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Chinese Radish Juice as a Growth Substrate for the Production of *Candida utilis* ATCC 42416 Biomass

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Abstract

Chinese radish juice (CRJ) was used as a culture medium for the production of *Candida utilis* ATCC 42416 biomass. Soluble solid and total sugar contents of Chinese radishes were in the range between 5.5 and 8.8° Brix and 3.5 and 6.5%, respectively. Since sugar in radishes are in readily usable forms, pretreatm ent procedures were not necessary after the extraction of juice from fresh radishes. In shake flask experiments, *C. utilis* ATCC 42416 grew well in CRJ and completed growth in 24 hrs at 30°C and 200rpm. Maximum cell dry weight obtainable from a liter of CRJ (1.0% sugar DCRJ×5) was 21.5g, when the yeast was grown on CRJ diluted 5 times or more with tap water to make sugar content to be equal to or less than 1.0%. Supplementation of 5-fold diluted CRJ with some nutrients did not greatly influence the growth rate, yeast biomass production, or cell protein content significantly, indicating that CRJ itself was a good substrate for the production of biomass by *C. utilis* ATCC 42416.

Key words : Chinese radish juice, *Candida utilis* ATCC 42416

INTRODUCTION

There have been world-wide efforts to produce single cell protein (SCP) from agricultural byproducts or food process wastes. Whey and molasess have been proven to be economically feasible in some countries in the world (1). However, it has been unsuccessful in Korea to find a Korean indigenous and readily usable carbon source available in quantities for the purpose of SCP production. There have seen reports in Korea to produce SCP from agricultural byproducts or food process wastes, generated in the country, such as rice and wheat straws (2-4), distiller's waste (5,6), citrus peel press liquor (7), ginseng cake extract (8), sweet potato starch cake (9), Chinese cabbage juice (10), etc.

A new renewable resourc, Chinese radish, which can be produced in great quantities in Korea was tested as a substrate for SCP production. Chinese radish is produced in Korea for the principal purpose of preparing kimchis. It is frequently overproduced too much to be consumed by the conventional purpose of use. An alternative way of consuming the surplus agricultural product is tested to produce microbial cells as SCP.

Especially significant is the sugar content of Chinese radish because sugar serves as an important nutrient for microorganisms. Sugar content of Chinese radish range from 3.1 to 6.1% depending on the varieties of Chinese radish (11). Radishes consists of 70% glucose and two other compounds are mannose and fructose (12). In addition to fermentable sugars, Chinese radish must contain nutrients supporting microbial growth, judging from the fact that lactic acid bacteria which are nutritionally fastidious grow well on it.

In the present study, we examined the potential of Chinese radish, which is a renewable resources and can be produced in great quantities in Korea, as a novel substrate for growth of *Candida utilis* ATCC 42416 for the purpose of producing biomass.

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MATERIALS AND METHODS

Microorganism and growth condition

Candida utilis ATCC 42416 obtained from the Korea Federation of Culture Collection, Seoul, was maintained on yeast extract (0.3%)-malt extract (0.3%)-peptone (0.5%)-glucose (2.0%) agar (YMPG agar) slants. Preculture was prepared by shake flask culture at 30° C and 200rpm (Low Temperature Shaking Incubator KSI-200L, Korea Instruments Co., Seoul) CRJ (diluted or undiluted, nutrients supplemented or unsupplemented) was dispensed into 500ml flask in 100 ml amounts and sterilized. The flask inoculated with 1ml of the preculture and shaken at 200rpm at 30° C.

Chinese radish juice (CRJ) preparation

Washed in 47 different lots of autumn-sowing Chinese radishes were cut and ground by a Waring blender to puree before strained through two layers of cheese-cloth. The strained fresh juice was heated for 10min. at 100° C and cooled to room temperature. The cooled CRJ was centrifuged at 3500rpm (Hanil Centrifuge Co.) for 10min to get clear liquid and kept frozen until used. This upper liquid did form precipitation upon sterilization.

Before each experiment, the frozen CRJ was thawed, and it was used as aliquots which were diluted with tap water to give desired sugar contents. Nutrients such as glucose (2.0%), ammonium sulfate (0.2%), yeast extract (0.2%) and peptone (0.2%) were added to 5-fold diluted CRJ before sterilization to test the supplementary effects of each of them on the growth of *C. utilis* ATCC 42416. KH_2PO_4 (0.2%) was separately sterilized before it was added to CRJ.

Analysis

Absorbance at 550nm (Spectronic 20D, Milton-Roy Co., Rochester, N.Y.) was employed to determine the growth of the yeast. For cell dry weight measurements, aliquots of the growth medium were sampled at 4hr intervals and centrifuged at 3500rpm (Hanil Centrifuge Co.) for 10 min. Washed yeast cells were transfered to preweighed weighing pan and dried at 105 °C overnight and then weighed the dried yeast cells. pH was measured at 4hr intervals by using a pH meter (Dong-Woo Medical Co.). Total sugar and soluble solids contents were measured by Anthrone method (13) and by a refractometer (Atago Hand Refractometer, Atago Co., Japan), respectively. Protein contents of dried yeast cells were measured by microKjeldahl technique (14).

RESULTS AND DISCUSSION

Sugar contents of Chinese radish

Soluble solids contents of 47 different lots of Chinese were in the range from 5.0 to 8.8° Brix and the total sugar contents of them ranged from 3.5 to 6.5% (Fig. 1) which was in the similar range as that reported earlier (11). In this research, results were effected by environmental conditions such as weather. Fluctuating temperatures produced uneven growth in Chinese radish. This influence had only a direct effect on the soluble solid contents but not on the permability of the radishes (15).

The relationship between soluble solids(X) and total sugar (Y) contents was Y=0.735X − 0.135.

Effect of dilution of CRJ on cell dry weight production and on the substrate yield coefficient

C. utilis ATCC 42416 was grown on undiluted CRJ (UNCRJ) and on YMPG broth (YMPGB) for the pur-

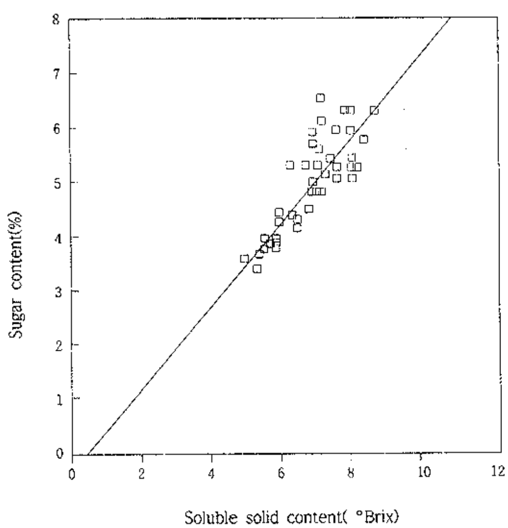

Fig. 1. Relationship between soluble solid and total sugar contents of Chinese radish juice.

pose of comparison (Fig. 2). A similar result was reported that there was not much difference in the growth pattern when *C. utilis* and *Saccharomyces cerevisiae* were grown on Chinese cabbage juice and on YMPGB (15).

Fig. 3 shows the growth curves of *C. utilis* on UDCRJ and 2- to 7-fold diluted CRJ (DCRJ). From this result Chinese radish was thought to contain growth inhibitory substance (s) against *C. utilis* as it was in Chinese cabbage juice (10) and in alfalfa residual juice (16). There was no difference in growth rate in DCRJ regardless of extent of dilution as long as CRJ was diluted more than twice. Maximum cell dry weight attainable from unit volume of CRJ was accomplished when the yeast was grown on CRJ diluted more than 5 times (Table 1) to make total sugar content which was approximately 1.0% or less. Most or all of the sugars in UDCRJ and DCRJ was depleted in about 20hr of cultivation (Fig. 4) regardless of the degree of dilution. The pattern of pH change was obvious from Fig. 4, as the degree of dilution increased, pH increased more rapidly from the 12th hr of *C. utilis* cultivation on UDCRJ and DCRJ. This can be explained during the earlier phase of cultivation, acids were produced from sugars which in turn was used by the yeast as fermentable sugars were depleted. As CRJ was diluted

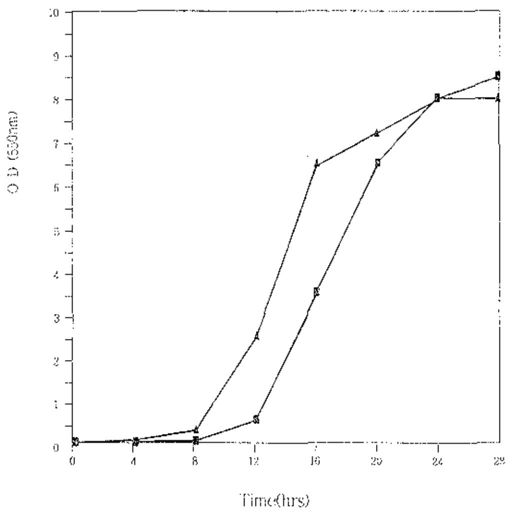

Fig. 2. Growth of *Candida utilis* ATCC 42416 on Chinese radish juice and YMPG broth.
■ : Chinese radish juice (undiluted), ▲ : YMPG broth.

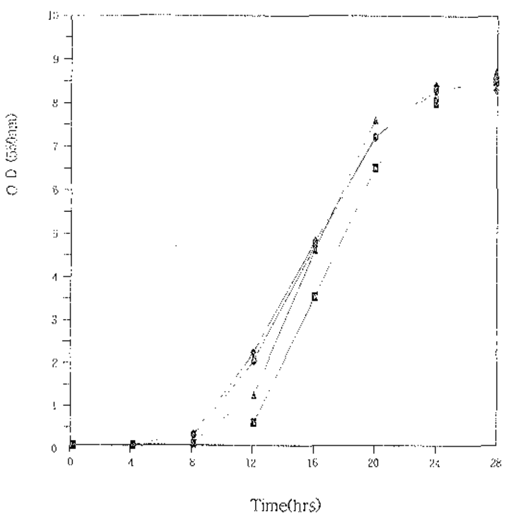

Fig. 3. Growth of *Candida utilis* ATCC 42416 on Chinese radish juice of different strength.
■ : UDCRJ, ▲ : 3X DCRJ
● : 5X DCRJ, ◆ : 7X DCRJ.

Table 1. Cell dry weight production and substrate yield coefficients of *C. utilis* ATCC 42416 grown on Chinese radish juice (CRJ) with different degrees of dilution

	Total sugar (%)	Cell dry weight (g/L)		Substrate yield coefficient (%)
		DCRJ	UDCRJ[1)]	
UDCRJ (control)	5.0	5.7	5.7	11.4
2× DCRJ	2.5	4.8	9.3	19.2
3× DCRJ	1.7	4.6	13.8	27.1
4× DCRJ	1.5	4.5	18.0	20.0
5× DCRJ	1.0	4.3	21.5	43.0
6× DCRJ	0.8	3.6	21.6	42.4
7× DCRJ	0.7	3.0	21.0	42.9

DCRJ : diluted CRJ, UDCRJ : undiluted CRJ
[1)]Cell dry weight calculated as from UDRJ after *C. utilis* was grown on DCRJ (DCRJ × dilution ratio)

Table 2. Effect of nutrient supplementation on the production of cell dry weight and cell protein content when *C. utilis* ATCC 42416 was grown on 5-fold diluted Chinese radish juice (DCRJ)

Nutrients	Cell dry weight (g/L)		Protein content (dried W%)
	DCRJ	UDCRJ[1)]	
5× DCRJ (control)	4.3	21.5	45
KH_2PO_4 (0.2%)	4.0	20.0	35
$(NH_4)_2SO_4$ (0.2%)	3.9	19.5	53
Yeast extract (0.2%)	4.6	23.0	50
Peptone (0.2%)	4.1	20.5	52
Glucose (2.0%)	3.8	19.0	46

DCRJ : diluted CRJ, UDCRJ : undiluted CRJ
[1)]UDCRJ=DCRJ × dilution ratio

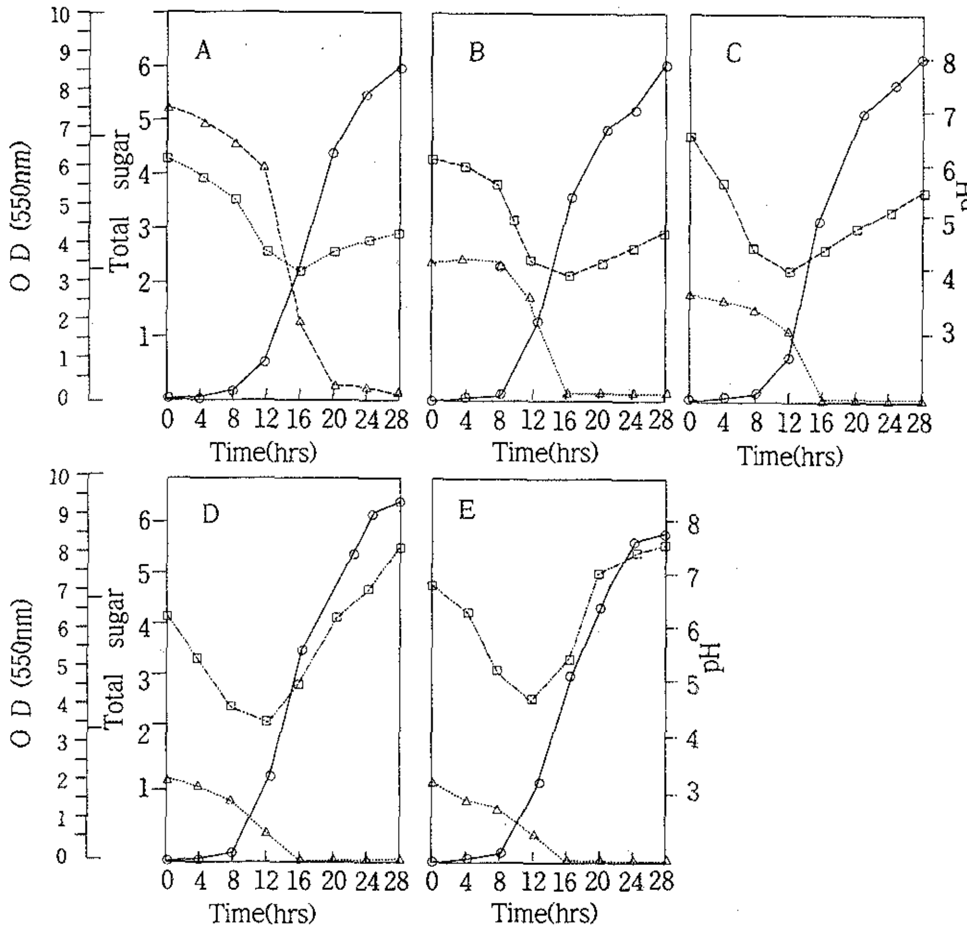

Fig. 4. Time course of changes of total sugar content, pH and optical density during the growth of *Candida utilis* ATCC 42416 on CRJ of different degrees of dilution.
A : undiluted, B : 2 fold diluted, C : 3 fold diluted, D : 4 fold diluted, E : 5 fold diluted.
△ : Total sugar content, □ : pH, ○ : Optical density.

up to 5 times both the cell productivity and substrate yield coefficient increased dramatically (Table 1). Further dilution did not improve neither one. From the view point of operational costs and efficiency of sugar utilization, diluting CRJ of 5.0% total sugar content 5 times was found to be optimal.

Effect of supplementary nutrients

Table 2 and Fig. 5 show the effects of various supplementary nutrients on the growth, the final cell dry weight and the protein content of the dried cell of *C. utilils* grown on 5-fold diluted CRJ. There was not much difference noted in the growth rate except during the initial growth phase (Fig. 5) and in the attainable final cell dry weight. There were more or less 10 % differences in cell dry weight production when nutrients were supplemented to 5-fold diluted CRJ (Table 2), which was believed to be within the experimental error range.

Nitrogenous nutrients such as yeast extract, ammonium sulfate and peptone increased cell protein contents by up to 18% while KH_2PO_4 decreased it more than 10%. This phenomenon was different from the result with a *Candida* sp. on alfalfa residual juice where KH_2PO_4 was stimulatory (17). This should be due to the compositional difference in the media and the yeast strain. The enhancement of cell dry weight production and cell protein content by supplementing

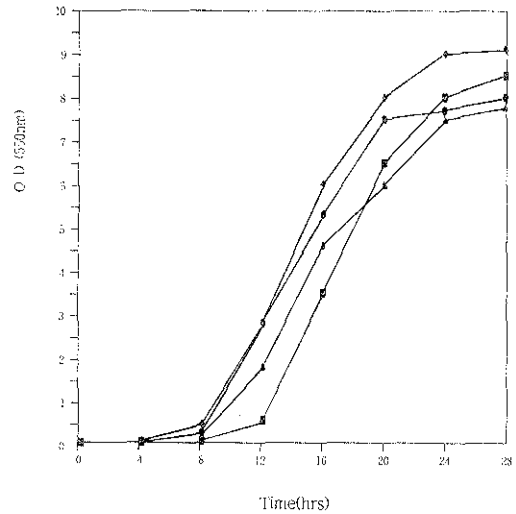

Fig. 5. Effects of supplementary nutrients on the growth of *Candida utilis* ATCC 42416.
■ : control, ▲ : Glucose
● : peptone, ◆ : yeast extract

such nitrogenous nutrients as yeast extract, ammonium sulfate and peptone was not significant compared to the amounts of nutrients supplemented. Overall, CRJ itself is an excellent substrate for the production of yeast cell mass by *C. utilis*.

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무즙을 이용한 *Candida utilis* ATCC 42416균체 생산

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요 약

단세포 단백질 자원으로 이용 가능한 균주로 알려진 *Candida utilis* ATCC 42416 균체를 생산하기 위하여 무를 파쇄하여 얻은 무즙을 배지로 균체배양 실험을 하였다. 무즙의 수용성 고형물과 총 당량은 각각 5.0~8.8 Brix와 3.5~6.5%였다. *C. utilis* ATCC 42416 균주를 무즙에 접종하여 30° C에서 200rpm으로 진탕 배양하였을 때 24시간 이내에 증식이 끝났으며, 단위 소모당에 대한 최대 균체 생산량은 당농도 1% 희석 무즙에서 L당(5 × DCRJ) 21.5g의 건조균체를 생산하였다. 5배 희석무즙에 약간의 영양소 glucose(2.0%), yeast extract(0.2%), peptone(0.2%), ammonium sulfate(0.2%) 및 KH_2PO_4(0.2%) 등을 각각 보강하여도 증식 속도, 균체생산 및 세포 단백질 함량 등에 차이가 거의 없어서 무즙자체가 *C. utilis* ATCC 42416 균체 생산에 좋은 기질이었음을 알 수 있다.