STUDIES OF EFFECTS ON COPPER RESISTANCE IN YEAST

AS INFLUENCED BY DESOXYRIBONUCLEATES

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SUMMARY

1. Study was made to investigate effects of desoxyribonucleates on copper-resistance in yeast.
2. It is found that the resistant to copper is a type of mutant, which is under-grown than the sensitive in multiplication in non-copper-media.
3. In the occurrence of the resistant strain there exists the phenomic lag.
4. Desoxyribonucleate isolated from copper resistant culture is capable of inducing the resistant strain, which is the same type as donor of the resistant type. It accelerated the rate of variation to the resistant, but it is of no effect on the resistant strain.
5. Desoxyribonucleate derived from non-resistant type inhibits growth of the resistant strain and delays the initial phase of growth. However, it is of no effect on the sensitive strain.

It is concluded that desoxyribonucleate derived from resistant culture is capable of inducing the resistance, however, nonresistant type desoxyribonucleate is of no effect on inducing the resistance.

INTRODUCTION

Various studies on drug-resistance in microorganisms have been carried out not only a phenomenon of bacterial variation but also practical application of antibiotics and bacterial bioassay. On the original mechanism of resistance, Hinshelwood\(^1\) indicated that the resistance is a result of adaptation; on the other hand Luria\(^2\) reported that the resistant strain is a type of mutant. Subsequently Lea and Coulson\(^3\) and Ryan reported that the variant is a mutant, which became to be a dominant through selection. Demerec\(^4\) found that copper sulphate is a mutagen to microorganism. The phenomena of copper resistance in yeast had been described by J. Ashida\(^5\) and Yanagijima\(^6\): copper resistance of yeast is a outcome of mutation and selection, the substance derived from resistant culture is capable of inducing the similar resistance. The latter opinion was recently supported by Lee et al\(^7\). Recently Avery and McCarty\(^8\) succeeded in inducing transformation of Pneumococcus type with desoxyribonucleic acid. Hotchkiss\(^9\) also reported the transfer of penicillin resistance with desoxyribonucleate. Lederberg reported transmissibility of some unknown inheritable substance in microorganism.

The present study is to investigate the transferability of resistance and its hereditability under the influence of desoxyribonucleic acid considered as the substance of genic system.

The major interest lies in the attempt to survey the effect of desoxyribonucleate on the occurrence of the resistance, and hereditability of desoxyribonucleate in copper resistance of yeast, to discuss induced mutation and adaptive variation.

The authors are grateful to Dr. K. N. Lee for assistance in planning the experiment and to M. K. Chyung for assistance in performance of experiment.
METHODS AND MATERIALS

Pure strains of Saccharomyces cerevisiae* and Saccharomyces sake** which are sensitive to copper were isolated and cultured. Henneberg's and Malt-Henneberg's media were used in the cultivation. The growth of yeast was estimated by the counting of cell numbers with Thomahemacytometer.

The extraction of deoxyribonucleic acid from yeast was done according to the method of Chargaff and Zamenhoff³. Approximately 20 mg. of sodium deoxyribonucleate was obtained as a coarse fibroid precipitate. The precipitate was tested for and it was found to be deoxyribonucleic acid by diphenylamin reaction of Dische's.

In order to investigate specific activity of deoxyribonucleic acid two types of deoxyribonucleates were used: one of which was derived from the resistant culture (resistant type deoxyribonucleate) and the other was a chemical product of Nutritional and Biochemical Co. in U. S. A. (labeled as non-resistant type deoxyribonucleate.). Each deoxyribonucleate 0.5 µg/cc and 5 µg/cc was added into every group of culture prior to the treatment.

EXPERIMENTS AND RESULTS

EXPERIMENT I. Occurrence of the resistant and its hereditability

The sensitive strain was transferred into agar medium with copper sulphate. The observed results are as following: (Table 1)

(1) The inhibitory action of copper against growth of the sensitive was initiated by the concentration of 0.2 millimole(mM), and was completely inhibited by 3 mM.

(2) The gradual increase in concentration of copper solution (such as 0.1 mM→0.2 mM→0.4 mM→0.6 mM→1 mM→2mM) permitted the growth and formation of its colonies, however, growth was completely inhibited and was killed thoroughly at 6 mM concentration of copper.

(3) The resistant strain*** at 1 mM concentration of copper manifested the ability of brown pigment production, and the rate of separation from mother cell in reproduction was slower than that of the sensitive. Furthermore, the resistant strain was able to grow in higher concentration of copper solution such as 4 mM and 5 mM.

Table 1 Ability of colonization of yeast as gradually inoculated in copper-media.

<table>
<thead>
<tr>
<th>Strain Conc.</th>
<th>Saccharomyces cerevisiae Sensitive</th>
<th>Res. Var.*</th>
<th>Rlb(O)</th>
<th>Saccharomyces sake Sensitive</th>
<th>Res. Var.*</th>
<th>Rlb(O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 mM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.4 mM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 mM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 mM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3 mM</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>4 mM</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>5 mM</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>6 mM</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>7 mM</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>8 mM</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>9 mM</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* Res. Var. Resistant Variant.

* This was kindly donated by Mr. Seong of O. B., Co.
** This was kindly donated by Mr. Tae of S. R. I., M. N. D.
*** It is hereafter called Rl(b) strain.