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Induction of Melibiase in Yeast*

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朴商信：酵母에 있어서 meliabiase의 誘導生成

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ABSTRACT

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Exposing yeast cells with a certain genotype to different inducers, the ability of the yeast cells (*Saccharomyces cerevisiae*) to obtain enhanced fermentation for carbohydrates was observed. Regardless of the preexposure to any substrate, the inherent character incapable of fermenting a certain carbohydrate was maintained, while utilization of carbohydrates by the cells with a certain gene markers was varied by the previous conditions where they were exposed. Galactose was the best inducer for the cells to elaborate melibiase, even the galactose was not utilized as a substrate. Preexposure to galactose seemed to be necessary for the cells to utilize galactose and melibiose. Galactose fermentation by GA cells was enhanced by the exposure of the cells to galactose, but not to melibiose, raffinose, sucrose or glucose. Delayed fermentation of sucrose by the cells exposed to glucose or melibiose, but not to galactose, was observed. Raffinose fermentation was obtained by the cells with either SU RAF or GA ME genes, but the enhanced fermentation of raffinose seemed to be dependent on which inducer the cells were exposed previously and enzymes induced by the inducer to break either one of the linkages of raffinose molecule, the alpha-galactosidic or the beta-fructo-furanosidic.

INTRODUCTION

The specificity in the induction of an enzyme by an inducer appears to be limited with in a certain range as well as the specificity in the role of an enzyme, which seems to be confined to a certain structure of a molecule of a subsrate which combines with the enzyme. Melibiase (alphagalactosidase) is inducible by exposing yeast cells to a certain substance such as galactose, but not glucose, sucrose, raffinose and etc.

Lindegren(1949) and Winge and Losada(1957) described the utilization of melibiose and raffinose, but they did not regard the induction of melibiase in the presence of various substances in relation to genetic character.

The present study is intended to find out the best inducer for melibiase which can be elaborated by the adapted yeast cells and to test the ability of the cells to ferment different carbohydrates with regard to the molecular structure of inducers and substrates.

MATERIALS AND METHODS

Carbondale breeding stock *Saccharomyces cerevisiae* 23970, 23971, 23972 and 23973 were used through out this study. The phenotype of the cultures was as follows:

*This experiment was carried out in the Biological Research Laboratory, Southern Illinois University, Carbondale, Illilois, U.S.A. and the result was presented in part at the Annual Meeting of the Botanical Society of Korea, Seoul, 9-11 November, 1963.

TABLE 1. The phenotypes of a tetrad

Strain No.	Substrates			
	Sucrose	Raffinose	Galactose	Melibiose
23970	+	+	−	−
23971	+	+	−	+
23972	−	+	+	+
23973	+	+	+	−

"+" indicates the fermentation of the respective substrate in Durham tubes in 48 hours.

The yeast strain used here was grown continuously in the nutrient broth composed of glucose, 40 g; $MgSO_4$, 1g; KH_2PO_4, 2g; Yeast extract, 4g; peptone, 10g; and distilled water, 1000ml. A loop of cells was inoculated into 500ml Erlenmyer flask containing 100ml of nutrient broth and the flask was placed on a shaker in a walk-in-incubator at 30°C for 24 hours. The cells were usually inoculated from a fresh culture grown on agar slant for 24 hours.

For adaptation the cultures were exposed to each one of inducers for five hours which was the optimal adaptation period for the previous experiments and the results with different cultures were compared. For adaptation, the suspension of glucose-grown cells was centrifuged, washed with 0.067 M aqueous KH_2PO_4, and incubated in adaptation medium with an inducer. The adaptation medium was the nutrient broth from which glucose had been omitted and to which one percent of the desired inducer had been added together with a small amount of an energy source (0.1 per cent glucose). Unless otherwise specified, all adaptation experiments were carried out as described.

After adaptation, the yeast cells were harvested by centrifugation, washed twice and suspended in the phosphate buffer solution to a population density of $8x10^8$ cells/ml. For the use of Syringe Gasometer, the number of cells was adjusted to be $4x10^8$/ml by adding 1 ml of the cells suspension to 1 ml of substrate solution in a Syringe Gasometer.

Measurement of gas production by the cells was made with a Syringe Gasometer (Baird, Hestrin and Lindegren, 1952) flushed with or without CO_2 at 30°C and with Warburg respirometer, suspending the cells in 0.067 M phosphate buffer solution with substrates added. For general studies of fermentation Syringe Gasometer was used. The measurement of gas production with Warburg-respirometer was carried out according to the Warburg technique (Umbreit et al, 1959) using 15% KOH with a top-cut filter paper.

EXPERIMENTAL RESULTS

The experimental results from the cultures 23970, 23971, 23972, and 23973 could be summarized as follows.

1. The culture 23970(SUC.RAF. gal. meb.)

The culture cells were tested with 2% of glucose, sucrose, melibiose and raffinose after the cells were grown in nutrient broth for 24 hours and exposed to each substance for five hours, expecting effects on the fermentation by the cells.

Regardless of whether the cells were exposed to any inducer or not the cells could ferment only sucrose and raffinose, but not galactose and melibiose indicating the ability of the cells to elaborate an enzyme, beta-fracto furanosidase which break beta-furacto-franosidic linkage of the substrate and their inborn

characteristics.

The ability of the cells to ferment 2% of glucose, sucrose, galactose, melibiose and raffinose was shown in Table 2.

TABLE 2. The fermentability of the culture 23970 in 0.067 M KH_2PO_4 Solution

Substrates / Inducers	Glucose	Sucrose	Galactose	Melibiose	Raffinose
Glucose (1%)	+	+	−	−	+
Suc. Gal. Mel. (1.5% of each)	+	+	−	−	+
Sucrose (1%)	+	+	−	−	+
Melibiose (1%)	+	+	−	−	+
Galactose (1%)	+	+	−	−	+

The cells were grown in nutrient broth for 24 hrs. and exposed to respective inducers. "+" indicates the fermentation of each substrates by the cells exposed to each inducer from the beginning in Syringe Gasometers. "−" indicates the failure of gas production by the respective cells within 9 hours duration. The concentration of substrates was 2% in final concentration.

2. The culture 23971 (SUC. RAF. gal. MEB.)

In contrast to the culture 23970, the results obtained from the culture 23971 showed the diversity in the fermentation of raffinose and melibiose depending upon the preadaptation to each sugars used as inducers. The fermentability of the cells for sucrose did not vary appreciably with Syringe Gasometer and for galactose it was same as the phenotype without having any change due to preadapation.

TABLE 3. The fermentability of the culture 23971 in 0.067 M KH_2PO_4 solution

Substrates / Inducers	Glucose	Sucrose	Galactose	Melibiose	Raffinose
Glucose (1%)	+	+	−	−	$+^2$
Suc. Gal. Mel. (1.5% of each)	+	+	−	+	+
Sucrose (1%)	+	+	−	−	+
Melibiose (1%)	+	+	−	−	−
Galactose (1%)	+	+	−	+	+
Raffinose (1%)	+	+	−	−	−

"$+^2$" indicates the beginning of the fermentation in the Syringe Gasometer in 2 hours.

As shown in Table 3, however, the fermentation of melibiose by the cells exposed to 1% of glucose, sucrose, melibiose and raffinose could not be observed in nine-hour tests with Syring Gasometers. Whenever the cells were exposed to 1% galactose, even with other substrates, sucrose and melibiose, the cells could ferment melibiose vigorously indicating that galactose is a good inducer for the elaboration of melibiase, alpha-galactosidase.

With regard to the fact that the fermentation of raffinose is due to the break of beta-fractofuranosidic linkage of raffinose molecule as well as that of alphagalactosidic linkage, rapid fermentation of raffinose could be expected as the result of the fermentation of sucrose. However, the fermentation of raffinose by the cells exposed to glucose, melibiose or raffinose was rarely observed in nine-hour tests. On the other hand, by the cells exposed to sucrose or galactose the rapid fermentation of raffinose was observed. This might be due to the difficulties in permeation or adaptation, and that the preadaptation to sucrose or galactose might help to solve the difficulties. Since the fact that galactose was found to be a good inducer for melibiase the enhanced fermentation of raffinose by the galactose exposed cells might be due to the elaboration of melibiase which might break up the alpha-galactosidic bond and remaining sucrose molecule.

If it is assumed that the sucrase, beta-fracto-furanosidase, was also an inducible enzyme, above explanation for raffinose fermentation by galactose-exposed cells might also apply to the rapid fermentation of raffinose by the sucrose-preadapted cells.

More clear-cut results were obtained when the cells were exposed to each inducer of synthetic medium (Middlekauff et al., 1956) for two hours and the ability of the cells to ferment each substrate was examined with Warburg respirometer. Figure 1 shows the ability of the cells, grown on nutrient broth for twenty-four hours, to ferment 2% of glucose, sucross, melibiose, raffinose and 1% galactose. Since production of carbondioxide by the cells in the presence of sucrose was slow in the beginning glucose might affect on the utilization of sucrose. Regarding to the glucose effect on the metabolic process(Englesberg. 1961) glucose seems to affect on the sucrose utilization some way.

The production of carbon-dioxide by the cells in the presence of melibiose and raffinose was not observed. It showed the necessity for the cells to be exposed to some inducers. In the presence of galactose, the cells were also incapable of producing carbondioxide.

Similar results were obtained when the cells were exposed to 1% melibiose: the production of carbondioxide in the presence of sucrose was also delayed in the beginning, and those of melibiose and raffinose were negligible and that of galactose was negative as expected, as shown in the Figure 2.

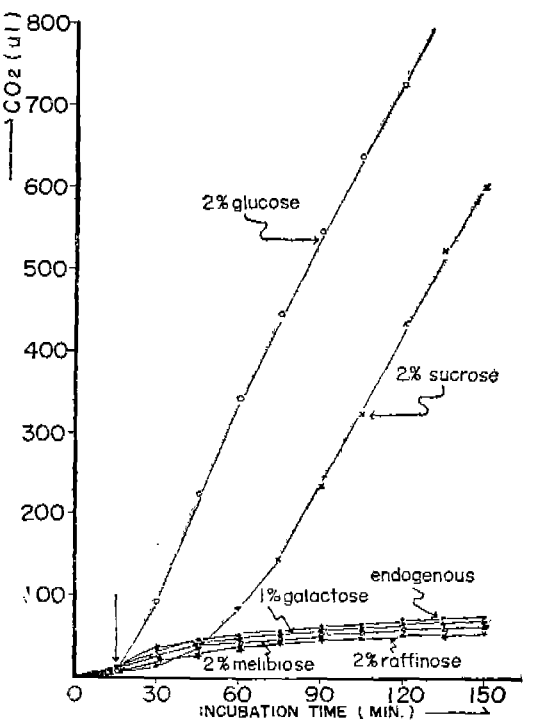

Fig 1. Carbondioxide production by the culture 23971 (1.5×10^8 cells/ml), grown in nutrient broth for 24 hrs., in 0.067M P-buffer, pH 4.5 at 30°C

In contrast to those cells grown on glucose nutrient broth and exposed to melibiose, the cells exposed to galactose previously could produce carbondioxide in the presence of sucrose as well as in the presence of glucose from the beginning (Fig.3). This result shows that preexposure of the cells to galactose does not influence on the utilization of sucrose by the cells at all, and that the preexposure of the cells to glucose or melibiose affect on it someway. Furthermore vigorous production of carbondioxide by the galactose exposed cells in the presence of melibiose and raffinose were also distinctly observed. This showed that galactose played a role better as an inducer for melibiase which would attack both on melibiose and on raffinose than melibiose or raffinose agreeing with previous syringe test. However, the production of carbondioxide in the presence of galactose was not observed at all, even though galactose was the best inducer for the cells to elaborate melibase. From this result it could be concluded that an inducer not necessarily be a substrate.

3. The culture 23972 (suc. RAF. GAL. MEB.)

As shown in Table 4, this culture was incapable of utilizing sucrose, therefore, the fermentation of raffinose by the culture cells must be due to the utilization of galactose followed the break of alpha-galactosidic linkage of raffinose molecule.

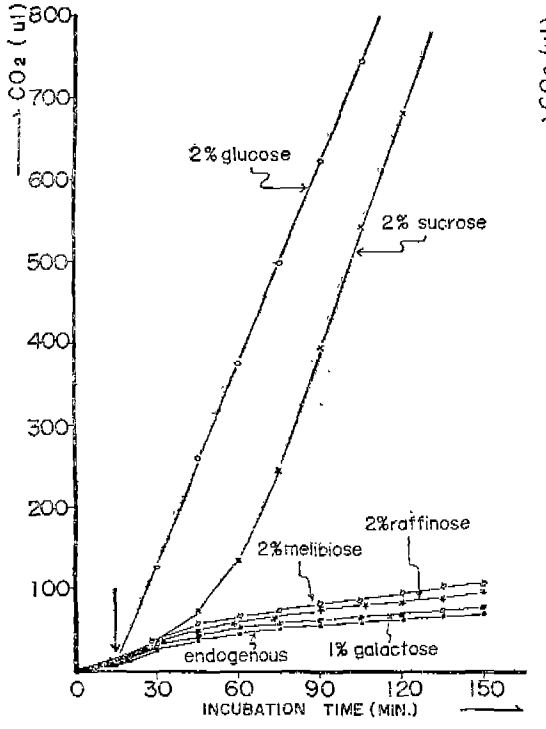

Fig. 2. Carbondioxide production by the culture 23971 (1.5×10^8 cells/ml), grown in nutrient broth for 24 hrs., and exposed to 1% melibiose for 2 hrs., in 0.067M P-buffer, pH 4.5 at 30°C.

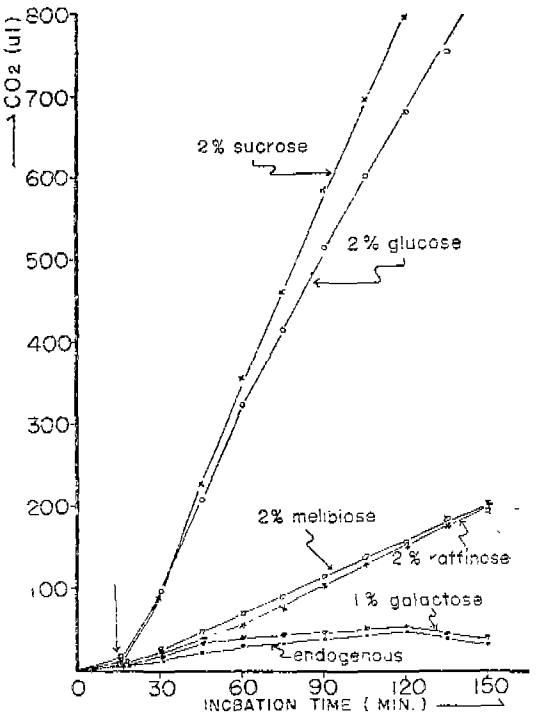

Fig 3. Carbondioxide production by the culture 23971 (1.5×10^8 cells/ml), grown in nutrient broth for 24 hrs. and exposed to 1% galactose for 2 hrs., in 0.067M P-buffer, pH 4.5 at 30°C.

When the cells were exposed to 1% glucose, there was no indication of fermentation in the presence of sucorse, galactose, melibiose and raffinose as shown in Table 4, showing that for any one of these substances glucose could not give rise any enhanced fermentation of galactose, melibiose or raffinose. By the exposure of the cells to sucrose similar results also were obtained.

However, by exposing the cells to 1% of melibiose or galactose the cells could ferment galactose, melibiose and raffinose all of them. Since galactose is the best inducer, the fermentation of melibiose and raffinose by the cells, for which melibiase is responsible, could be expectable. But the capacity of the cells to ferment melibiose might be due to the inherent capacity of the cells to utilize galactose which could be released by melibiase where the galactose acted as both an inducer and an energy source.

TABLE 4. The fermentability of the culture 23972 in 0.067M KH_2PO_4 solution

Substrates / Inducers	Glucose	Sucrose	Galactose	Melibiose	Raffinose
Glucose (1%)	+	−	−	−	−
Suc. Gal. Mel. (1.5 of each)	+	−	+	+	+
Sucrose (1%)	+	−	−	$+^2$	−
Melibiose (1%)	+	−	+	+	+
Galactose (1%)	+	−	+	+	+
Raffinose (1%)	+	−	$+^6$	$+^6$	−

"$+^2$" and "$+^6$" indicate the beginning of the fermentation in the Syringe Gasometers in 2 hours and in 6 hours respectively.

With regard to the enhanced ability of the galactose-exposed cells to ferment galactose, galactose also seemed to induce the enzymes which were responsible for the utilization of galactose. For the culture 23971, raffinose seemed not to have any effect on the enhanced fermentation of galactose, melibiose and raffinose. The incapability of the cells to use sucrose showed their inherent character.

4. The culture 23973 (SUC. RAF. GAL. meb.)

Like the previously known phenotype, these cells did not have any dificulties to utilize sucrose and raffinose regardless of the previous condition of exposing, except the fermentation of galactose. The results was shown on Table 5.

TABLE 5. *The fermentability of the culture 23973 in 0.067M* KH_4PO_2 *solution*

Substrates / Inducer	Glucose	Sucrose	Galactose	Melibiose	Raffinose
Glucose	+	+	−	−	+
Suc. Gal. Mel. (1.5% of each)	+	+	+	−	+
Sucrose (1%)	+	+	−	−	+
Melibiose (1%)	+	+	−	−	+
Galactose (1%)	+	+	+	−	+

The cells exposed to glucose, sucrose or melibiose could not ferment galactose with enhanced rate, rapidly in nine hours duration of the experiment. Only after the exposure of the cells to galactose, even without having any interference by other substrates such as sucrose and melibiose, the cells could ferment galactose. But fermentation of galactose by melibiose exposed cells was not observed. It indicates that melibiose can not induce the enzyme which is responsible for the fermentation of galactose. This result lead to suggest the induction of melibiase in the presence of melibiose by the culture 23972 must be due to the fact that the actual induction of melibiase by melibiose was caused by galactose delibered from melibiose by ME gene.

DISCUSSION

Through all the adaptation experiments two distinct results were observed. First, the ability of the cells to ferment carbohydrates depend upon the genetic character coincided with previous results. Second, the readiness of the fermentability with a certain genetic character depend upon the environmental factors, such as chance to be exposed to an inducible substance and interference with other substance.

In the fermentation of sucrose and raffinose which is due to sucrose part of raffinose molecule by the culture 23970 and by the culture 23973, the cells ferment sucrose and raffinose reggardless of the preexposure of the cells to glucose or the other substances. However, by the culture 23971 preexposed to glucose and melibiose, the fermentation of sucrose and raffinose was distinctly delayed but not by the cells preexposed to galactose or sucrose. The effect might be due to the well known glucose effect (Englesberg, 1961). The problem to detect any substance which is derived from glucose and effective on the utilization of sucrose remain to be studied.

The galactose fermentation by the cells with GA required that the cells must be preexposed to galactose for the readiness of the fermentation.

By exposing the cells to melibiose the enhanced fermentation of melibiose was observed by the cells capable of using both galactose and melibiose(23972), but not by the cells capable of using melibiose alone (23971).

By exposing the cells to galactose the enhanced fermentation of melibiose was also observed with both cultures. There seemed to be intergenic problems, but its mechanism could not be clearified at present.

For the fermentation of raffinose there were two ways to utilize raffinose——utilization of sucrose

part of raffinose molecule by beta-fracto-furanosidase and the utilization of melibiose part of raffinose molecule by alphagalactosidase. Both phenomena were observed with the cultures 23971 and 23972. With the culture 23971 the rapid fermentation of raffinose was observed by exposing the cells either to sucrose or galactose. This results lead to suggest that by exposing to sucrose or galactose the cells were adapted to elaborate sucrase or melibiase which break either one of the linkages of raffinose molecule, beta-fracto-furanosidic linkage or alpha-galactosidic linkage. The fermentation of raffinose by the culture 23972, incapable of using sucrose but capable of using galactose and melibiose, was obtained distinctly by exposing the cells to galactose which was the best inducer for melibiase. Melibiase was induced by galactose with the culture capable of using both galactose and melibiose and melibiose alone. This indicated that the inducer not necessarily be the substrate for the cells. The glucose effect on the utilization of sucrose with the cells (23971) exposed to glucose was obsrved, but the mechanism was not known.

The further study which would clear the mechanisms involved in induction and interference of substances in the utilization of substrates was proposed on the basis of molecular level with regard to the function of genic material and its messengers.

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摘 要

一定한 遺傳型을 갖인 酵母(*Saccharomyees cerevisiae*)를 여러 酵素誘導物質에 適應시키면서 炭水化物에 對한 醱酵能을 살펴 보았다. 一定한 遺傳型에 따라서 일어나는 一定한 炭水化物에 對한 醱酵能은 그 能率이 培養條件에 依해 變化를 가져오고 있었다. 그러나 醱酵能이 없는것은 어느 基質에 露出하여도 그劣性의 特徵을 維持하고 있었다. Melibiase의 生成을 誘導하는데는 Galactose에 露出하는것이 가장 有効하였으며 이 誘導物質의 效力은 酵母가 Galactose를 基質로 使用할 수 있건 없건 無關하였다. Galactose나 Melibiose를 使用하도록 하기 爲하여서는 酵母를 미리 Galactose에 適應시켜야만 했다. 그러나 Melibiose나 Raffinose, glucose 및 Sucrose에 露出시키면 아무런 效果가 없었다. 酵母를 Glucose나 Melibiose에 露出시키면 例外로 Sucrose의 醱酵가 遲延되었다. 그러나 Galactose에 露出시켰을 때는 그런 抑制된 影響을 볼수가 없었다. Raffinose의 醱酵는 SU RAF 遺傳子를 갖인 酵母와 GA ME 遺傳子를 갖인 酵母로써 볼수가 있었는데 그 醱酵能은 Raffinose 分子를 이루는 α-galactosidic 및 β-fructofuranosidic 結合을 끊는 酵素를 誘導生成케하는 誘導物質에의 露出 與否에 달려 있었다.

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