strain 1L.5 carried a single dominant gene for resistance to 0.5N LiCl was obtained from the results of a cross of this strain with one of the nonresistant segregants, 23-9b, from H23. Segregant 23-9b did not show visible growth on medium containing LiCl in concentrations higher than 0.2N. The hybrid from this cross, H60, grew as well on 0.5N medium as 1L.5. Eight asci from this hybrid were dissected and in each of these two of the segregants were resistant to 0.5N LiCl and two were not (table 3).

The conclusion that strain 1073 carried a gene for slow growth in the presence of 0.5N LiCl was tested by crossing 1073 with the nonresistant segregant 23-16d. The hybrid from this cross resembled 1073 in its response to LiCl. In eight asci from H78, two of the segregants were slow growers on 0.5N LiCl and two did not grow.

The cross 1L.5 X 1073 may thus be represented genotypically as L, L, × l, l,. L, and l, being two dominant nonallelic genes for rapid and slow growth, respectively, in response to 0.5N LiCl. Hybrid 23, being L,, l, l,, would be expected to produce asci of the following three types of segregation: The parental ditype: L,, l,, l,, l,.; the nonparental ditype: L,, L,, l,, l,.; and the tetratype: L,, L,, l,, l,. The first of these has two rapid and two slow-growing segregants, the second has two rapid growers and two nongrowers, and the third has two rapid growers, one slow grower, and one nongrower. These are the types which were actually obtained from H23 (table 2).

A final test of two-gene segregation in the asci from hybrid H23 was made by crossing the three resistant segregants of a tetratype ascus (23-9) with nonresistant segre-
TABLE 4

Results of crosses of nonresistant strains by three resistant segregants of a tetratype ascus from H23. Segregant 23-9a was a slow grower on 0.5N LiCl; 23-9c and 23-9d were rapid growers

<table>
<thead>
<tr>
<th>Cross Nonresistant X Resistant</th>
<th>Number of asci</th>
<th>Segregation for resistance to 0.5N LiCl</th>
<th>Genotype of resistant parent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rapid growth</td>
<td>Slow growth</td>
</tr>
<tr>
<td>23-9b X 23-9c</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>23-9b X 23-9d</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>23-16d X 23-9a</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

gants of genotype $l_zl_y$. It was expected that the cross involving the slowgrowing segregant ($l_zl_y$) would yield asci with a 2:2 segregation for slow growth vs. no growth. The crosses involving the rapid growers should produce segregations like those obtained from H23 if the resistant parent were $L_xL_y$, or 2:2 segregations for rapid growth vs. no growth if the parent were $L_yL_y$. These expectations were realized (table 4).

Of the asci in which both $L_x$ and $L_y$ were segregating, five were parental ditypes, two were nonparental ditypes, and twelve were tetratypes. If the two genes were not linked and if only one or neither was linked to the centromere, the asci would be expected in the proportion 1:1:4 (Lindegren 1949). Thus, $L_x$ and $L_y$ are either loosely linked or not linked at all.

Resistance to concentrations of LiCl below 0.5N

The results presented above indicated four degrees of resistance to LiCl, exemplified by strains 1L.5, 1073, 1, and the nonresistant segregants of genotype $l_zl_y$. Strain 1, which grew slowly on 0.3N LiCl medium and not on higher concentrations, was crossed with the nonresistant segregant 23-9b, which did not grow at concentrations above 0.2N. The hybrid H77 was like strain 1 in its response to LiCl. In each of the eight asci from this hybrid two of the segregants grew slowly in the presence of 0.3N LiCl and two did not grow at all. Thus strain 1 carries a single dominant gene, $L_x$, for growth at this concentration.

Since $L_x$ arose in strain 1, it was of interest to determine whether $L_x$ and $L_z$ are at different loci. This was ascertained by retesting on 0.3N LiCl medium the segregants obtained from hybrid H60. If $L_x$ and $L_z$ are nonallelic, the genotypes of the parents of H60 may be presented by $L_d,l_yL_x(1L.5) \times l_yl_zl_x(23-9b)$, and two-gene segregation would be expected on 0.3N LiCl. The results presented in table 3 indicate that $L_z$ and $L_x$ are at different loci and that there is little if any linkage between them.

As a further test of two-gene segregation, the resistant segregants of the tetratype ascus 60-1 were crossed with nonresistant segregants. Since 60-1a grew slowly on 0.3N LiCl medium and therefore should be $l_zl_yL_x$, the cross 60-1a $\times$ 60-2c ($l_yl_zl_x$) was expected to yield 2:2 segregations for slow growth vs. no growth on 0.3N. Segregants 60-1b and 60-1d each grew rapidly on 0.5N LiCl; both therefore should carry $L_x$ and one should have $L_z$ as well. These were crossed with 60-1c ($l_yl_zl_x$), a
TABLE 5
Results of crosses of nonresistant strains by three resistant segregants of a tetratype ascus from H60.
Segregant 60-la grew slowly on 0.3N LiCl and did not grow on 0.5N LiCl; 60-lb and 60-lc grew rapidly on both concentrations. Symbols as in table 3

<table>
<thead>
<tr>
<th>Cross</th>
<th>Hybrid number</th>
<th>Number of asci</th>
<th>Segregation for growth on 0.3N LiCl</th>
<th>Segregation for growth on 0.5N LiCl</th>
<th>Genotype of resistant parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-la × 60-lb</td>
<td>82</td>
<td>3</td>
<td>RG</td>
<td>2</td>
<td>SG</td>
</tr>
<tr>
<td>60-la × 60-lc</td>
<td>83</td>
<td>8</td>
<td>RG</td>
<td>2</td>
<td>SG</td>
</tr>
</tbody>
</table>

TABLE 6
Results of cross between 1073 (L) and I (L) to determine whether L and L are alleles

<table>
<thead>
<tr>
<th>Number of asci</th>
<th>Segregation for growth on 0.3N LiCl</th>
<th>Segregation for growth on 0.5N LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RG</td>
<td>SG</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

nonresistant segregant from the same ascus. From the cross 60-lb × 60-la (hybrid H82), nine asci were obtained which exhibited 2:2 segregation when the segregants were tested on 0.5N LiCl medium and two-gene segregations when they were tested on 0.3N LiCl (table 5). Thus 60-la is Ia lal L2. The cross 60-la × 60-la (hybrid H83) yielded eight asci, in each of which two of the segregants grew on 0.3N and 0.5N LiCl and two did not grow at either of these concentrations. The genotype of 60-lb is therefore Ia lal l2. In summary, these results confirm the previous evidence that 1L,5 carries both L and L.

To determine whether L and L are allelic, 1073 was crossed with strain I to produce the hybrid H24. Eight asci were obtained from this hybrid (table 6) and the 2:2 and 3:1 segregations of resistance:nonresistance exhibited in some of these asci when tests were made on 0.3N medium indicates that L and L are at different loci. Strain 1073 is thus lal lal l2.

Resistance to 1.0N LiCl

The resistant mutant 1011L1, which grew rapidly on media containing 1.0N LiCl, was obtained in two steps from the unadapted strain 1011, which is itself capable of slow growth on 0.5N LiCl. A mutant for rapid growth on 0.5N, 1011L5, which also grew slowly on 1.0N, was first obtained from 1011. The mutant 1011L1 was in turn obtained from 1011L5 (see table 1 for the range of tolerance of 1011 and the two mutant strains). It should be mentioned that resistance to 1.0N LiCl has also been obtained in a single step from nonresistant strains but has not been analyzed genetically.
A cross was made between 1011L1 and the nonresistant strain 1101-8d to produce hybrid H89, which was like 1011L1 in its response to LiCl. The segregants of eight asci were tested on 1.0N LiCl medium (table 7). Rapid growth was obtained from two segregants of each ascus and, in seven of the eight asci, one or both of the remaining segregants grew slowly. The latter exhibited rapid growth on 0.5N LiCl. Thus it is reasonable to assume that 1011L1 arose in the following way: First a mutation to \( L_r \) in the original strain 1011 for rapid growth on 0.5N LiCl medium and slow growth on 1.0N LiCl medium; followed by mutation at another locus to \( L_{rs} \), for rapid growth on 1.0N LiCl medium.

Since \( L_r \) and \( L_{rs} \), previously identified in 1L.5, were similar in their response to LiCl, a cross was made between 89-5a (\( l_rL_s \)) and 60-8a, which was known to carry \( L_s \) (table 3). The hybrid H90 grew rapidly in the presence of 0.5N LiCl. In the six asci from this hybrid, segregations of 2:2 (2 ascis), 3:1 (3 ascis), and 4:0 (1 ascus) were obtained from rapid growth on 0.5N LiCl medium. \( L_r \) and \( L_s \) are therefore at different loci.

Tests of LiCl-resistant strains on other salt media

To determine whether resistance to LiCl is specific or is a general resistance to salts, several strains of different genotype were plated on media containing potassium chloride, sodium chloride, or manganese chloride. The results are summarized in table 8. All of the strains grew when exposed to KCl in concentrations as high as 2N.
but not on 2.5N. On NaCl medium, growth was observed on 1.5N but not on 2N, except for 101J11 (L\textsubscript{a}L\textsubscript{a}) which grew slowly at the latter concentration. However, there was no indication that the slow growth of this strain was correlated with resistance to LiCl since the two segregants 89-5a (L\textsubscript{a}L\textsubscript{a}) and 89-5b (L\textsubscript{a}L\textsubscript{a}) did not exhibit growth on 2N NaCl medium. None of the strains grew on 0.25N MnCl\textsubscript{2} medium; this concentration was chosen because mutants resistant to 0.25N and 0.5N have been obtained and will be described elsewhere. In summary, there is no evidence of augmentation in general resistance in those strains selected for resistance to LiCl.

**DISCUSSION**

The evidence which has been presented indicates that resistance to LiCl is genetically determined, and that several loci are involved. Genes for resistance to relatively low concentrations were present in strains which had not previously been exposed to LiCl; those for resistance to higher concentrations were found in cultures which arose after exposure to the salt. The possibility that LiCl served as a mutagenic as well as a selective agent in these experiments remains to be investigated. It is likely that modifiers affecting resistance were also segregating in the crosses reported above since segregations for slight differences in growth rate on LiCl medium were frequently observed. The degree of resistance of a cell to LiCl depends on the allele for maximum resistance which is present in the cell; i.e., the genes for resistance to LiCl are not cumulative in their effects.

The fact that resistance to LiCl appears to be specific raises the question of whether LiCl exerts a specific effect on the cell or is selectively excluded from the resistant cell by a change in its permeability. The following scattered observations indicate the directions which are being followed in studying this problem. From the results of a spectrophotometric analysis of resistant and nonresistant strains exposed to LiCl, it is unlikely that Li\textsuperscript{+} ion penetrates the cell. However, both types of cells, when suspended in LiCl medium for 24–48 hours, lose up to 70 percent of their potassium content, as compared to controls not exposed to salt (LASKOWSKI 1955). Thus, the presence of Li\textsuperscript{+} ion in the medium causes a considerable decrease in the K\textsuperscript{+} ion concentration normally present in the cell. Several investigators (MUNTZ 1947; SCOTT et al. 1950; MEYERHOFF and KAPLAN 1951) have shown that the K\textsuperscript{+} ion plays an essential role in carbohydrate metabolism in yeast and that neither growth nor fermentation can occur in media lacking this ion. In experiments with K\textsuperscript{+}-deficient medium (LASKOWSKI 1955), it was found that growth and fermentation were inhibited in LiCl-nonresistant strains but were not inhibited in resistant strains. The K\textsuperscript{+}-deficient medium was not freed of traces of potassium and it may be that even the resistant strains require small amounts of this ion. These results taken together suggest that the genes for resistance to LiCl may be regarded as genes which suppress the dependence of the cell on potassium, and thus that the selection of resistant mutants is actually a selection for cells with a reduced potassium requirement.

**SUMMARY**

Several strains of Saccharomyces have been isolated which differ in their resistance to lithium chloride. Mutants resistant to concentrations of 0.5N and 1.0N were ob-
tained by exposure to these concentrations of LiCl. Five genes for resistance have
been identified; all of these are dominants and at least three are nonallelic. Resistance
to LiCl did not carry with it an augmentation of resistance to other salts, i.e., to
potassium chloride, sodium chloride, or manganese chloride. The effect of the Li+
ion on carbohydrate metabolism and the potassium content of the cells is discussed.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to DR. HERSHEY ROMAN for the op-
opportunity of working in his laboratory and for invaluable discussions during the
progress of this investigation. He is also indebted to DR. HOWARD C. DOUGLAS for
his advice and criticism. It is a pleasure to acknowledge further the helpful technical
assistance of MISS ALCETTA GILBERT.

LITERATURE CITED

GRAY, W. D., 1945 The sugar tolerance of four strains of distiller’s yeast. J. Bacteriol. 49: 445-
452.
KROEMER, K., and G. KREUMBLOH, 1932 Untersuchungen über osmophile Sprossspilze. V. Mitte-
lung: Das Verhalten von Sprossspilzen in Nährlösungen mit hohen Neutralsalzkonzentrationen.
Arch. Mikrobiol. 3: 384–396.
LASKOWSKI, W., 1955 Resistance of Saccharomyces to high concentrations of lithium chloride.
Science 121: 299–300.
LODDER, J., 1932 Über einige durch das “Centralabureau voor Schimmelcultures” neuerworbene
MEYERHOF, O., and A. KAPLAN, 1951 The speed-controlling reactions in fermentation of quickly
Abt. II 100: 289–294.
Polytech., Osaka City Univ. Ser. D 4: 35–42.
PERLMAN, D., and E. O’BRIEN, 1954 Characteristics of a cobalt tolerant culture of Saccharomyces
SCARR, M. P., 1951 Osmophilic yeasts in raw beet and cane sugars and intermediate sugar-refining
SCOTT, G. T., M. A. JACOBSON, and M. E. RICE, 1950 The influence of glycolytic factors on the
SUSSMAN, M., and S. G. BRADLEY, 1953 Mutant yeast strains resistant to arsenate and azide. J.
TAKADA, H., 1953 Protoplasmological studies on the yeast cell adapted to high osmotic environ-
YANAGISHIMA, N., 1952 Studies on the adaptation of yeast to copper V, VI and VII. J. Inst. Poly-
1954 Growth and variability of yeast on some media of high sodium chloride content. J. Inst.
Polytech., Osaka City Univ. Ser. D 5: 29–44.