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Strain Improvement of *Candida tropicalis* for the Production of Xylitol: Biochemical and Physiological Characterization of Wild-type and Mutant Strain CT-OMV5

Ravella Sreenivas Rao[1,3], Cherukuri Pavana Jyothi[1], Reddy Shetty Prakasham[2], Chaganti Subba Rao[2], Ponnupalli Nageshwara Sarma[2] and Linga Venkateswar Rao[1,*]

[1]*Department of Microbiology, Omania University, Hyderabad - 500 007, India*
[2]*Biochemical and Environmental Engineering Centre, Indian Institute of Chemical Technology, Hyderabad - 500 007, India*
[3]*W113, Centre for Cellular and Molecular Biology (CCMB), Uppal Road, Hyderabad - 500 007, India.*

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***Candida tropicalis* was treated with ultraviolet (UV) rays, and the mutants obtained were screened for xylitol production. One of the mutants, the UV1 produced 0.81g of xylitol per gram of xylose. This was further mutated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and the mutants obtained were screened for xylitol production. One of the mutants (CT-OMV5) produced 0.85g/g of xylitol from xylose. Xylitol production improved to 0.87 g/g of xylose with this strain when the production medium was supplemented with urea. The CT-OMV5 mutant strain differs by 12 tests when compared to the wild-type *Candida tropicalis* strain. The XR activity was higher in mutant CT-OMV5. The distinct difference between the mutant and wild-type strain is the presence of numerous chlamydospores in the mutant. In this investigation, we have demonstrated that mutagenesis was successful in generating a superior xylitol-producing strain, CT-OMV5, and uncovered distinctive biochemical and physiological characteristics of the wild-type and mutant strain, CT-OMV5.**

Keywords: *Candida tropicalis*, biochemical and physiological characterization, MNNG, mutagenesis, strain improvement, UV, xylitol, wild, mutant, yeast

Xylitol is a naturally occurring sugar alcohol that dissolves readily in water. It is as sweet as sucrose and approximately twice as sweet as sorbitol. Additionally it gives a pleasant, cool, fresh sensation due to its high negative heat of solution (Pepper and Olinger, 1988). Xylitol has broad applications, either as a sole sweetener or in conjunction with the other sweeteners in the preparation of wide variety of full and reduced energy, sugarless confectionery products, which are especially suitable for infants and diabetics (Fran Gare, 2003). It also finds its application potential in confectionery products, especially in spices and relishes, jams, jellies, marmalades, and desserts. The most notable property of xylitol is that it is not harmful to the teeth. It is widely used in oral hygiene products and pharmaceuticals to reduce tooth decay and ear infection, especially in children (Pepper and Olinger, 1988; Makinen, 2000; Lynch and Milgrom, 2003). Jannesson *et al.* (2002), studied the effect of the combination of triclosan and xylitol in toothpaste (Colgate Total with the addition of 10% xylitol) on *Streptococcus mutans* in saliva and dental plaque, the researchers concluded that the addition of 10% xylitol to a triclosan-containing dentifrice reduces the number of *Streptococcus mutans* in saliva and dental plaque.

Xylitol is currently produced chemically on a large scale. The chemical method of xylitol production is based on the catalytic hydrogenation of xylose or xylose-rich hemicellulose hydrolysate (Jyri-Pekka *et al.*, 2001). Chemical synthesis requires high temperature, high pressure, and extensive purification of the substrate (Hyoenen *et al.*, 1983). Despite its wide range of applications, the use of xylitol as a sweetener is limited. Its comparatively high production cost, $7 per kg, appears to be responsible for its limited market share. This has led to the development of improved technologies that reduced production costs. The existing drawbacks of conventional xylitol production methods motivated researchers to seek alternative methods of production. Biotechnological production

* To whom correspondence should be addressed.
(Tel) 91-40-27682246; (Fax) 91-40-27019020
(E-mail) vrlinga@yahoo.com, rao_micro@yahoo.com

has, of late, become more lucrative, since the cost of downstream processing has been reduced over time (Whistler and Bemiller, 1993; Kim *et al.*, 1999).

Considerable efforts were made to maximize the microbial production of xylitol from xylose. Several bacteria (Yoshitake *et al.*, 1971; Izumori and Tuzaki, 1988), filamentous fungi (Dahiya, 1991), and yeasts (Gong *et al.*, 1983; Nishio *et al.*, 1989; Meyrial *et al.*, 1991; Vandeska *et al.*, 1996) are known to convert xylose to xylitol. Special attention was paid to the fundamentals of xylose metabolism, especially with respect to the regulation of xylose to xylitol-catalyzing enzyme, as it was a key factor affecting the feasibility of the biotechnological method of xylitol production.

Induced mutagenesis using physical and chemical mutagens seems to be a simple and rational approach to the improvement of yeast strains. The method was used earlier to improve D-xylose fermentation (Lachke and Jeffries, 1986; Jeffries *et al.*, 1993) for an improved ability to utilize xylose (Tantirungkij *et al.*, 1994; Schmiedel *et al.*, 1996; Wahlbom *et al.*, 2003(a,b); Sonderegger and Sauer, 2003) and for Kefir production (Petsas *et al.*, 2002). Mutagenesis on *Candida tropicalis* was also reported for xylose utilization (Suzuki *et al.*, 1991).

In continuation of our earlier efforts for the isolation and characterization of a xylitol-producing *Candida tropicalis* strain (Sreenivas Rao *et al.*, 2004), the present investigation was aimed at further improvement of the strain by simultaneous treatment with UV rays and MNNG mutagenesis and characterization. Characterization of the wild strain also was done; the wild-type strain was then compared with the mutant strain in terms of physiological, morphological, and biochemical properties in addition to xylitol production titers.

Materials and Methods

Microorganism and culture conditions

A yeast strain isolated from a soil sample was used in this study. This strain was identified as *Candida tropicalis* using the CANDIFAST® KIT (International Microbio, Stago Group, France) and further confirmed by IMTECH, Chandigarh, India. The susceptibility of the strain was evaluated using actidione. Using Hi Media carbohydrates the fermentation of different sugars was observed according to a change in the colour of the indicator to yellow due to acidification of the medium. *Candida tropicalis* and the mutant strain, CT-OMV5, were maintained on YM (Yeast extract-Maltose) agar and sub cultured every 4 weeks.

Inoculum preparation and fermentation

Inoculum (25ml) was prepared in 100ml Erlenmeyer flasks, using media consisting (in gram per litre) of: xylose, 30; yeast extract, 10; peptone, 20; glucose, 10; KH_2PO_4, 0.5; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5, pH 5.0. Fermentation was carried out by placing the inoculated flasks on a rotary shaker (250 rpm) at 30°C. After 24 hours, the cells were recovered by centrifugation. All of the experiments were conducted in triplicate, and the average results were reported in this investigation. The observed variation was approximately 2.0 – 2.5%.

Fermentation medium

Fermentation medium (100 ml) was prepared in 250 ml Erlenmeyer flasks with the following media components (in gram per litre): xylose, 30; yeast extract, 5; peptone, 10; glucose, 10; KH_2PO_4, 0.5; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5. Mutated cells (5%, 1.0 OD600nm) were used for inoculation and were incubated for 48 hours at 33°C on a rotary shaker at 250 rpm. When required, different carbon sources (D-mannitol, maltose, ribitol, sucrose, D-galactose, D-glucitol) and nitrogen sources (casein hydrolysate, yeast extract, urea, sodium nitrate, ammonium chloride, ammonium sulfate) were supplemented to the medium to test the xylitol production.

Preparation of cell-free extract

After the yeast cells were grown in YEPX (yeast extract peptone xylose) medium, they were harvested by centrifugation at 5,000 rpm at 4°C for 10 min and washed twice with sterile distilled water. The cell-free extract was prepared by suspension of 20 g of cells (wet weight) in 15 ml of 0.5 M potassium phosphate buffer (pH 7.5), followed by the disruption of cells. Cell disruption was performed in a homogenizer for 15 min with liquid nitrogen in the presence of 0.5 g of acid-washed glass beads (425-600 microns). After disruption, the slurry was centrifuged (12,000 rpm at 4°C for 20 min) to separate out the cell debris; and the supernatant was used for enzyme analysis. The protein concentration was determined by the Lowry method using bovine serum albumin as the standard.

Enzyme assay

Xylose Reductase enzyme (XR) activity was determined spectrophotometrically using a UV-Visible (BECKMAN DU 7400) spectrophotometer. The final reaction mixture contained (in 3 ml) 1.5 ml of 0.1 M potassium phosphate buffer (pH 7.5), 0.3 ml of 0.1 M mercaptoethanol, 0.3 ml of 0.5 M D-Xylose, 0.3 ml of distilled water, 0.3 ml of 3.4 mM NADPH, and 0.3 ml of enzyme. At 30°C, the enzyme assay was followed by measurement of the decrease in optical density at 340 nm over a period of time. One unit of activity was defined as the amount of enzyme oxidizing

1 µmol of NADPH per minute. Specific activity was expressed in units/mg protein.

Scanning electron micrograph (SEM) analysis

In order to conducted SEM analysis, 24-hour-old yeast cells grown in YEPX medium was used. The cells were harvested (5,000 rpm at 4°C for 10 min) by centrifugation, rinsed twice with sterile double-distilled water, and distributed on a 12 mm glass coverslip coated with poly-L-lysine (Sigma Diagnostics). The cells were then fixed for 50 min by incubating in a solution containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer. The cells and glass coverslips were washed twice in 0.1 M Sodium cacodylate buffer and post-fixed using 10% OsO_4 in 0.1 M cacodylate buffer. To improve the surface architecture, post-fixed cells were rinsed thoroughly using 0.1 M cacodylate buffer, and treated with 6% thiocarbohydrazide. The cells were finally washed with double-distilled water and dehydrated through a graded series of ethanol solutions. These were then dried, mounted on aluminium stubs, and sputter-coated with a gold layer (Hitachi HUS-5GB) and used for scanning. Samples were scanned with a Hitachi S-520 scanning electron microscope.

Analytical procedures

Sugar and sugar alcohols in the culture broth were measured by High Performance Liquid Chromatography (HPLC) fitted with an ion-moderated partition chromatography sugar column SHODEX SC 1011 (8mm X 300 mm). The samples were eluted with HPLC grade water at a flow rate of 0.5 ml/min at 80°C and detected with a differential refractometer (WATERS 410).

UV mutagenesis

UV mutagenesis was carried out according to the method of Winston and Ausubel (1990). A culture grown overnight in 5 ml of *Candida tropicalis* culture was washed twice with sterile distilled water and then re-suspended in phosphate buffer (pH 7.0; 0.1 molar concentration) in order to achieve 10^8 cells per ml. Two ml of the above cell suspension was placed in a sterile Petri dish and exposed to UV rays (235 nm) at a distance of 20 cm. At regular intervals, the samples were collected and plated on plates containing YEPX agar medium. Serial dilutions were done using sterile water so that each plate had 200 to 300 viable cells. The plates were then incubated at 33°C for 48 hours.

MNNG mutagenesis

Five ml of UV1 culture was grown overnight and centrifuged to collect the cell mass. The cell mass was washed twice with sterile distilled water. The selected cell mass was then re-suspended in 10 ml phosphate buffer (pH 7.0; 0.1 molar concentration) to obtain10^8 cells per ml. One ml of the above culture was transferred to a sterile microcentrifuge tube. Cells were pelleted in a microcentrifuge for 5-10 seconds at maximum speed. The supernatant was discarded and the cells were resuspended in 1.0 ml of sterile 0.1 M phosphate buffer, pH 7.0. Ten microcentrifuge tubes were prepared by the above method. Two mM stock solution of MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) was prepared by dissolving 2.94 mg of MNNG in 10 ml distilled water. Fifty microlitres of MNNG was added to the cells, dispersed by vortexing, and incubated for different time intervals. Every 15 minutes, the cells were washed with sterile distilled water, and plated on YEPX agar, and incubated at 33°C. After 60 minutes of exposure, this treatment eliminates 40% of the cells when compared with control, (Winston and Ausubel 1990). Several morphologically different colonies were selected and investigated for xylitol production using YEPX medium.

Results

Xylitol production by the wild-type and mutant strains

To obtain a strain with better xylitol yield, the wild organism was treated with UV rays and inoculated on sterilized agar-based plates for colony development. After 24 hours of incubation at 33°C, fifty colonies with different colony morphologies from the wild-type were selected (wild strain showed wrinkles, crepe, and large-sized colony morphology, while colonies selected after mutation had craters, concentric shape, and small-sized colony morphology). The selected colonies were inoculated in 5 ml sterile YEPX medium contained in test tubes and incubated at 33°C and 250 rpm. After 24 hours of incubation, the cells were transferred to 100 ml xylose-containing medium in 250 ml conical flasks and incubated at 33°C and 250 rpm for 48 hours. The cell-free broth was then analyzed for xylitol production and the best performer was designated as UV1. This strain showed higher xylitol production (0.81 g xylitol/g of xylose) than that of the wild strain. To further improve production, the strain (UV1) was mutated with MNNG.

Twenty-five colonies from MNNG plates exposed for different time intervals were selected, based on colony morphology and size. Upon analysis of xylitol production capability, the strain with the highest xylitol yield was selected and repeatedly grown on yeast extract, peptone, and xylose medium (YEPX-medium) in order to assess the consistency. The strain was then designated to be CT-OMV5. Xylitol production studies revealed that this CT-OMV5 showed enhanced xylitol production compared to the UV1 strain. The xylitol yield from one gram of D-xylose under similar

Table 1. Biochemical and physiological characteristics of wild and mutant strain, CT-OMV5

Test	Wild-type	Mutant CT-OMV5
Growth on		
D-Galactose	+	+
L-Sorbose	−	+
D-Ribose	−	−
D-Xylose	+	+
L-Arabinose	−	−
D-Arabinose	−	−
L-Rhamnose	−	−
Sucrose	+	+
Maltose	+	+
Alpha.alpha-Trehalose	+	+
Me alpha-D-Glucoside	+	+
Cellobiose	+	−
Salicin	+	−
Arbutin	−	−
Melibiose	ND	−
Lactose	−	−
Raffinose	−	−
Melezitose	+	+
Inulin	−	−
Starch	+	−
Glycerol	−	−
Erythritol	−	−
Ribitol	+	+
Xylitol	−	−
L-Arabinitol	−	−
D-Glucitol	+	+
D-Mannitol	+	+
Galactitol	−	−
Myo-Inositol	−	−
D-Glucono-1,5-lactone	+	−
2-keto-D-Gluconate	+	−
D-Gluconate	+	−
D-Glucuronate	−	−
DL-Lactate	+	−
Succinate	+	−
Citrate	−	−
Methanol	−	ND
Ethanol	+	ND
Growth at		
25°C	+	+
30°C	+	+
37°C	+	+
42°C	+	+

Test	Wild-type	Mutant CT-OMV5
Growth on		
0.01% Cycloheximide	−	−
0.1% Cycloheximide	−	−
50% D-Glucose	+	+
60% D-Glucose	+	−
Production of		
Acetic acid	−	−
Diazonium Blue B reaction	−	−
Physiological		
Pink colonies	−	−
Budding cells	−	+
Splitting cells	−	−
Filamentous	+	+
Pseudohyphae	−	−
Septate hyphae	+	+
Arthroconidia	−	−
Ballistoconidia	+	+
Chlamydospores	−	+
Ascospores	−	−
Fermentation of		
D-Galactose	+	ND
Maltose	+	ND
Me alpha-D-Glucoside	−	ND
Sucrose	+	ND
Alpha.alpha-Trehalose	+	ND
Melibiose	−	ND
Lactose	−	ND
Cellobiose	−	ND
Raffinose	−	ND
Inulin	−	ND
Starch	−	ND
XR specific activity	9.74 U/mg protein	12.03 U/mg protein

fermentation conditions was found to be 0.85, 0.81, and 0.77 g/g xylose with the mutant (CT-OMV5), UV1, and wild strain, respectively. Therefore, further characterization of CT-OMV5 and wild strains with respect to physiological and biochemical parameters was undertaken and is noted in Table 1.

Difference between the wild and mutant strain of Candida tropicalis OMV-5

The biochemical and physiological characteristics given in Table 1 indicate that these strains, wild-type and CT-OMV5, belong to the members of the genus, *Candida*, and show more similarity to *Candida tropicalis*. Based on morphological (Fig. 1) and physiological characterization (Table 1), there were five discrepancies noticed in tests between the isolate and the standard *Candida tropicalis* reference strain. This could be due to strain variation occurring during mutation. Mutant strain CT-OMV5 differed from the reference *Candida tropicalis* in 8 tests, and differed from the wild-type in 12 tests, thereby indicating the influence of mutagenesis. The distinct difference between the mutant and the wild strain was the presence of numerous chlamydospores (Fig. 1 - D) in the mutant strain. Table 2 summarizes the differences between the wild and mutant (CT-OMV5) strains of *Candida tropicalis*.

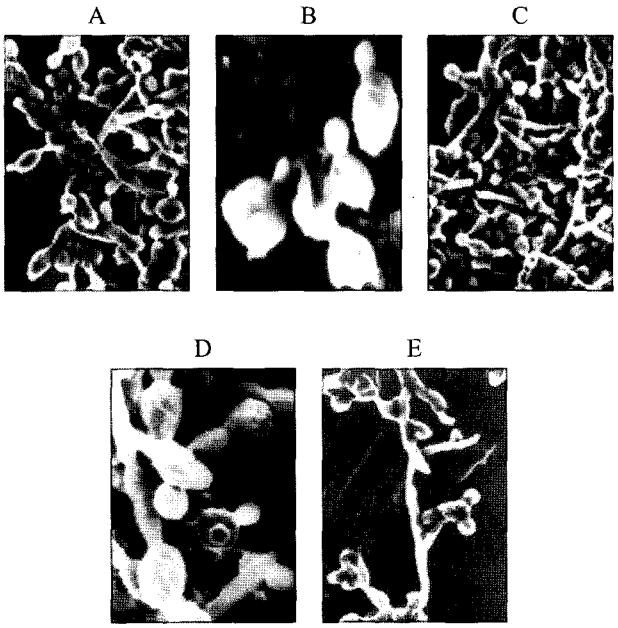

Fig. 1. Scanning electron microscopy, photographs of mutant (A-D) and wild (E) yeast strains.
A, mutant strain showing filamentous growth and budding cells. B, budding cells of the mutant strain. C, mutant strain with filamentous growth, budding cells, and chlamydospores. D, chlamydospores of the mutant strain. E, wild strain with septate hyphae.

Influence of different carbon sources on biomass and xylitol production by wild and mutant (CT-OMV5) strains

The impact of different carbon sources on biomass and xylitol production by the wild-type strain and mutant strain, CT-OMV5, was studied by inoculating the 5 ml of 1.0 OD(600 nm) culture in 100 ml YEPX-medium supplemented with 0.5% different carbon sources. After 48 hours of incubation at 250 rpm at 33°C, the contents of the flasks were individually centrifuged and analyzed for biomass and xylitol production. The biomass and xylitol production data of the wild-type and mutant, CT-OMV5, under various carbon-supplemented conditions are given in Table 3. It was clear from this data that the wild-type strain showed no improvement in xylitol yield, but the biomass increased when the medium was supplemented with carbon sources, whereas the mutant strain produced more xylitol than the wild-type strain

Table 2. Difference between the wild and mutant strains of *Candida tropicalis* OMV-5

S. No.	Difference test	Wild-type	Mutant
1	Starch growth	+	−
2	D-Glucono-1, 5-lactone growth	+	−
3	2-keto-D-Gluconate growth	+	−
4	Succinate growth	+	−
5	60% D-Glucose growth	+	−
6	Budding cells	Few	Numerous
7	Chlamydospores	−	Numerous
8	L-Sorbose growth	−	+
9	Cellobiose growth	+	−
10	Salicin growth	+	−
11	D-Gluconate growth	+	−
12	DL-Lactate growth	+	−

Table 3. Influence of different carbon sources on biomass and xylitol production by wild-type and mutant (CT-OMV5) strains

S. No.	Supplemented Carbon source	Biomass production (g/l)		Xylitol production (g/g xylose)	
		WT	Mutant	WT	Mutant
1	Control (YEPX medium)	6.44	5.50	0.77	0.85
2	D-Mannitol	6.36	7.50	0.74	0.70
3	Maltose	7.50	6.62	0.64	0.77
4	Ribitol	7.62	7.82	0.68	0.73
5	Sucrose	7.51	6.50	0.64	0.71
6	D-Galactose	6.84	6.35	0.69	0.77
7	D-Glucitol	7.45	6.25	0.68	0.77

with less biomass, indicating an improvement of specific productivity.

Effect of different nitrogen sources on biomass and xylitol production by wild-type and mutant (CT-OMV5) strains

The role of different nitrogen sources on the biomass and xylitol production of the wild-type and mutant strains was studied by supplementing YEPX-medium with 0.5% different nitrogen sources. After 48 hours of incubation at 250 rpm at 33°C, the contents of the flasks were individually centrifuged and analyzed for biomass and xylitol production. The biomass and xylitol production capabilities of the wild and mutant CT-OMV5 strains under various nitrogen-supplemented conditions are shown in Table 4.

Significant variations in biomass and xylitol production parameters were noticed between the mutant and wild-type strains. The addition of yeast extract increased biomass production in the wild-type strain. Similarly, supplementation of inorganic nitrogen sources (ammonium chloride and ammonium sulfate) in the fermentation medium enhanced biomass and xylitol production in the mutant strain compared to that of the wild-type. A maximum improvement (0.87 g/g of xylose) in xylitol yield with the mutant strain observed when the medium was supplemented with urea, but no improvement in biomass was observed when compared to the control.

To further evaluate the relationship, the first enzyme involved in xylose to xylitol conversion, xylose reductase, in the wild and mutant strains was estimated after growing both strains in similar fermentation environments. The enzyme analysis data revealed that, xylitol reductase activity was greater in the mutant strain as compared to the wild-type. Such data indicated that the mutant strain has more potential for xylitol production.

Table 4. Effect of different nitrogen sources on biomass and xylitol production by wild and mutant (CT-OMV5) strains

S. No.	Supplemented Nitrogen source	Biomass production (g/l)		Xylitol production (g/g xylose)	
		WT	Mutant	WT	Mutant
1	Control (YEPX medium)	6.44	5.50	0.77	0.85
2	Casein hydrolysate	7.24	7.26	0.65	0.76
3	Yeast extract	7.55	6.14	0.66	0.79
4	Urea	5.32	5.46	0.68	0.87
5	Sodium nitrate	5.12	5.26	0.49	0.55
6	Ammonium chloride	6.64	7.84	0.65	0.70
7	Ammonium sulfate	5.28	6.68	0.60	0.72

Discussion

In the present study, physical and chemical mutagenesis was used to improve the xylitol production of a *Candida tropicalis* strain. Phenotypic characterization was done for both the wild and mutants strains.

The distinct difference between the mutant and wild strains is the presence of numerous chlamydospores and budding cells in the mutant strain. Mutagenesis has affected the properties of the mutant strain, CT-OMV5. When Suzuki *et al.* (1991) irradiated the asexual yeast with UV rays, variations in colony morphology and chromosomal rearrangements were observed in *Candida tropicalis* pK233. In another study, Mahmoud (1999) demonstrated that large doses of ethyl methane sulphonate greatly increased the induction of auxotrophic mutants in *Candida tropicalis*. The maximum yield of biomass and protein was recorded in some mutants isolated after EMS treatment. Recently, Sonderegger and Sauer (2003) reported multiple mutations with EMS, which, helped to shift the metabolism of D-xylose from aerobic to anaerobic metabolism. They claimed that the yeast strain, which they had grown under strictly anaerobic conditions on xylose as the sole carbon source, was the first strain. These studies indicate that physical and chemical mutagenesis helped to improve the yeasts, D-xylose metabolism was especially important.

In the literature, the xylitol yield has been reported to range from 0.62 to 0.87 g/g of xylose depending on the microbial system. Working with *Candida mogii,* Sirisansaneeyakul *et al.* (1995) reported a xylitol yield of 0.62 g per gram of xylose. In another study, the xylitol yield was found to be 0.80g per gram of xylose with *Candida guilliermondii* (Preziosi-Belloy *et al.*, 2000). Lu *et al.* (1995) reported a xylitol yield of 0.87 g per gram of xylose with *Candida* sp. L-102 strain. Working with *Candida tropicalis*, Kim *et al.* (2002) reported only 0.75 g per gram of xylose. It was observed in the present investigation that the xylitol yield was found to be improved from 0.77 g/g of xylose with wild *Candida tropicalis* (Sreenivas Rao *et al.*, 2004), to 0.87 g/g of xylose with mutant, CT-OMV5, indicating an increase of 10% with the mutant strain.

The first enzyme in xylose metabolism is xylose reductase (XR), which converts xylose to xylitol. An improved XR specific activity (9.74 to 12.03 U/mg) was observed in the mutant strain. Jeffries *et al.* (1993) obtained mutants of *Pichia stipitis* that produced up to 55% more ethanol than the wild strain on xylose-contaning medium. In their study, another mutant of *Candida shehatae* was obtained that fermented

xylose more rapidly than the wild strain. Increased XR activity was noted with these strains. Working with a mutant of *Pachysolen tannophilus*, Y-2460, Lachke and Jeffries (1986) observed that the levels of oxidoreductive enzymes involved in converting D-xylose to D-xylulose via xylitol were 1.5-14.7 fold higher than those in the wild-type starin. An increase in the specific activity of XR of about 1.5-5.2 fold was observed in this mutant. All of these studies reveal the imperative role of mutations in improving D-xylose metabolism and xylitol production.

Biochemical studies of xylose reductase (XR) levels in *Candida shehatae* grown on several different carbon and nitrogen sources showed that urea, peptone, and most other organic nitrogen sources induce these enzymes. Paul *et al.* (1988) also reported the induction of XR activity in *Pachysolen tannophilus* and *Pichia stipitis* on xylose medium supplemented with different carbon sources. In the present investigation, the supplementation of different carbon sources has not helped to improve the xylitol yield, but improvements in biomass have been observed. Among the various nitrogen sources supplemented, urea enhanced the xylitol production (0.87 g/g of D-xylose) with the mutant, CT-OMV5. Lu *et al.* (1995) tested *Candida sp.* L-102 for xylitol production from different nitrogen sources; they reported that urea and glycine gave the highest average specific productivity, and ammonium phosphate gave the highest yield. When Suryadi *et al.* (2000) tested 11 nitrogen sources for xylitol production, urea, $(NH_4)_2SO_4$, and NH_4NO_3 gave the highest yields of xylitol (0.55 g/g of xylose) with *Hansenula polymorpha.* Paltnitkar and Lachke (1992) also reported improved xylitol yield with organic nitrogen sources and more XR activity in the medium supplemented with urea, asparagines, or peptone.

Based on the results obtained, it can be concluded that an increase in xylitol production can be achieved with a mutant strain (CT-OMV5) of *Candida tropicalis* obtained by the treatment with UV rays and MNNG, the resultant mutant (CT-OMV5) strain will be more useful for xylitol production. These results are in agreement with the reported literature on xylitol production by *Candida* sp. (Sirisansaneeykul *et al.*, 1995; Preziosi-Belloy *et al.*, 2000; Kim *et al.*, 2002).

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