<Original article>

STUDIES OF EFFECTS ON COPPER RESISTANCE IN YEAST AS INFLUENCED BY DESOXYRIBONUCLEATES

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李敏哉·金鍾協 : *Saccharomyces cereisiase*의 銅抵抗變異에 미치는 DNA의 影響에 關한 硏究

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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[8] indicated that the resistance is a result of adaptation; on the other hand Luria[15] reported that the resistant strain is a type of mutant. Subsequently Lea and Coulson[14] and Ryan reported that the variant is a mutant, which became to be a dominant through selection. Demerec[3] found that copper sulphate is a mutagen to microorganism. The phenomena of copper resistance in yeast had been described by J. Ashida[10] and Yanagijima[21] : 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[16]. Recently Avery and McCarty[1] succeded 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 heredtiability of desoxyribonucleate in copper resistance of yeast, to discuss induced mutation and adaptive variation.

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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 desoxyribonucleic acid from yeast was done according to the method of Chargaff and Zamenhoff[2]. Approximately 20 mg. of sodium desoxyribonucleate was obtained as a coarse fibroid precipitate. The precipitate was tested for and it was found to be desoxyribonucleic acid by diphenylamin reaction of Dische's.

In order to investigate specific activity of desoxyribonucleic acid two types of desoxyribonucleates were used: one of which was derived from the resistant culture (resistant type desoxyribonucleate) and the other was a chemical product of Nutritional and Biochemical Co. in U. S. A. (labeled as non-resistant type desoxyribonucleate.). Each desoxyribonucleate 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 ara 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 menifested 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.

	Saccharomyces cerevisiae			Saccharomyces sake		
Strain / Conc.	Sensitive	Res. Var.*	Rlb(O)	Sensitive	Res. Var.*	Rlb(O)
0.2mM	+	+	+	+	+	+
0.4mM	+	+	+	+	+	+
1 mM	+	+	+	+	+	+
2 mM	+	+	+	+	+	+
3 mM	−	+	+	−	+	+
4 mM	−	+	+	−	+	+
5 mM	−	+	+	−	+	+
6 mM	−	−	−	−	+	+
7 mM	−	−	−	−	+	+
8 mM	−	−	−	−	−	−
9 mM	−	−	−	−	−	−

* 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.

(4) The Rl(b) strain which had been cultured in non-copper medium for six days never lost its resistant property and it was able to grow readily in copper medium without any inhibitory action.

(5) Scores of hours were needed for occurrence of the resistant strain, this phenomenon hereafter is called phenomic lag or α-phase(Fig. 1).

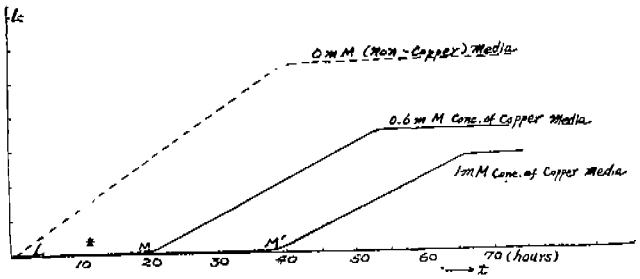

Fig. 1 Growth curve of yeast in copper-media and non-copper-media.

EXPERIMENT II. Extraction of desoxyribonucleic acid from resistant culture

The method of extraction was that of Chargaff and Zamenhoff's, in order to protect nature of nucleic acid from decomposition and denaturalization sodium citrate was added to the condensed wet yeast, then sodium chloride method and chloroform method were applied. With addition of alcohol a coarse fibroid precipitate of sodium desoxyribonucleate was obtained, and it weighed about 20 mg. in dehydrated form. It was found to be the nucleic acid of desoxyribose type through diphenylamin reaction of Dische's.

EXPERIMENT III. Effects of desoxyribonucleates on the sensitive which is in the process of resistant induction in copper-media

Desoxyribonucleate(non-resistant type) was added to C_1 and C_2 groups of sensitive culture which is in the process of resistant induction. It was found that occurrence of the resistant was inhibited and there were considerable phenomic lag, that is, α-phase. These phenomena resembled to those of the control.

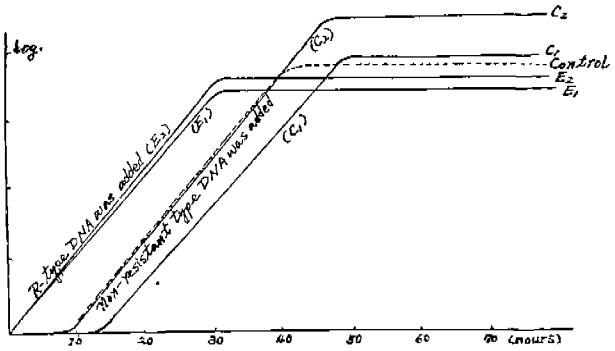

Fig. 2 Effects of desoxyribonucleates on resistance (Behaviour of the sensitive strain in copper-media)

When resistant-type desoxyribonucleate was added to E_1 and E_2 groups vigorous growth has been observed, and the progeny was found to be the similar resistant type(Fig. 2).

The groups of control is exclusive of any desoxyribonucleate, E_1 and E_2 groups are treated with resistant type desoxyribonucleate, and C_1 and C_2 groups are treated with non-resistant type desoxyribonucleate.

EXPERIMENT IV. Effects of desoxyribonucleates on the resistant strain in copper-media

A fraction of 0.5 μg and 5 μg of desoxyribonucleate(nonresistant type) was added to C_1 and C_2 groups, and to E_1 and E_2 groups 0.5 μg and 5 μg of resistant type desoxyribonucleate was added. Then the behaviour of the resistant strain under influence of desoxyribonucleate was observed. Similar to the control in E_1 and E_2 groups it was found that the resistant strain grew normally, however, in C_1 and C_2 the

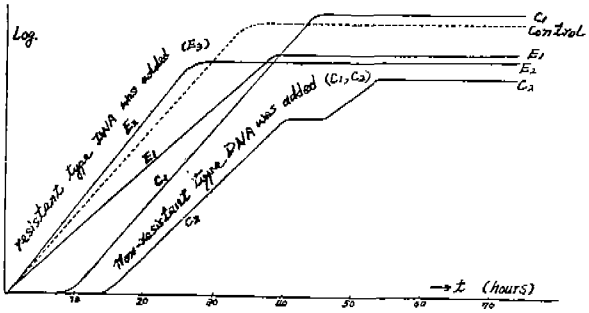

Fig. 3 Effects of desoxyribonucleates on the sensitive strain in copper-media.

growth were considerably inhibited(Fig. 3).

EXPERIMENT V. Effects of desoxyribonucleates on the resistant strain in non-copper-media

0.5 μg and 5 μg of desoxyribonucleate(non-resistant type) was added to groups of C_1 and C_2. 0.5 μg and 5 μg of resistant type desoxyribonucleate was added to group E_1 and E_2. The control was excluded from any nucleic acid.

It was observed that non-resistant type desoxyribonucleate inhibited growth of the resistant strain in C_1 and C_2 groups. The amounts of growth in each of C_1 and C_2 were found to be two and a half times more than those of E_1 and E_2 respectively. The pattern of growth in the control group was similar to C_1 and C_2 groups, but the amount of growth differed. The yield of the control was considerably, about one half times, less than C_1 and C_2 groups.

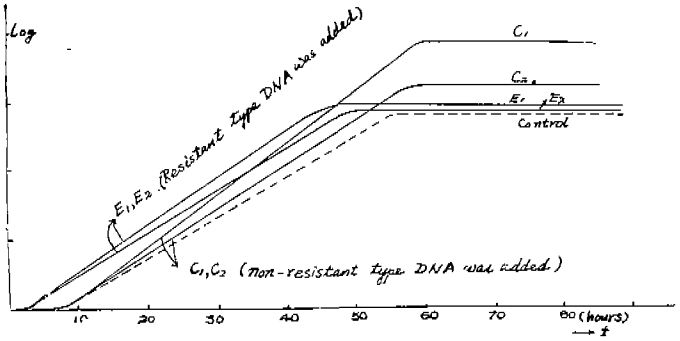

Fig. 4 Growth of the resistant strain in non-copper-media under influence of desoxyribonucleates.

In group of E_1 and E_2, the resistant grew regularly under influence of resistant type desoxyribonucleate except for a slight delay of initial stationary phase(Fig. 4).

DISCUSSION AND CONCLUSION

The results from Experiment 1. indicate that the variant strain which is resistant to copper is a type of mutant for its phenotypes are specific and different from those of the sensitive strain and never lose its resistant characteristics, these findings support those previously reported by Demerec(1953), Ashida (1955) and Lee(1956).

The results from Experiment 2. is significant in that the delay of initial stationary phase of growth in C_1, C_2 and control groups is a type of phenomic lag for resistance(Demerec). The growth curves of C_1 and C_2 were compared with those of E_1 and E_2 and analysed mathematically.

Differential equation for bacterial growth is

$$\frac{dy}{dt}=Ky(L-y) \qquad \text{and} \qquad y=\frac{L}{1+e-r(t-a)}$$

y ; Cell numbers at a certain time

K; A proportional constant

L ; Cell numbers at saturated stationary phase

r ; $\left(\frac{dy}{dt}\right)t=a\times\frac{4}{L}$

From above equations the "first order different equation" is derived,

$$e^{-rh}\frac{L}{y(t)}=\frac{L}{y(t+h)}=e^{-rh}-1$$

$$y(t+h)+a_1y(t)-a_2=0$$

If yeast continued to multiply at normal condition then values such as $1/y(t+h)$ and $1/y(t)$ would locate on a straight line in discriminative different diagram of two dimensional reciprocals(Diagram 1).

The results of the present investigation were analysed and the non-resistant type desoxyribonucleate has no effect on inducing resistance in C_1 and C_2 groups, for C_1 and C_2 pass through phenomic lag. It

seems that the resistant type desoxyribonucleate affects positively on the sensitive strain and induces the sensitive to the resistant, consequently there is no phenomic lag.

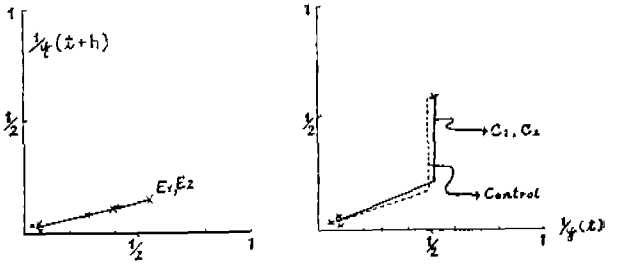

Diagram 1

These phenomena are considered as specific activities, that is, the specific type of desoxyribonucleate participates solely in the same specific type of metabolic pool of desoxyribonucleic acid, therefore menifests the same specific physiological phenotype. This suggestion was made by Hotchkiss and Avery in their studies of transformation. In the results from C_1 and C_2 groups indicate that the non-resistant type desoxyribonucleate may not participates in the metabolic pool which is operated by the resistant type nucleic acid. Therefore the non-resistant type desoxyribonucleate was of no effect on activation of resistance.

Regard to the results of group C_1 and C_2 in experiment 4. it is suggested that the non-resistant type desoxyribonucleate is able to participate in the metabolic pool of nucleic acid and, therefore, would change the resistant type metabolism to non-resistant type or induce to backmutation such as from the resistant to the sensitive since phenomic lags exist in C_1 and C_2. However, it may be reasonable to consider that the non-resistant type desoxyribonucleate may inhibites growth of the resistant strain in copper-media. In group E_1 and E_2 the results indicate that the resistant type desoxyribonucleate has no effect on the resistant type strain.

In Experiment 5. it is indicated that the non-resistant type desoxyribonucleate participates in metabolic pool of the resistant type nucleic acid and may causes the disorder of the metabolic pool, or may induces the mutation. However, the growth activity of the non-resistant type desoxyribonucleate seems to be great. In general growth of the strain in C_1 and C_2 is accelerated by the non-resistant type desoxyribonucleate greately in non-copper-media but the resistant type desoxyribonucleate doesn't affect on growth.

The question whether non-resistant type desoxyribonucleate acts as a accelerating agent for growth of the resistant strain or as a mutagenic agent which may produce a type of mutant of a well growing type is to be answered.

In general, through this study, it is established that characteristics of desoxyribonucleate is specific to metabolism, and resistance is a result of either transformation or transduction as pointed out by Avery, Hotchkiss and Lederberg.

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摘 要

麥酒酵母菌이 硫酸銅溶液에 抵抗性을 나타내는 現象이 一時的 適應變異이냐 또는 遺傳的 突然變異이냐 하는 點을 硏究하였다. DNA는 遺傳因子의 基礎物質이므로 DNA를 變異過程에 投與하였을 때 變異의 機作이 解明될 것이라는 意圖下에 實驗이 實施되었다.

DNA는 酵母核酸抽出法에 依하여 變性을 防止하면서 Na鹽의 DNA를 抽出하였고, 抵抗菌의 DNA를 R-type DNA라 하였다. 非抵抗性의 DNA는 化學試藥用의 DNA-Na鹽을 使用하며 對照하였다.

實驗結果는 下記와 같다.

1. 銅抵抗性의 酵母菌은 無銅인 正常培地에 있어서 非抵抗菌보다 發育이 不振하였다.
2. 銅抵抗菌의 發生過程에 있어서는 初期段階에서 遲滯期가 長期間 介在하였다.
3. 銅抵抗性菌으로부터 抽出된 DNA는 非抵抗菌으로 하여금 抵抗菌으로 轉換시켰다.
4. 銅抵抗性菌으로부터 抽出된 DNA는 抵抗菌自體에는 아무런 影響도 미치지 않았다.
5. 非抵抗性 DNA는 抵抗菌에 對해서 發育을 抑制하였으며, 初期段階의 發育도 역시 抑制하였다.
6. 非抵抗性 DNA는 非抵抗菌 自體에 對해서는 影響을 미치지 않았다.

따라서 抵抗菌의 DNA는 非抵抗菌으로 하여금 抵抗性菌으로 誘導轉換시키는 事實은, DNA가 遺傳子系物質인 點으로 보아서 抵抗性變異現象이 遺傳場內에서 進行함을 알수 있다.

이와같은 結論은 非抵抗性 DNA가 抵抗變異를 誘起시키지 못하는 事實(實驗結果)로서 더욱 確固해진다.