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The Growth Characteristics of *Candida* sp. JY-Cells on Ethanol, Acetic acid and Acetaldehyde Substrate

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Ethanol, Acetic acid 및 Acetaldehyde기질에 대한 *Candida* sp. JY-5 효모의 증식 특징

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The growth characteristics of *Candida* sp. JY-5 were examined on ethanol, acetic acid, or acetaldehyde as sole source of carbon by batch culture. The specific growth rates (μ) of this strain on an ethanol during the exponential period were changed depending upon the initial concentrations at above 0.5 g/l, but not in the proportion. The highest μ value was 0.291 hr^{-1} and the maximum growth yield was 61.2% at the concentration of 10 g/l. The μ values on an acetic acid substrate were constant regardless of the initial concentrations presenting 0.106 hr^{-1} in the highest value. The maximum growth yield was shown as 46.8% at the initial concentration of 10 g/l. The μ value on an acetaldehyde during the exponential period was 0.063 hr^{-1} and the maximum growth yield was 44.9% at the initial acetaldehyde concentration of 0.2 g/l.

The researches on the ethanol-, acetic acid-, or acetaldehyde-assimilating yeasts among C_2-compounds by batch culture were largely focused upon the cultural conditions, or cell yields. Some examinations were done on the optimal condition and cell yield of *Candida brassicae* nov. sp.[1,2] and *Pichia guilliermondii* Wickerham.[3] There are also reports regarding the application of substrate consumption and respiration from *Saccharomyces cerevisiae*[4], or the activities of several key enzyme from the genus *Candida*.[5]

And the batch cultures of *Candida methanolica* and *Torulopsis methanolovescens*[6] as acetic acid-assimilating yeasts and of *Candida utilis* MG-I[7], as an acetaldethyde-assimilating yeast were discussed.

Besides these experiments, the cell yields of *Saccharomyces cerevisiae* FRI 14[8], *Hansenula miso* IFO 0176[9] and *Candida tropicalis*[10] as acetic acid-assimilating yeasts were also reported. The present study was concentrated on the growth characteristics of the isolated strain, *Candida* JY-5, including cell yield, and specific growth rate in ethanol-, acetic acid-, or acetaldehyde-substrate among C_2-compounds by the batch culture and the results of the work were compared with those of the previously studied yeast strains.

MATERIALS AND METHODS

Microorganism.

The isolated strain, *Candida* sp. JY-5, was maintained by subculture in medium containing glucose 1%, ammonium sulfate 0.3%, sodium phosphate monobasic 0.1%, magne-

sium sulfate 0.3% and agar 1.5% (pH 6.0) and used throughout the experiments.

Cultivation method.

The cultivation was carried out in 750 ml of medium of jar fermentor (Bioflow model-30, New Brunswick Scientific Co., Inc.) possessing 375 ml of working volume for the culture of higher cell concentration at 30°C and pH 5.5. As subculture for the inoculation into jar fermentor, the media with the composition of ethanol 0.5%, acetic acid 0.5%, or acetaldehyde 0.02%, ammonium sulfate 0.3% and basal salts (Table 1 in assimilability of ethanol, acetic acid and acetaldehyde in *Candida* sp. JY-5) were dispensed in test tube and cultivated at 30°C for 30 hours on the reciprocal shaker. The media for jar fermentor was the same as those of subculture and the inoculum size was 2 ml of an exponential phase yeast culture that had been adapted to ethanol, acetic acid, or acetaldehyde, respectively. The aeration rate was controlled as 0.5 v.v.m. and the agitator speed was maintained at 400 r.p.m. As an antifoaming agent, silicone oil was incorporated.

The pH of media for the optimal growth was automatically monitored and controlled with 1 N HCl or 1 N NaOH solution.

Analytic method.

Apparent cell growth yield was expressed as the ratio of maximum dry cell quantity formed to the substrate quantity incorporated initially. For the dry cell quantity, the samples were centrifuged at 12,000 r.p.m. for 15 min. and resuspended in distilled water.

Then the resulting washed cells were recentrifuged and transferred to the previously dried and weighed dishes and dried to constant weight at 105°C for 24 hours on drying oven. To determine the specific growth rate (μ) during the exponential growth, the data of absorbance were plotted on semilogarithmic paper and a straight line was graphically fitted and then the specific growth rate was calculated.

RESULTS

Batch culture experiments were exerted to establish the effects of various concentrations of ethanol, acetic acid, or acetaldehyde as a sole source of carbon on cell growth.

Growth on ethanol substrate.

Fig. 1 showed the growth curves of *Candida* sp. JY-5 in various initial concentrations of ethanol.

Candida JY-5 strain could grow in as much as below about 40 g/l of initial ethanol concentration and the maximum absorbance increased generally with the initial ethanol concentration, while no growth was observed at ethanol concentration above about 40 g/l. Furthermore, when growth occurred, the exponential growth took place commonly for this strain after any lag periods. The growth characteristics of the strain on ethanol observed in this experiment were summarized in Table 1. The specific growth rates (μ) during the exponential growth for the strain on ethanol at various concentrations decreased generally in proportion to the increase in the initial concentration.

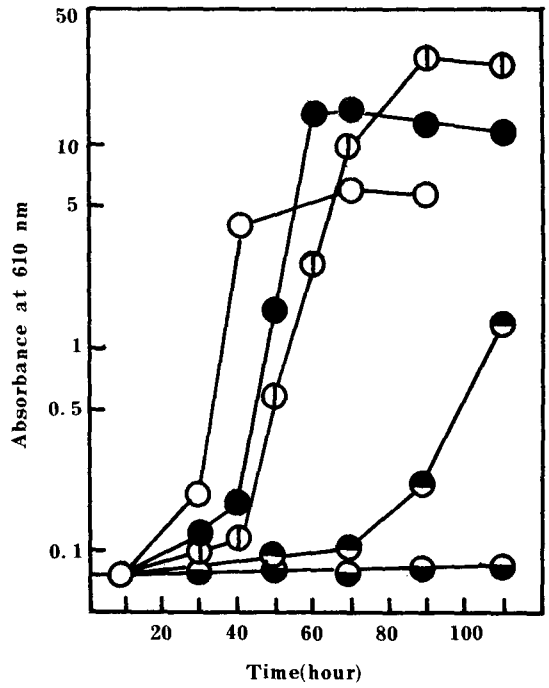

Fig. 1. Growth curves of *Candida* sp. JY-5 obtained from batch culture with various initial concentrations of ethanol(g/l).
○—○ 5 : ●—● 10 : ⦶—⦶ 20 : ◓—◓ 40 : ◒—◒ 80.

The growth yields decreased and the lag time was prolonged proportionally the increase in the initial ethanol concentrations. The highest yield obtained on ethanol was 61.2% in this strain at an initial ethanol concentration of 10 g/l.

Growth on acetic acid substrate.

The growth curves of *Candida* sp. JY-5 in acetic acid substrate were presented in Fig. 2 at various initial concentrations. This strain could grow at various initial concentrations below about 80 g/l, but it was likely that this strain

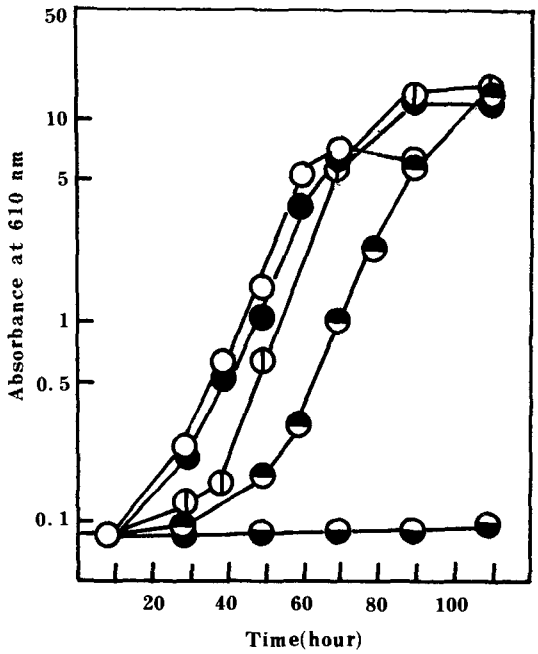

Fig. 2. Growth curves of *Candida* sp. JY-5 obtained from batch culture with various initial concentrations of acetate (g/l).
○—○ 5 : ●—● 10 : ①—①— 20 : ◓—◓ 40 : ◒—◒ 80.

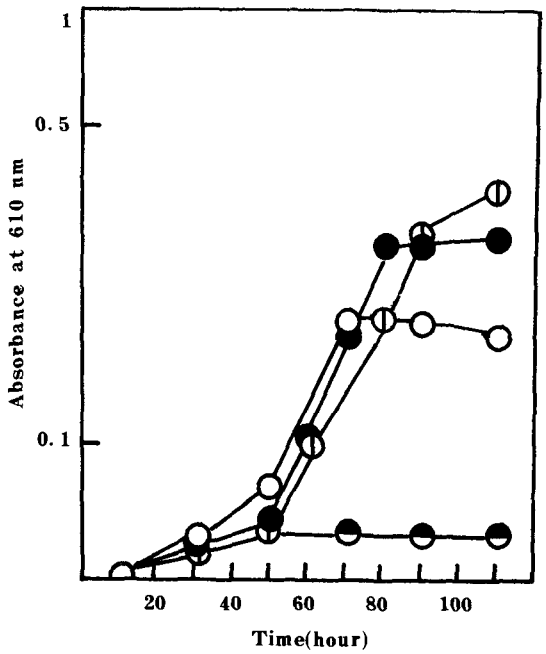

Fig. 3. Growth curves of *Candida* sp. JY-5 obtained from batch culture with various initial concentrations of acetaldehyde (g/l).
○—○ 0.2 : ●—● 0.5 : ①—① 1.0 : ◓—◓ 5.0.

couldn't grow at above about 80 g/l. The maximum absorbance generally increased in proportion to the initial acetic acid concentrations. (Fig. 2)

The growth characteristics of this strain on acetic acid substrate were also summarized in Table 1. As the initial concentration of acetic acid arised, the maximum dry cell weight increased. The specific growth rates during the exponential growth were observed.

As long as the growth proceeded, the μ values remained constant regardless of the initial concentrations of acetic acid. The μ of the strain was 0.106 hr^{-1}. The highest growth yield was achieved generally at lower initial concentration of acetic acid. The growth yields decreased in general and the lag time was prolonged for the strain in proportion to the initial concentrations of acetic acid. The highest yield obtained was 46.8% at 10 g/l in this strain.

Growth on acetaldehyde substrate.

The growth curves of this strain in acetaldehyde at various initial concentrations were shown as in Fig. 3. An acetaldehyde substrate inhibited the growth of the acetaldehyde-assimilating yeast. The results obtained presented the typical patterns of inhibition on cell growth. Therefore it was assumed that this strain employed was capable of utilizing only small degree of acetaldehyde, but an acetaldehyde was not utilized in the concentrations which are toxic. (Fig. 3)

This *Candida* species was able to grow at the initial acetaldehyde concentrations lower than 1 g/l of the concentration, but by increasing the initial concentrations the lag time was prolonged and the maximum absorbance generally increased. At the initial concentrations of this substrate higher than 5 g/l, the growths of this strain were stopped. Accordingly 1 g/l of acetaldehyde was considered the threshold concentration of this strain for the batch culture. The growth characteristics of this strain in an acetaldehyde substrate at various initial concentrations were summarized also in Table 1. The μ values for this strain in an acetaldehyde substrate were changed with the initial concentrations of acetaldehyde and the values decreased, or increased with the increase in the initial concentrations of this substrate. The growth yields decreased in proportion to the increase in the initial concentrations of acetaldehyde as observed in an

Table 1. Growth characteristics of *Candida* sp. JY-5 on ethanol, acetic acid and acetaldehyde substrate with its various concentrations obtained by batch culture.

Substrate	Initial conc. (g/l)	Maximum dry cell weight (g/l)	Apparent overall growth yield (%)	μ (hr^{-1})
Ethanol	5.0	0.800	36.0	0.291
	10.0	6.123	61.2	0.216
	20.0	10.811	54.0	0.170
	40.0	2.883	7.2	0.089
	80.0	–	–	–
Acetic acid	5.0	1.872	37.4	0.106
	10.0	4.681	46.8	〃
	20.0	5.760	28.8	〃
	40.0	9.362	23.4	〃
	80.0	0.081	0.01	–
Acetaldehyde	0.2	0.090	44.9	0.061
	0.5	0.121	24.1	0.063
	1.0	0.151	15.1	0.040
	5.0	–	–	–

ethanol substrate. The maximum growth yield obtained from the curves was 44.9% in this *Candida* strain at 0.2 g/l.

DISCUSSION

Candida JY-5 could grow at below 40 g/l of the initial concentration of ethanol, but not grow at above the concentrations, this result indicated that this strain was considered to have higher affinity, or tolerance to ethanol in comparison with *Candida methanolica* and *Torulopsis methanolovescens*.[6] The finding that the lag time was prolonged with the increase in the initial concentration was in good agreement with the results of *Candida* and *Torulopsis* species. Furthermore the specific growth rates inclined to reduction with the increase in the initial concentrations of ethanol and this result was similar to those of the above *Candida* and *Torulopsis* species. In the cases of cell yields, the results of *Candida* JY-5 were compared with 78.2% in *Torulopsis methanolovescens* from initial ethanol concentration of 4.02 g/l containing malt extract, yeast extract and peptone, 64.4% in *Candida methanolica*, about 75% in *Candida utilis* MG-1[7], 68% in *Candida utilis*[11], 56.6% in *Hansenula miso*[9], 27% in *Pichia guilliermondii* Wickerham[3], and 65.3% in *Candida brassicae* sp.[1] under the condition with yeast extract and peptone.

This comparison indicated that *Candida* sp. JY-5 was lower in growth yield to ethanol than in those of *Torulopsis methanolovescens*, *Candida methanolica*, *Candida utilis*, and *Candida brassicae* sp. In the case of μ value, the results showing differences in values accompanied by the initial ethanol concentrations corresponded with those of Amano *et al.*[1], Urakami[7] and Goto *et al.*[6] And the isolated strain JY-5 showed a little difference in the μ value comparing with *Candida* and *Torulopsis* strain.

This meant that this strain possessed comparatively strong resistance to ethanol and overcame the substrate inhibition of ethanol. And *Candida* JY-5 strain showed the stronger adaptability to acetic acid. The results that μ values of this strain examined appeared to be constant without regard to the initial concentration of acetic acid agreed with that of Goto *et al.*[6], but that of Cama and Edwards[12] was not the case.

When the results of other reports in maximum μ value, 0.45 hr^{-1} in *Candida utilis*, 0.25 hr^{-1} in *Candida methanolica* and 0.248 hr^{-1} in *Torulopsis methanolovescens* were in comparison with that of 0.106 hr^{-1} in *Candida* JY-5 strain, it was shown that the μ value of this strain was more or less lower than those of the previously studied strains.

In the case of the acetaldehyde-assimilation, *Candida* JY-5 strain seemed to utilize small amounts of acetaldehyde for their cell growth and to become weaker in tolerance to the compound. Finally the cell number inclined to reduction by the toxicity of the compound. It was thought that it was essential to screen the powerful strains tolerant to this compound. From the above examinations, *Candida* JY-5 strain was generally excellent in growth rate and cell yield. Accordingly the regard could be given that *Candida* JY-5 strain was a good yeast for the microbial cell production.

요 약

Ethanol, Acetic acid 및 Acetaldehyde를 단일 탄소원으로 하여 *Candida* sp. JY-5 효모의 증식특성을 batch culture를 통해 검토하였다. Ethanol 상에서 이 효모의 지수적 증식기 동안의 비증식도 (μ) 값은 농도가 0.5g/l 이상일 경우 그 초기 농도에 따라 변화하나 피례하지는 않았다. 최대 비증식도는 0.291hr^{-1}이었고 최대 증식수율은 10g/l 농도에서 61.2%이었다. Acetic acid상에서의 비증식도는 이

기질의 초기 농도에 관계없이 일정하였으며 그 값이 0.106 hr^{-1}, 최대 증식수율은 10g/*l* 농도에서 46.8%로 나타났다.

한편 Acetaldehyde 기질에서의 지수적 증식기 동안의 최대 비증식도는 0.063 hr^{-1}이었고 최대 증식수율은 0.2 g/*l* 농도에서 44.9 %였다.

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