Journal of Microbiology and Biotechnology
Vol. 4, No. 1, 56-62, 1994

Direct Fermentation of D-Xylose to Ethanol by *Candida* sp. BT001

LEE, SANG-HYEOB AND WON-GI BANG*

Department of Agricultural Chemistry, College of Natural Resources, Korea University, 1 Anam-Dong, Sungbuk-Ku, Seoul 136-701, Korea

A yeast strain, BT001, which can directly ferment D-xylose to ethanol was isolated from forest soils, and then identified as *Candida* sp. Cultural conditions for the optimum ethanol production, along with the effects of aeration on cell growth and ethanol production were investigated. Aeration stimulated the cell growth and the volumetric rate of ethanol production, but decreased the ethanol yield. Optimum temperature and initial pH for the ethanol production were 33°C and 6.0, respectively. In a shake flask culture, this strain produced 52.3 g ethanol per liter from 12%(w/v) D-xylose after incubation for 96 hours. Ethanol yield was 0.436 g per g D-xylose consumed. This corresponds to 85.8% of theoretical yield. Also, this yeast strain produced ethanol from D-galactose, D-glucose and D-mannose, but not from L-arabinose and L-rhamnose. Among these sugars, D-glucose was the fastest in being converted to ethanol sugars.

Hemicellulose is one of the major components of renewable biomass produced by photosynthesis. Annual production of the biomass is 1.5×10^{11} tons on a global basis (25). Hemicellulose is easily hydrolyzed to a mixture of hexoses and pentoses by dilute acid under mild conditions (14). D-Xylose is a major component of the pentoses. The pentosan content of some agricultural residues such as corn cobs or husks may reach as high as 35% of the total carbohydrate content (11). A biological method which converts monomeric sugars of hemicellulose, especially D-xylose, to ethanol could reduce the overall process cost for ethanol production in bioconversion of biomass. Hence, the ethanol production processes could become more feasible economically.

At present, ethanol is utilized as an additive to gasoline because it improves the anti-knock quality of fuel and reduces the CO content of the exhaust gases (9).

Until the beginning of 1980's, yeasts, including *Saccharomyces* genus, have been considered unable to ferment D-xylose to ethanol, even though many yeasts can assimilate D-xylose oxidatively (1). At present, the D-xylose fermentation by anaerobic or facultative bacteria is not satisfactory due to the simultaneous production of many by-products (19). Several fungal systems that ferment D-xylose to ethanol are not practical due to their low growth and conversion rate (26). From this point of view, it would be ideal to use a single yeast which can convert both hexoses and pentoses to ethanol in high yield, as the yeast fermentation of D-glucose is a well-established industrial process. It is known that several microorganisms ferment D-xylose directly to ethanol in significant quantities with yields greater than 0.2 g ethanol per g D-xylose. These include *Pachysolen tannophilus* (3-6, 17, 20-22), *Candida* sp. (5, 7, 10), *Pichia stipitis* (5, 7), *Kluyveromyces* sp. (15, 16), and *Clavispora* sp. (18), as well as a mold *Fusarium* sp. (13, 23, 24) and *Paecilomyces* sp. (29).

Apart from D-xylose, other hemicellulose hydrolytic products include D-galactose, D-glucose, D-mannose, L-arabinose, L-rhamnose, and D-cellobiose, depending on the nature of the plant materials and hydrolysis procedures (8, 17). Therefore, the extent to which these sugars could be fermented to ethanol by the microorganisms is of interest to the commercial exploitation of lignocellulosic biomass.

In this paper, we report the production of ethanol from D-xylose and other sugars by the newly isolated yeast *Candida* sp. BT001.

MATERIALS AND METHODS

Microorganism

Candida sp. BT001 was isolated from forest soils. This strain was subcultured at 30°C on a YM agar slant (28) containing 3 g yeast extract, 3 g malt extract, 5 g pep-

*Corresponding Author
Key words: D-xylose, fermentation, ethanol, *Candida* sp. BT001

tone, 10 g D-glucose and 20 g agar per liter and stored at 4°C.

Isolation and Screening of the Yeast Capable of Directly Fermenting D-Xylose to Ethanol

The forest soil samples were suspended in a physiological saline solution and filtered through a Whatman No.1 paper. The filtrate was inoculated into the isolation medium (pH 7.2) containing 0.66 g $(NH_4)_2SO_4$, 1.36 g K_2HPO_4, 0.12 g $MgSO_4 \cdot 7H_2O$, 0.017 g $CaSO_4 \cdot 7H_2O$, 0.06 g $FeSO_4 \cdot 7H_2O$ and 30 g D-xylose per liter, and then an enrichment culture was carried out at 30°C on a reciprocal shaker (120 2.5 cm-strokes/min) more than 3 times. The diluted culture was spread on the isolation agar plate and incubated at 30°C. The grown colonies were isolated with a transfer needle and subcultured on YM agar plate for pure cultures.

To screen a yeast which shows high D-xylose-fermenting ability, a loopful of each of the isolated yeasts was inoculated into screw-capped tube (125×25 mm) containing 5 ml of the sterilized screening medium (pH 6.5) containing 1 g KNO_3, 0.66 g $(NH_4)_2SO_4$, 0.75 g $MgSO_4 7H_2O$, 0.07 g $CuSO_4 \cdot 5H_2O$, 0.07 g $ZnSO_4 \cdot 7H_2O$, 10 g yeast extract and 10 g D-xylose per liter, and then incubated at 30°C for 6 days in a static culture. Ethanol in culture broth was determined, and the yeast which showed the highest ethanol productivity was selected.

Identification of the Isolated Yeast

The isolated yeast, strain BT001, was identified following the standard method reported by Van Der Walt and Yarrow (27).

Growth and Fermentation Conditions

The fermentation medium for shake flask cultures contained 1.5 g $(NH_4)_2HPO_4$, 1.0 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.2 g $MgCl_2$, 5.0 g yeast extract and carbon sources. The initial pH of the medium was adjusted with 1N HCl and 1N NaOH solution. The carbon sources were autoclaved separately. For the preparation of inoculum, a loopful of active slant culture was transferred into a 50 ml Erlenmeyer flask containing 10 ml of YM broth with 1%(w/v) D-xylose, and incubated at 30°C for 24 hours in a reciprocal shaker. 1%(v/v) preculture was then inoculated into a 500 ml Erlenmeyer flask with silicone rubber stopper, in which a glass tube (10×7 mm) stuffed with a cotton was inserted for CO_2 and air ventilation. Unless otherwise stated, each flask contained 200 ml of the fermentation medium and the fermentation was carried out on a reciprocal shaker at 100 2.5 cm-strokes/min.

Analytical Procedures

Cell growth was determined by optical densities of culture samples at 600 nm on Beckman DB spectrophotometer. Dry biomass concentrations and optical densities were correlated by the following equation.

Cell Dry Weight (mg/L)=(225×Optical Density−1.33)×Dilution Rate

Cell-free samples were assayed for ethanol on a Hewlett Packard 5890A Series gas chromatograph equipped with 6 feet Porapack Q column operating at 185°C. D-Xylose and other carbohydrates were determined by gas chromatography of the aldononitrile acetate derivatives following the method reported by Chen and McGinnis (2).

RESULTS AND DISCUSSION

Isolation and Screening of the Yeast Capable of Directly Fermenting D-Xylose to Ethanol

Thirty yeast strains which could assimilate D-xylose aerobically were isolated from the soil samples. Of these strains, only 6 strains were able to ferment D-xylose to ethanol in static culture (Table 1). Among these yeasts, the strain BT001 and BT026 showed higher abilities of fermenting D-xylose to ethanol than the other strains. The strain with the highest ability, BT001, was selected for further experiments.

Identification of the Yeast Strain BT001

Morphological, cultural, physiological and biochemical properties of the yeast strain BT001 were investigated. After incubation for 3 days at 25°C in glucose-yeast extract-peptone water, the cells were mainly ovoid, (2.5−4.7)×(3.5−7.0) μm, and occurred singly, budding, or in pairs (Figure 1A). The elongated pseudohyphal cell might occur. A sediment was strongly formed, while a thin pellicle was not present. After incubation for 1 month at 25°C on morphological agar (Difco Co.), the streaked culture was dull, the colony texture changed from coherent or butyrous to white or cream-colored, the elevation from raised to umbonate, and the surface from smooth to rough with a filamentous margin. A well-developed pseudomycelium with blastospores occurring singly, or in short chains and clusters was formed abundantly on the corn meal agar (Figure 1B). Vegetative cells were reproduced by multilateral budding and they were not triangular. Acetic acid was not formed from

Table 1. Ethanol production from D-xylose by the isolated yeast strains

Strains	Ethanol, g/l
BT001	2.84
BT008	0.94
BT013	0.86
BT016	0.85
BT024	0.91
BT026	1.32

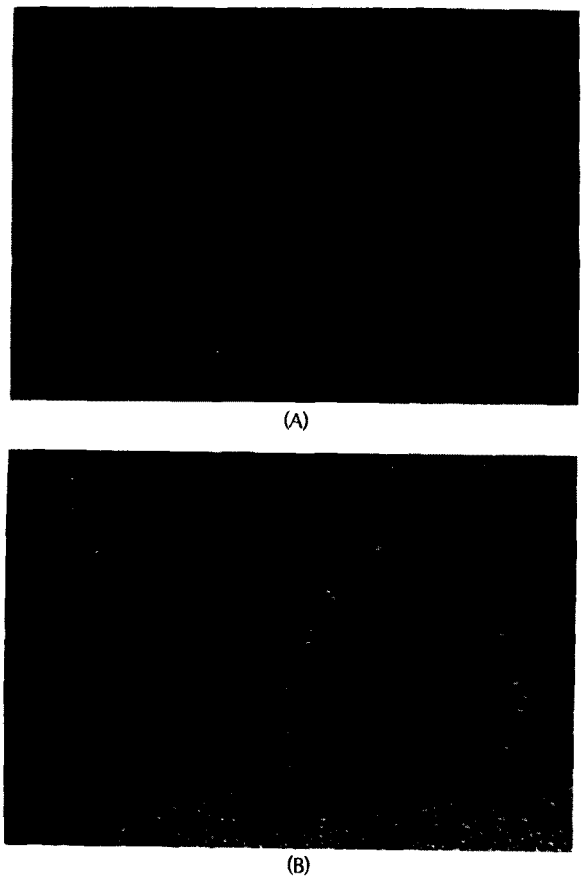

Fig. 1. Photomicrographs of the yeast strain BT001.
(A) bright-field microscopy (×1000), (B) phase-contrast microscopy (×800).

D-glucose. Ascospores, ballistospores, teliospores and needle-shaped arthrospores were not formed. Inositol, nitrate, nitrite, erythritol and raffinose were not assimilated, whereas maltose was assimilated. Table 2 showed the physiological and biochemical properties of the strain BT001. These results indicate that the strain BT001 belongs to the genus *Candida*. Therefore, the strain BT 001 was named *Candida* sp. BT001.

Effects of Aeration on the Cell Growth and Ethanol Production

Aeration was varied randomly by employing 100, 200, 300, and 400 m*l* of fermentation medium with 2%(w/v) D-xylose in 500 m*l* Erlenmeyer flask. As shown in Figure 2, *Candida* sp. BT001 required oxygen for growth, while ethanol production occurred in both aerobic and anaerobic conditions. Cell growth rate decreased as aeration decreased. Under a more oxygen-limited condition, a higher amount of ethanol was produced, but the fermentation time to reach the maximum ethanol production was delayed. Because this strain assimilated ethanol (See Table 2), the aeration stimulated the ethanol uptake

Table 2. Physiological and biochemical properties of the strain BT001

Fermentation of carbon compounds:					
D-Glucose	+	D-Xylose	+	D-Galactose	+w
D-Mannose	+	Maltose	+	Sucrose	−
Assimilation of carbon compounds:					
D-Glucose	+	D-Fructose	+	D-Galactose	+
L-Sorbose	+	D-Xylose	+	D-Arabinose	−
L-Arabinose	−	D-Mannose	+	D-Cellobiose	+
Melibiose	−	Maltose	+	Sucrose	+
Trehalose	+	Melezitose	+	Raffinose	−
L-Rhamnose	−	Lactose	−	Erythritol	−
Inositol	−	Galactitol	−	Glycerol	+
Xylitol	−	Ribitol	+	Sorbitol	+
Mannitol	+	Ethanol	+	Salicin	+
Soluble starch	+	Citric acid	+	Lactic acid	−
Succinic acid	+				
Assimilation of KNO_3 and $NaNO_2$: −					
Growth in Vitamin free medium: +					
Growth on 50%(w/v) glucose-yeast extract agar: −					
Growth 10% NaCl plus 5% glucose in yeast nitrogen base: −					
Growth at 37°C: +					
Urease: −					
Gelatin liquefaction: −					
Diazonium blue B color test: −					
Coenzyme type: CoQ_9					
Arbutin: +					

+, Positive; −, Negative; +w, Weakly positive

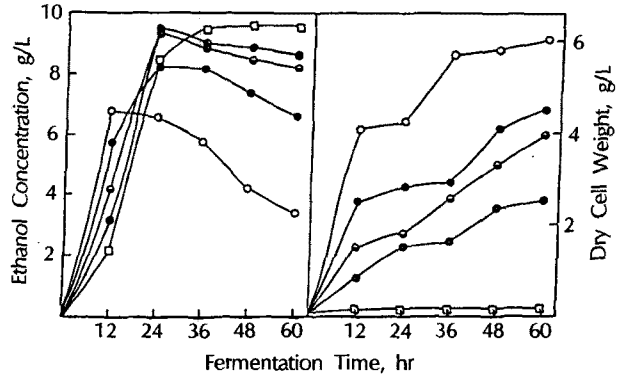

Fig. 2. D-Xylose fermentation by Candidaa sp. BTOOL under the aerobic and anaerobic condition.
[○—○: 100/500, ●—●: 200/500, ◒—◒: 300/500, ⊙—⊙: 400/500 m*l* broth in Erlenmeyer flask, □—□: 200/500 anaerobic conditions]. The initial pH and temperature were 5.0 and 30°C, respectively.

rate of ethanol when D-xylose became depleted. The diauxy phenomenon in cell growth reflects a catabolite repression in ethanol assimilation. Under anaerobic condition (N_2-flushing and tight-sealing), neither growth nor ethanol utilization was observed. The maximum ethanol concentration was slightly higher under anaerobic condition. However, about twice the fermentation

time was required to reach maximum production.

From the comparison of fermentation parameters (Table 3), which were obtained by monitoring cell growth and ethanol production at intervals of 2 hours with 3% (w/v) D-xylose, the degree of aeration exerted a considerable influence on the fermentation parameters. The maximum specific growth rate (μ_{max}, hr^{-1}) and cell yield ($Y_{x/s}$, g dry biomass per g D-xylose consumed) were reached under the more aerobic condition, whereas the ethanol yield ($Y_{p/s}$, g ethanol per g D-xylose consumed) was the highest under the aeration-limited condition. Also, aeration influenced the maximum specific rate of ethanol production (q_p, g ethanol per g dry biomass per hour) and the maximum volumetric rate of ethanol production (Q_p, calculated from the slope of the ethanol vs. time, g ethanol/l/hr). The q_p was 2.023 hr^{-1} when 400 ml of medium was used, almost twice the value obtained from 100 ml of the working volume. However, Q_p was highest (1.053 g/l/hr) when 100 ml of medium was used.

Table 3. Effects of aeration on cell growth and ethanol production in the D-xylose fermentation by *Candida* sp. BT001

Working volume*	Kinetic parameters				
	μ_{max}	q_p	Q_p	$Y_{p/s}$	$Y_{x/s}$
100	0.437	1.040	1.053	0.359	0.127
200	0.263	1.447	0.758	0.468	0.076
300	0.209	1.452	0.660	0.475	0.066
400	0.098	2.023	0.245	0.486	0.052

*Working volume (ml) per ca. 500 ml Erlenmeyer flask.
Fermentation temperature and initial pH were 30°C and 5.0, respectively.

These results indicate that aeration (oxygen) stimulates *Candida* sp. BT001 cell growth and maximizes its volumetric rate of ethanol production (Q_p), while decreasing the ethanol yield. These results are in accordance with those reported by Schneider *et al.* (20) and Delgenes *et al.* (5).

Effect of Initial pH on D-Xylose Fermentation

The influence of initial pH on the D-xylose fermentation was investigated. Table 4 shows that the initial pH of fermentation medium obviously affected the fermentation parameters. Higher ethanol yield, cell yield and volumetric ethanol productivity (final ethanol concentration divided by the time from incubation to the delivery of the batch, g ethanol/l/hr) were obtainable at the initial pH of 5.0 and 6.0. The highest ethanol yield was obtained at pH 6.0 when ethanol concentration was 13.9 g/l, corresponding to 90.8 % of the theoretical ethanol yield.

An initial pH of 4.5 had been reported for ethanol production from D-xylose by *Candida shehatae* (7). However, the initial pH of 6.0 in this experiment is similar to that reported for *Candida* sp. XF217, in which the initial pH was 5.8 (10).

Effect of Temperature on D-Xylose Fermentation

To determine the optimum temperature for ethanol production, shake flask cultures were carried out at different temperatures. As shown in Table 5, the ethanol yield increased gradually up to 33°C. The highest ethanol yield was obtainable between 30°C and 33°C at which ethanol concentrations were 13.9 g/l and 14.0 g/l, respectively. The cell yield reached its maximum at 30 °C.

Table 4. Effect of initial pH on ethanol production from D-xylose by *Candida* sp. BT001

Initial pH	Final pH	Ethanol g/l	$Y_{p/s}$	$Y_{x/s}$	Volumertic ethanol productivity g/hr/l
3.0	2.8	10.5	0.455	0.078	0.435
4.0	3.3	12.7	0.454	0.080	0.529
5.0	4.2	13.5	0.463	0.086	0.563
6.0	5.2	13.9	0.466	0.090	0.579
7.0	5.8	12.5	0.454	0.086	0.521

Initial D-xylose concentration and fermentation temperature were 3%(w/v) and 30°C, respectively. Ethanol concentration and cell growth were estimated at 24 hr.

Table 5. Effect of temperature on ethanol production from D-xylose by *Candida* sp. BT001

Temperature °C	Ethanol g/l	$Y_{p/s}$	$Y_{x/s}$	Volumetric ethanol productivity g/l/hr
24	8.3	0.458	0.087	0.348
27	12.1	0.459	0.089	0.506
30	13.9	0.466	0.090	0.579
33	14.0	0.468	0.083	0.584
36	13.2	0.442	0.074	0.553

Initial D-xylose concentration was 3%(w/v) and the initial pH was 6.0. Ethanol concentration and cell growth were estimated at 24 hr.

Table 6. Effect of substrate concentration on ethanol production from D-xylose by *Candida* sp. BT001

Substrate % (w/v)	Fermentation time (hr)	Ethanol g/l	$Y_{p/s}$	$Y_{x/s}$	Volumertic ethanol productivity g/l/hr
3	24	14.0	0.467	0.082	0.583
6	48	28.1	0.468	0.069	0.585
12	96	52.3	0.436	0.045	0.545
18*	144	68.1	0.415	0.043	0.473

The fermentation temperature was 33°C and initial pH was 6.0. *The residual D-xylose concentration was 1.6%(w/v).

These results contradict those reported for *Candida shehatae* (7) and *Candida* sp. XF217 (10), in which the optimum temperature for D-xylose fermentation was 30°C.

Effect of D-Xylose Concentration on Ethanol Production

To investigate the effect of substrate concentration on the D-xylose fermentation, the D-xylose concentration in shake flask cultures was varied from 3 to 18% (w/v). As shown in Table 6, the ethanol yield and cell yield decreases as the initial D-xylose concentration increases. However, the ethanol concentration increases when the initial D-xylose concentration is increased. At the concentration of up to 6%(w/v), both the ethanol yield and volumetric ethanol productivity were nearly the same, but the cell yield reached maximum at 3% (w/v) D-xylose. Ladish and Dyck (12) reported that at least, an ethanol concentration of 4%(w/v) in a liquor was a requisite for economically viable product separation and recovery process. Thus, the concentration of ethanol accumulated was a key point of interest. *Candida* sp. BT001 maintained a high ethanol yield of over 0.415 g/g up to a concentration of 18%(w/v) D-xylose. Although the maximum ethanol concentration of 68.1 g/l was produced with 18%(w/v) D-xylose, the ethanol yield (0.415 g/g) and volumetric ethanol productivity (0.473 g/l/hr) were lower than those from 12%(w/v) D-xylose.

Figure 3 shows the time course of ethanol production by *Candida* sp. BT001 on 12%(w/v) of D-xylose. *Candida* sp. BT001 produced 52.3 g/l of ethanol when D-xylose was completely consumed after incubation for 96 hours and the ethanol yield was 0.436 g/g. A pH decline, from 6.0 to near 4.0, coincided roughly with the period of D-xylose uptake. Xylitol which was known as a main by-product was not produced, although trace amounts of xylitol (<0.02%, w/v) was detected in the broth when the autolysis of biomass occurred. This may have contributed to a high ethanol concentration. Initial D-xylose concentration of 12%(w/v) showed no substrate inhibition as evidenced by the rapid initial fermentation rate, although there might be a question of product inhibition at concentration of ethanol above 40 g/l.

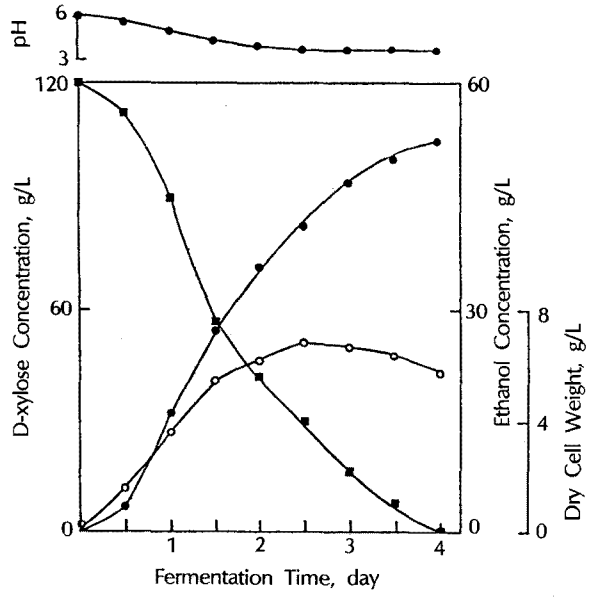

Fig. 3. Time course of ethanol production on 12%(w/v) D-xylose by *Candida* sp. BT001.

The two yeast strains, *Candida shehatae*, which produced 17~37 g/l of ethanol (ethanol yield; 0.29~0.44 g/g) from 15%(w/v) D-xylose, and *Pichia stipitis*, which produced 33~52 g/l of ethanol (ethanol yield; 0.34~0.42 g/g) from 15%(w/v) D-xylose (22), have been known as promising yeasts for D-xylose fermentation due to their rapid rate of fermentation and high ethanol yield. However, the results obtained in this experiment showed *Candida* sp. BT001 as the better strain.

Ethanol Production from Different Sugars

Besides D-xylose, *Candida* sp. BT001 can ferment D-glucose, D-mannose and D-galactose to ethanol (Table 7). The μ_{max}, Q_p, q_p and $Y_{x/s}$ obtained from D-glucose were obviously higher than the values obtained from other sugars. These values for D-glucose: 86% of μ_{max}, 41% of Q_p, 46% of q_p and 50% of $Y_{x/s}$, were higher than the values obtained for D-xylose. The maximum volumetric rate of ethanol production with D-mannose was similar to that with D-glucose. However, the ethanol yield from D-xylose was the highest. This phenomenon may be due to the fermentation condition used in this

Table 7. Comparison of kinetic parameters for the individual fermentation of 30 g/l each of different sugars by *Candida* sp. BT001

Sugars	Parameters					
	μ_{max}	Q_p	q_p	$Y_{x/s}$	$Y_{p/s}$	F_{max}*
D-Glucose	0.39	1.62	2.14	0.12	0.45	12
D-Mannose	0.30	1.62	1.45	0.10	0.47	18
D-Galactose	0.16	0.64	0.40	0.10	0.45	44
D-Xylose	0.21	1.15	1.47	0.08	0.48	24

The fermentation temperature was 33°C and initial pH was 6.0.
*Time required for the maximum ethanol concentration to be reached.

experiment. That is, aerobic condition is more favorable to D-xylose. The fermentation parameters obtained with D-galactose were the least, compared with those obtained from other sugars. This may be due to a long lag phase for D-galactose metabolism.

In summary, *Candida* sp. BT001 can ferment all major monomeric sugars produced by the acid hydrolysis of plant materials, namely D-glucose, D-mannose, D-galactose and D-xylose. L-Arabinose and L-rhamnose, which are usually produced at very low concentrates (17), were not utilized by *Candida* sp. BT001 as shown in Table 2.

The newly isolated *Candida* sp. BT001 may be the most promising yeast for fermentation of D-xylose to ethanol, if D-xylose could be produced inexpensively from the plant residues in the future.

Acknowledgment

This study was supported by a research grant (Subject Number 891-152-019-1) from the Korean Science and Engineering Foundation, for which authors are very grateful.

REFERENCES

1. Barnett, J.A. 1976. The utilization of sugars by yeasts. *Adv. Carbohyd. Chem. Biochem.* **32**: 125-228.
2. Chen, C.C. and G.D. McGinnis. 1981. The use of 1-methylimidazole as solvent and catalyst for the preparation of aldononitrile acetates of aldoses. *Carbohydr. Res.* **90**: 127-130.
3. Debus, D., H. Mether, D. Schilze and H. Dellweg. 1983. Fermentation of xylose with the yeast *Pachysolen tannophilus*. *Eur. J. Appl. Microbiol. Biotechnol.* **17**: 287-291.
4. Dekker R.F.H. 1982. Ethanol production from D-xylose and other sugars by the yeast *Pachysolen tannophilus*. *Biotechnol. Lett.* **4**: 411-416.
5. Delgenes, J.P., R. Moletta and J.M. Navarro. 1986. The effect of aeration on D-xylose fermentation by *Pachysolen tannophilus, Pichia stipitis, Kluyveromyces marxianus* and *Candida shehatae*. *Biotechnol. Lett.* **8**: 897-900.
6. Dellweg H., M. Rizzi, H. Mether and D. Debus. 1984. Xylose fermentation by Yeast: 3. Comparison of *Pachysolen tannophilus* and *Pichia stipitis*. *Biotechnol. Lett.* **6**: 395-400.
7. Du Preeze J.C., M. Bosch and B.A. Prior. 1986. Xylose fermentation by *Candida shehatae* and *Pichia stipitis*: Effects of pH, temperature and substrate concentration. *Enzyme Microb. Technol.* **8**: 360-364.
8. Du Toit, P.J., S.P. Oliver and P.L. Van Biljon. 1984. Sugar cane bagasse as a possible source of fermentable carbohydrates: 1. Characterization of bagasse with regard to monosaccharide, hemicellulose, and amino acid composition. *Biotechnol. Bioeng.* **26**: 1071-1078.
9. Esser, K. and T. Karsch. 1983. Bacterial ethanol production: Advantage and disadvantages. *Process Biochem.* **18**(August): 116-121.
10. Gong, C.S., L.D. McGracken and G.T. Tsao. 1981. Direct fermentation of D-xylose to ethanol by a xylose-fermenting yeast mutant, *Candida* sp. XF217. *Biotechnol. Lett.* **3**: 245-250.
11. Krull, H.L. and G.E. Inglett. 1980. Analysis of neutral carbohydrates in agricultural residues by gas chromatography. *J. Agric. Food Chem.* **26**: 917-919.
12. Ladisch, M.R. and K. Dyck. 1979. Dehydration of ethanol: New approach gives positive energy balance. *Science* **205**: 898-900.
13. Lee, S.H., W.S. Lee and W.G. Bang 1987. Production of ethanol from D-xylose by *Fusarium* sp. *Kor. J. Appl. Microbiol. Bioeng.* **15**: 340-345.
14. Lee, Y.Y., C.M. Lin, T. Johnson and R.P. Chamber. 1978. Selective hydrolysis of hardwood hemicellulose by acids. *Biotechnol. Bioeng. Symp.* **8**: 75-88.
15. Margaritis, A. and P. Bajpai. 1982. Direct fermentation of D-xylose to ethanol by *Kluyveromyces marxianus* strains. *Appl. Environ. Microbiol.* **44**: 1039-1041.
16. Morikawa, Y., S. Takasawa, I. Masunaga and K. Takayama. 1985. Ethanol productions from D-xylose and cellobiose by *Kluyveromyces cellobiovorus*. *Biotechnol. Bioeng.* **27**: 509-513.
17. Neirink, L., R. Maleszka and H. Schneider. 1982. Alcohol production from sugar mixtures by *Pachysolen tannophilus*. *Biotechnol. Bioeng. Symp.* **12**: 161-169.
18. Nigam, J.N., A. Margaritis and M.A. Lanchance. 1985. Aerobic fermentation of D-xylose by *Clavispora* sp. *Appl. Environ. Microbiol.* **50**: 763-766.
19. Rosenberg, S.L. 1980. Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. *Enzyme Microb. Technol.* **2**: 185-193.
20. Schneider H., P.Y. Wang, Y.K. Chan and R. Maleszka. 1981. Conversion of D-xylose into ethanol by the yeast *Pachysolen tannophilus*. *Biotechnol. Lett.* **3**: 89-92.
21. Slininger, P.J., R.J. Bothast, J.E. Van Cauwenberge and C.P. Kurtzman. 1982. Conversion of D-xylose to ethanol by the yeast *Pachysolen tannophilus*. *Biotechnol. Bioeng.* **24**: 371-384.
22. Slininger, P.J., R.J. Bothast, M.R. Okos and M.R. Sadisch. 1985. Comparative evaluation of ethanol production by xylose-fermenting yeasts presented high xylose concentration. *Biotechnol. Lett.* **7**: 431-436.
23. Suihko, M.L. and T.M. Enari. 1981. The production of etha-

nol from D-glucose and D-xylose by different *Fusarium strains. Biotechnol. Lett.* **3**: 723-728.
24. Suihko, M.L. 1983. The fermentation of different carbon sources by *Fusarium oxysporum. Biotechnol. Lett.* **5**: 721-724.
25. Tanaka, M. 1980. Reutilization of cellulosic resources. *Hakko Kogaku* **58**: 145-155.
26. Ueng, P.P. and C.S. Gong. 1982. Ethanol production from pentoses and sugar cane bagasse hemicellulose hydrolysate by *Mucor* and *Fusarium* species. *Enzyme Microb. Technol.* **4**: 169-171.
27. Van der Walt J.P. and D. Yarrow. 1984. Methods for the isolation, maintenance, classification and identification of yeasts, p.45-104. In Kreger-Van Rij N.J.W. (ed.), The yeast: A taxonomic study, Elsevier Publishers B.V., Amsterdam, Netherlands.
28. Wickerham, L.T. 1951. Taxonomy of yeasts. Techn. Bull. No.1029, U.S. department of Agriculture, Washington D.C.
29. Wu, J.F., S.M. Lastik and D.M. Updegraff. 1986. Ethanol production from sugars derived from plant biomass by a novel fungus. *Nature* **321**: 887-888.

(Received August 26, 1993)