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An Isolated *Candida albicans* TL3 Capable of Degrading Phenol at Large Concentration

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An isolated yeast strain was grown aerobically on phenol as a sole carbon source up to 24 mM; the rate of degradation of phenol at 30 °C was greater than other microorganisms at the comparable phenol concentrations. This microorganism was further identified and is designated *Candida albicans* TL3. The catabolic activity of *C. albicans* TL3 for degradation of phenol was evaluated with the K_s and V_{max} values of 1.7 ± 0.1 mM and 0.66 ± 0.02 μmol/min/mg of protein, respectively. With application of enzymatic, chromatographic and mass-spectrometric analyses, we confirmed that catechol and *cis,cis*-muconic acid were produced during the biodegradation of phenol performed by *C. albicans* TL3, indicating the occurrence of an ortho-fission pathway. The maximum activity of phenol hydroxylase and catechol-1,2-dioxygenase were induced when this strain grew in phenol culture media at 22 mM and 10 mM, respectively. In addition to phenol, *C. albicans* TL3 was effective in degrading formaldehyde, which is another major pollutant in waste water from a factory producing phenolic resin. The promising result from the bio-treatment of such factory effluent makes *Candida albicans* TL3 be a potentially useful strain for industrial application.

Key words: phenol degradation; phenol hydroxylase; catechol-1,2-dioxygenase; catechol; *cis,cis*-muconic acid

Phenol and phenolic compounds, hazardous pollutants in the environment throughout the last century, are present in effluents from many industries including petrochemical, coal-gasification, ceramic, pharmaceutical and dye manufacturing industries.[1] Physical and chemical methods to treat organic compounds require many processing steps, which are costly and typically produce also other toxic end products.[2] Biodegradation has been considered to be a highly effective method of decontamination of a fouled environment. The remarkable ability of microbes to decompose chemicals not only is useful in pollution remediation but also serves as a prospective tool to detect pollutants.

Many microorganisms, including bacteria,[3–12] yeasts,[13–17] algae[18] and aquatic fungi,[19] are found to be capable of degrading phenol at various concentrations. Since 1985, the catabolism of aromatic hydrocarbons by microbes has been widely discussed. The typical pathway of phenol degradation in microorganisms occurs *via* the formation of catechol derivative following by ring cleavage through the ortho-fission or meta-fission pathway.[20] Both pathways commonly use phenol hydroxylase, a monooxygenase, as catalyst at first step of degradation. However, they are different in the second degradation step (Fig. 1). In general, yeasts and bacteria utilize catechol-1,2-dioxygenase and 2,3-dioxygenase, corresponding to the ortho-fission and the meta-fission pathway, respectively, to perform the oxidative reaction of catechol.[21,22] Several reports stated that certain soil yeasts possess a great inductive capacity for degradation of diverse aromatic compounds of small molar mass,[23,24] but, based on currently available literature, we found that yeasts were less commonly reported. We are interested in how to isolate a naturally occurring yeast possessing a capacity to effectively degrade phenol at higher concentration for use as a prospective microorganism in waste water or treatment of soil. In this work, we characterized the capacity of an isolated strain, *Candida albicans*, to degrade not only phenol but also formaldehyde, another toxic pollutant. Although many microorganisms exhibit a catabolic power on formaldehyde,[25–28] a strain with effective operation on both toxic ingredients has been rarely reported. We discuss here the phenol catabolic pathway of *C. albican* that we investigated, and also evaluated the efficiency of *C. albicans* TL3 to treat waste water from a factory producing phenolic resin.

Materials and Methods

Chemicals. Chemicals (Sigma, St. Louis, MO, U.S.A., or Merck, Darmstadt, Germany), Yeast Nitrogen Base

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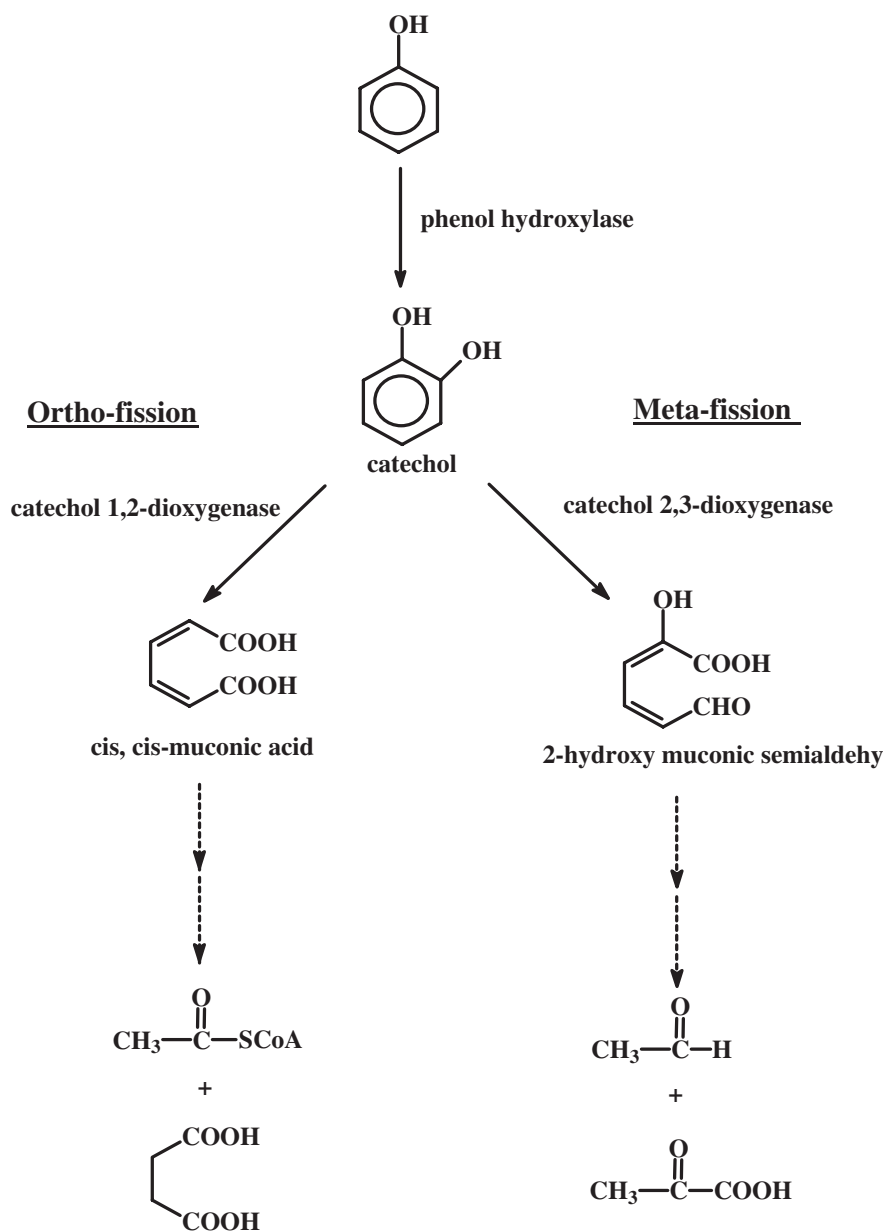
Fig. 1. Two Common Phenol Degradation Pathways, the Ortho- and Meta-Fission, Occur in Microorganisms.

(YNB) without amino acids (Difco, Detroit, MI, U.S.A.), a glucose oxidase dehydrogenase/phenol 4-aminoanti-pyrine (GOD–PAP) assay kit (Randox, Antrim, UK) were obtained from the indicated suppliers. All other chemicals used were of pure and reagent grades.

Media formulation and microorganism screening. The microorganism used in our work was isolated from the soil sample of a petrochemical plant in Taiwan. Soil sample was stirred for 2 h at room temperature in sterile distilled water. 5 ml of the above sample was inoculated in 150 ml of YNB medium (0.67% w/v) containing various phenol concentrations (10, 12, 14, 16, and 18 mM). After 2 weeks of cultivation (150 rpm, 30 °C), 0.1 ml of the resulting culture medium (OD$_{600}$ of 1.65) was subjected to spreading on agar plates containing the

same YNB medium with 15 mM phenol. Strains were obtained after purification by sequential cultures. A powerful phenol-degradation strain was identified as *Candida albicans* by CBS (Centralbureau voor Schimmelcultures, Boarn, Netherlands) based on its morphology, physiological and biochemical characteristics. To investigate a carbon source, we directly added a suitable amount of phenol, glucose, or *cis,cis*-muconic acid to a YNB culture medium (0.67%). All resulting media were sterilized by ultra-filtration before use. Unless otherwise stated, all flask cultures were incubated in a rotary shaker (150 rpm) at 30 °C.

Cell growth and Phenol degradation. The isolated strain was cultured at 30 °C in flask containing YNB medium (50 ml, 0.67%) with phenol at various concen-

trations (0–24 mM) and/or glucose (0.2%, w/v). To examine the prospective application of this microbe, we used waste water obtained from a local manufacturer of phenolic resin (Chang Chun Plastics Co., Ltd., Taiwan) as a carbon source and added it directly to a YNB culture medium (0.67%). We withdrew samples from the cultures at various intervals, and estimated the cell density by measurement of the absorbance of the sample at 600 nm using a UV–Vis spectrophotometer (Shimadzu, UV-1601) and with a colorimetric method, using 4-aminoantipyrene to estimate the residual phenol in the culture medium.[29] The residual glucose and formaldehyde were determined by GOD–PAP assay and Hantzsch reaction,[30] respectively. All cultures were cultivated with an initial cell density 0.02 (OD_{600}) per milliliter of medium in triplicate; the mean values are reported, for which values of standard error were less than 10%.

For measuring the kinetic parameters of the whole-cell, different phenol concentrations (0.5–5 mM) were prepared in 1 ml culture medium (50 mM Tris, pH 7.0) containing the final cell density (stationary phase) of OD_{600} of 1. The resulting mixture was incubated at 30 °C with an agitation rate of 150 rpm. The residual phenol concentration was monitored after the inoculation of microorganism, with which the initial rate of phenol degradation can be obtained. Data were analyzed by non-linear regression (Grafit program) using the Haldane's equation[31] shown as below.

$$\text{v} = [V_{\max} \cdot (S)]/[(S) + K_s + (S)^2/K_I]$$

Where v is the initial rate of degradation, (S) is the phenol concentration, K_s is the half-saturation constant (equivalent to K_m in enzyme kinetics), K_I is the inhibition constant, and $V_{\max}$ is the theoretical maximum degradation rate.

Enzyme activity assays. A cell culture (50 ml) was harvested from YNB medium (0.67%) containing phenol at various concentrations when the growth of *C. albicans* TL3 approached a stationary phase. After centrifugation, a cell pellet were washed twice with distilled water (2 ml) and then resuspended in the assay buffer (0.5 ml, 50-mM potassium phosphate buffer, pH 7.6, containing 1-mM β-mercaptoethanol, 0.1-mM EDTA and 10-μM FAD). After disruption by sonication, the cell debris was removed by centrifugation at 13700 *g* for 30 min at 4 °C. The supernatant (crude enzyme extract) was used for assay of enzymatic activity, metabolite preparation and determination of protein concentration. Phenol hydroxylase (EC 1.14.13.7) activity was assayed spectrophotometrically on monitoring the disappearance of NADPH in absorbance at 340 nm.[32] Catechol-1,2-dioxygenase (EC 1.14.13.1) and catechol-2,3-dioxygenase (EC 1.13.11.2) activities were assayed by determining the rate of accumulation of *cis,cis*-muconic acid (absorbance increase at 260 nm)[33] and 2-hydroxymuconic semialdehyde (absorbance in-

crease at 375 nm),[34] respectively. Control reactions (without substrate or crude enzyme extract) were performed for each assay. One unit of phenol hydroxylase is defined as the amount of enzyme that catalyzes the disappearance of NADPH at 1 μmol min^{-1}. One unit of catechol-1,2-dioxygenase is defined as the amount of enzyme that catalyzes the formation of *cis,cis*-muconic acid at 1 μmol min^{-1}. The concentration of protein was determined with a protein assay kit (Bio-Rad) with bovine serum albumin as standard; the specific activity is defined in units per mg protein.

Product analysis and identification. To prepare the metabolites, we added crude enzyme extract (described in the previous section, 30 μl) to reaction mixture (970 μl, 50-mM potassium phosphate buffer, pH 7.6, containing 170-μM phenol, 1-mM β-mercaptoethanol, 0.1-mM EDTA, 10-μM FAD and 170-μM NADPH). The resulting mixture was incubated for 10 or 25 min at 25 °C. The reaction was terminated on addition of methanol (1 ml). After removing the protein precipitant by centrifugation, we analyzed the supernatant by HPLC. The suspected product fractions were collected and used for GC–mass analysis. For analysis by HPLC (Lab Alliance series 4 pump, detector: GL Sciences UV-620) we used a reversed phase column (Waters, *u* Bondapak C_{18}, 3.9 × 300 mm; mobile phase, methanol: 1% acetic acid = 80:20 v/v), at a flow rate 1 ml min^{-1}, and monitored the compounds of the supernatant at 260 nm. To prepare the enzymatic products of catechol-1,2-dioxygenase, we added crude enzyme extract (10 μl) to reaction mixture (990 μl, 50-mM Tris–HCl buffer, pH 8.3, containing 5-mM β-mercaptoethanol, 20-μM $FeSO_4$, and 1-mM catechol) and incubated for 2 or 10 min at 25 °C. The reaction was terminated on addition of methanol (1 ml). The resulting protein precipitant was removed by centrifugation. We analyzed the supernatant by ion chromatography (IC), and collected the product fractions for use in LC mass and LC tandem mass-spectrometric analysis. We performed ion-chromatographic analysis (DX-500 with *Gp40* gradient pump) on a column (DIONEX, Ion Pac Asll, 4 × 250 mm; mobile phase, NaOH solution 0.01 M, flow rate 1.5 ml min^{-1}) and monitored the conductivity of compound with an electrochemical detector (ED40) and an anion self-regenerating suppressor set at 50 mA.

Mass-spectrometric analysis. For GC–MS (ion trap detector, Perkin-Elmer Instruments, Turbo Mass Gold Mass spectrometer, with a MDN-5 ms 30-mm column, inner diameter 0.25 mm, film thickness 0.25 μm, MDN, Supelco, PA, U.S.A.), the analytical conditions were 70 °C for 2 min, 70–250 °C at 20 °C per min and 250 °C for 5 min. The temperatures of the injector, ion source and transfer lines were 250 °C, 200 °C and 220 °C, respectively. The mass spectra were recorded on a time-of-flight mass spectrometer (Q-TOF Micromass) in the electrospray (−) mode. The mass analyzer was scanned

Table 1. Capability of Complete Degradation of Phenol by Various Yeasts

Strain	Limit of phenol degradation (mM)	Reference
Trichosporon cutaneum	< 5.3	17
Rhodotorula glutinis	5	35
Trichosporon dulcitum	15	3
Basidiomycetous yeast	15	3
Candida tropicalis	16	15
Trichosporon cutaneum sp. LE3	18	14
Candida maltosa	18	13
Candida tropicalis mutant	22	36
Candida albicans TL3	24	this work

over a ratio m/z of mass to charge 50–500 u, with a scan step 2 s and an inter scan 0.1 s/step. The source block temperature and desolvation temperatures were set at 80 °C and 150 °C, respectively. The rate of flow of the delivery solvent (10% acetonitrile containing 0.1% aqueous NH_3) was 1 ml min^{-1}.

Results and Discussion

Identification of the isolated strain and its tolerance against phenol

Because of the industrial demand of phenol degradation, many microbes have been screened. Potentially useful strains might be subjected to random mutagenesis to improve the degrading power.[6,36] Table 1 summarizes the capability of various yeasts to degrade phenol completely.[3,13–15,17,35,36] Most native yeasts can tolerate phenol at a concentration no greater than 18 mM. With much effort in mutagenesis, *Candida tropicalis* was improved to accept a phenol concentration up to 22 mM.[36] Using phenol enrichment, we screened a few microbial strains with varied ability to utilize phenol as a carbon source. Among them, *Candida albicans* was identified (further designated TL3) and evaluated as having the greatest potential to degrade phenol. Without further mutagenic treatment, *Candida albicans* TL3 tolerates phenol up to 24 mM (∼2400 ppm). This record is not only the greatest among yeasts but also greater than most bacteria isolated in the environment, according to current literature.[3,6,15,37] The ability of *C. albicans* to degrade phenol that we discovered is first reported here.

In addition to phenol, potential metabolites such as catechol and *cis,cis*-muconic acid were tested as a source of carbon for *C. albicans* TL3. The results show that catechol was effectively digested, but *cis,cis*-muconic acid remained intact.

Cell growth and phenol degradation

To understand further the catabolic property of *C. albicans* TL3, we investigated the microbial growth with various carbon sources. Figure 2 shows the temporal course of replicated biomass population and the simultaneous phenol degradation by *C. albicans*

TL3 in a liquid medium with phenol and/or glucose at various concentrations. As shown in Fig. 2(a), increasing the concentration (5–24 mM) of phenol in the culture medium evidently produced a prolonged lag for *C. albicans* TL3 to adapt to the growth environment and consequently resulted in a delay to attain the stationary phase of cell growth. The cell growth approached the stationary phase within four days with the amended substrates, except for the case of phenol (24 mM), for which incubation for ten days was required. The temporal course of substrate degradation [Fig. 2(b)] correlated well with cell growth. At the stationary phase, the biodegradation of phenol was nearly complete (>99%). When glucose (0.2%) was used as the sole nutrient, *C. albicans* TL3 grew rapidly with a log-phase feature, whereas in a mixed substrate system (15-mM phenol + 0.2% glucose) the cell grew with a two-stage feature, shown as Fig. 2(a). Glucose was consumed rapidly in the first stage of cell growth, whereas, phenol, serving as nutrient in the second growth stage, was nearly unchanged in the first stage [Fig. 2(b)]. Although *C. albicans* TL3 can use phenol as a source of energy, the presence of phenol could inhibit cell growth in a glucose medium [*c.f.* the growth curves of 0.2% glucose with and without phenol in Fig. 2(a)]. Based on the rate of substrate disappearance, we conclude that *C. albicans* TL3 favors utilization of glucose over phenol when both ingredients exist together. The effects of glucose on phenol degradation were, however, dependent on the inoculated microbial strains. For instance, the preference for glucose metabolism of *C. albicans* TL3 was similar to that of *Trichosporon cutaneum* sp. LE3,[14] but different from that of another strain of *T. cutaneum*[38] and *C. maltosa*.[39] As discussed in the literature,[40] the presence of glucose and thereafter its metabolites might inhibit *C. albicans* TL3 from the transport of phenol or the synthesis of such a transport system. Most mesophilic yeasts that are highly active in degrading phenol, such as *Candida tropicalis*,[15] *Candida maltosa*[13] and *Trichosporon* spp.[14] are reported to degrade phenol (16–18 mM) already within 5–6 days at 30 °C, but *C. albicans* TL3 needed only three days to consume phenol (20 mM) at the same temperature [Fig. 2(b)]. Some mesophilic bacteria with great activity to degrade phenol, such as *Burkholderia cepacia* PW3 and *Pseudomonas aeruginosa* AT2[5] were reported to degrade phenol (22 mM) within 11–15 days at 30 °C, but *C. albicans* TL3 required only four days at the same temperature and concentration of phenol [Fig. 2(b)]. These results show that the rate of degradation of phenol by *C. albicans* TL3 is clearly greater than that of other microorganisms effective in degrading phenol.

The kinetic property of phenol degradation catalyzed by *C. albicans* TL3 gave a K_s and a V_{max} value of 1.7 ± 0.1 mM and 0.66 ± 0.02 μmol/min/mg of protein, respectively. The K_s and V_{max} values are higher than those for several *Pseudomonas* strains[5,31] and *Burkholderia cepacia*.[5] Phenol also exhibited a weak inhibition
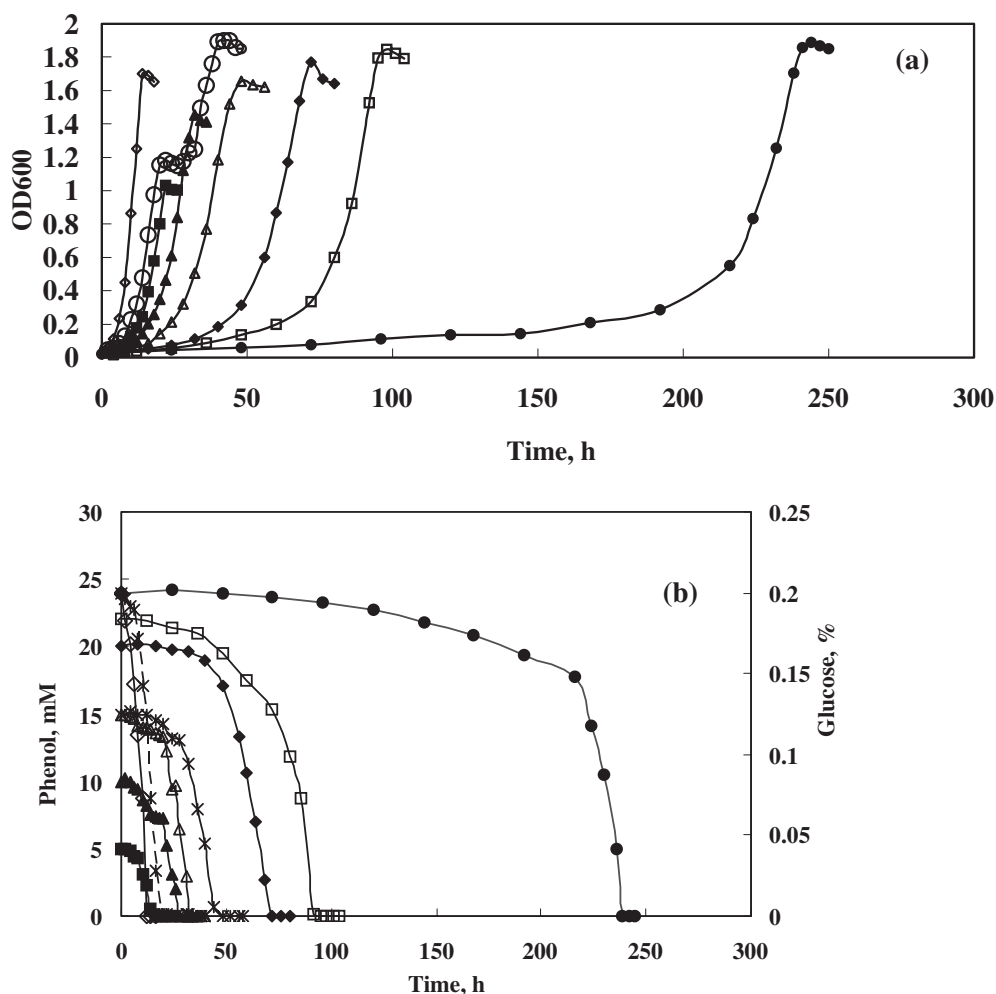
Fig. 2. Time-Course Profiles of Cell Growth (a) and Consumption of Phenol and Glucose (b) of *Candida albicans* TL3.

The cells were incubated at 30 °C with YNB medium (0.67%) containing phenol at various concentrations: 5 mM (-■-); 10 mM (-▲-); 15 mM (-△-); 20 mM (-◆-); 22 mM (-⊞-); 24 mM (-●-), 0.2% glucose (-◇-), and the mixture of 15-mM phenol + 0.2% glucose (-⊖-), and the residual concentrations of phenol (-✕-) and glucose (-✕-) in the mixture medium. Each data point represents the mean of triplicate independent measurements. Data shown are the mean of triplicate experiments with standard deviation within 10%.

with a K_I value of 40 ± 8 mM, which is much higher than those of *Pseudomonas* strains[5,31)] and *Burkholderia cepacia* ranging from 1–17.8 mM.[5)]

Effect of temperature on the growth of C. albicans TL3

The growth of *C. albicans* TL3 in phenol (15 mM) at 25, 30, 35 and 40 °C was monitored (data not shown). After incubation (48 h), the cell cultures at 30 and 35 °C approached a stationary phase, whereas the cell density of cultures at 25 and 40 °C were only approximately 33% and 7% of that at 30 °C (or 35 °C), respectively. The cell growth temperature was therefore controlled at 30 °C for further enzymatic tests.

Characterization of the pathway of phenol degradation by C. albicans TL3

To assay the activity of phenol-degrading enzyme, we prepared the crude enzyme extract by ultra-sonication of the *C. albicans* TL3 cell cultivated to the stationary

phase with phenol at various concentrations. The specific activities of phenol hydroxylase and catechol-1,2-dioxygenase of the crude enzyme extract were examined and are summarized in Table 2. In general, significant activities of phenol hydroxylase and catechol-1,2-dioxygenase are detectable except the case of using glucose (0.2%) alone as nutrient. The activity of catechol-2,3-dioxygenase was not detected throughout these tests. Although the strain required phenol as an inducer to initiate the biosyntheses of phenol hydroxylase and catechol-1,2-dioxygenase, phenol at a greater concentration suppressed the biosynthesis of both enzymes. For instance, phenol hydroxylase and catechol-1,2-dioxygenase activity began to decline when phenol was employed at concentrations >22 mM and >10 mM, respectively. When the strain was grown in a medium containing phenol (15 mM) and glucose (0.1%, 0.2% and 0.4%), the specific activities of phenol hydroxylase were approximately 70, 34 and 17% of that of the culture in medium with phenol as sole carbon

Table 2. Comparison of Enzyme Specific Activity of *Candida albicans* TL3*

Source of carbon	Specific activity (unit/mg protein)	
	phenol hydroxylase	catechol-1,2-dioxygenase
glucose, 0.2%	0	0
phenol, 5 mM	0.025 ± 0.0008	0.270 ± 0.01
phenol, 10 mM	0.044 ± 0.0021	0.734 ± 0.037
phenol, 15 mM	0.083 ± 0.0046	0.217 ± 0.019
phenol, 15 mM + glucose, 0.1%	0.057 ± 0.0027	0.174 ± 0.008
phenol, 15 mM + glucose, 0.2%	0.028 ± 0.0022	0.149 ± 0.012
phenol, 15 mM + glucose, 0.4%	0.014 ± 0.0018	0.128 ± 0.007
phenol, 20 mM	0.115 ± 0.0063	0.107 ± 0.009
phenol, 22 mM	0.156 ± 0.011	0.101 ± 0.005
phenol, 24 mM	0.129 ± 0.0045	0.087 ± 0.006

*Growth conditions: YNB medium (0.67%) containing phenol and/or glucose, at 30 °C. Data are expressed as mean ± standard deviation ($n = 3$).

Table 3. Effect of Temperature on Specific Enzyme Activity of *Candida albicans* TL3*

Growth temperature (°C)	specific activity (unit/mg protein)	
	phenol hydroxylase	catechol-1,2-dioxygenase
25	0.087 ± 0.0041	0.275 ± 0.012
30	0.083 ± 0.0046	0.217 ± 0.019
35	0.064 ± 0.0037	0.158 ± 0.007
40	0.061 ± 0.002	0.100 ± 0.003

*The microbe was grown with YNB medium (0.67%) containing phenol (15 mM). Data are expressed as mean ± standard deviation ($n = 3$).

source, respectively. Similarly, the specific activities of catechol-1,2-dioxygenase were 1.25, 1.46 and 1.70-fold smaller in a phenol medium containing glucose at 0.1%, 0.2% and 0.4%, compared with that without glucose. The biosynthetic paths of phenol hydroxylase and catechol-1,2-dioxygenase in this microbe are hence likely modulated by one or more additional regulatory mechanisms such as catabolite repression.

The effect of temperature on enzyme induction was also investigated. The activities of phenol hydroxylase and catechol-1,2-dioxygenase of *C. albicans* TL3 cultivated at temperatures 25, 30, 35 and 40 °C are summarized in Table 3. A trend is observable that a lower temperature seemed to be favorable to induce both enzyme activities. The optimal growth temperature for *C. albicans* TL3 in phenol was near 30–35 °C (data not shown), but both enzyme activities decreased inversely with growth temperature. As the temperature factor on the rate of cell growth and the induction of phenol hydroxylase and catechol-1,2-dioxygenase were discordant, both enzymes were unlikely to play a major role in rate of growth of *C. albicans* TL3.

To identify the phenol metabolic pathway in *C. albicans* TL3, we isolated the metabolites of phenol and analyzed them with a mass spectrometer. Figure 3 shows overlaid HPLC chromatograms with samples having various compositions (such as NADPH and phenol) and enzymatic product(s). The chromatograms labeled a, b and c in Fig. 3 exhibited the analytical outcomes of samples without both NADPH and phenol,

with NADPH, and with phenol, respectively. Discernible in Fig. 3(d–f), an additional feature with a retention period 3.1 min evolved when both NADPH and phenol were added in the assay solution. This feature is suggested to be catechol, according to spiking catechol (0.02 mM) in the sample (chromatogram e in Fig. 3). The area of the feature for a sample incubated 25 min (chromatogram f) is about triple that of a sample incubated for 10 min. To identify the enzymatic product, we collected the suspected product fractions and used them for GC–mass analysis. The result shows that a major peak with retention period near 6.31 min in the GC chromatogram; the corresponding mass analysis yielded m/z 39, 53, 64, 81, 92, 110 (Fig. 4). This fragmentation pattern is virtually identical to that of a pure catechol standard. To analyze the catalytic activity of 1,2-dihydrogenase, we treated the reaction mixture containing catechol with the crude enzyme extract. The resulting mixture was subjected to product analysis by ion chromatography as described in the method section. In Fig. 5, a new feature, as compared with both background chromatograms of catechol and crude enzyme sample, was observed at a retention period 2.2 min after the 2-min enzymatic catalysis of catechol. This feature was enhanced when *cis,cis*-muconic acid was spiked in the sample (chromatogram b) or when the duration of reaction was increased to 10 min. (chromatogram c). The eluent corresponding to the new peak area was collected and analyzed with electrospray ionization LC mass spectrometry (LC/MS) in a negative-charge mode and tandem mass spectrometry (MS/MS); the results are shown in Fig. 6(a) and (b). The signal at m/z 163 is consistent with the molar mass of monosodium muconate. The tandem mass analysis exhibited the following signals with m/z 97, 137 and 163, which are identical to the fragment pattern of pure *cis,cis*-monosodium muconate. Based on the enzymatic product and activity analysis, we conclude that an ortho-fission pathway is involved in the degradation of phenol by this isolated strain.

Although both catechol and *cis,cis*-muconic acid are intermediates in the ortho-fission pathway of phenol degradation, we found that *C. albicans* TL3 utilized
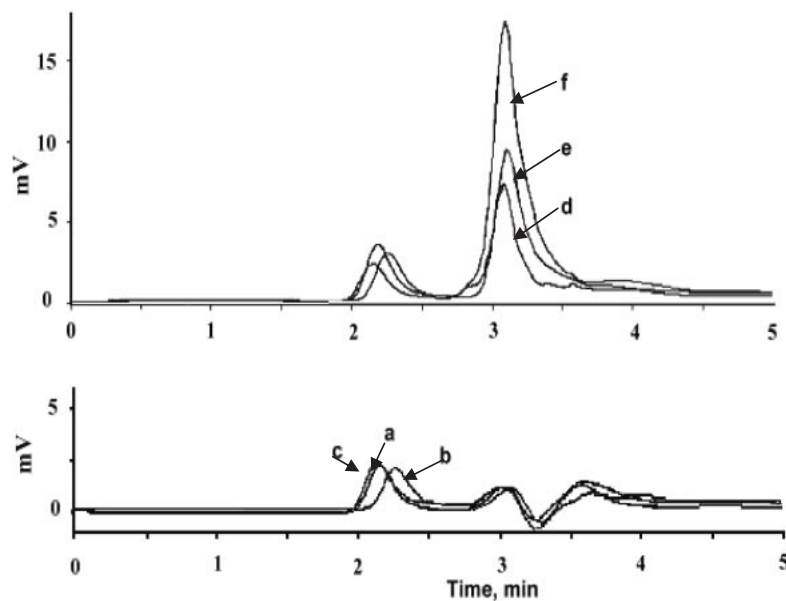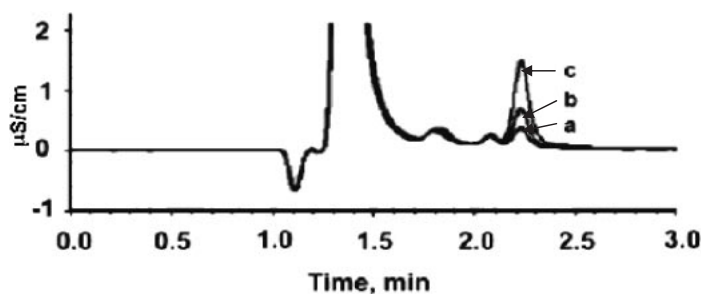
Fig. 3. HPLC Analysis of the Enzymatic Product of Degradation of Phenol Catalyzed by Crude Enzyme Extract.
    Reactions were terminated at 10 or 25 min on addition of excess methanol. Samples taken from the mixtures were then centrifuged to remove the precipitant before applying to a column. The resulting supernatants containing varied compositions were analyzed: (a) without addition of NADPH and phenol; (b) with phenol; (c) with NADPH; (d) 10-min reaction with both NADPH and phenol; (e) with spiking 0.02-mM catechol in the sample (d); (f) 25-min reaction with both NADPH and phenol. The extract medium of crude enzyme as described in the text.
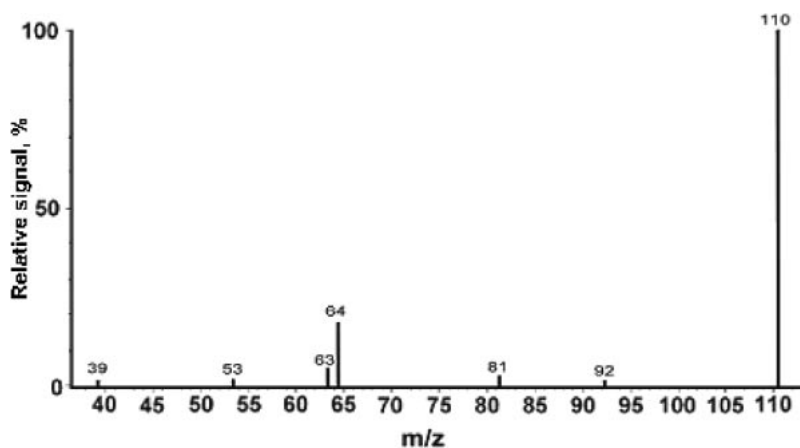

Fig. 4. GC–Mass Analysis of the Enzymatic Product of Degradation of Phenol Catalyzed by Crude Enzyme Extract.
    The sample containing catechol, the anticipated product of phenol hydroxylase-catalyzed reaction, was analyzed and confirmed by electron ionization mass spectrometry (EI/MS). The feature at m/z 110 corresponds to the molecular ion of catechol (M$^+$). All fragments shown are consistent with those of standard catechol.

Fig. 5. Ion-Chromatographic Analysis of the Product(s) of Catechol Catalyzed by Crude Enzyme Extract.
    Reactions were stopped at 2 or10 min on addition of excess methanol. Samples taken from the mixtures were then centrifuged to remove the precipitant before applying to a column. The figure contains the overlay of chromatograms with sample from (a) 2-min reaction mixture; (b) 2-min reaction mixture with spiking of 0.015 mM *cis,cis*-muconic acid; (c) 10-min reaction mixture.
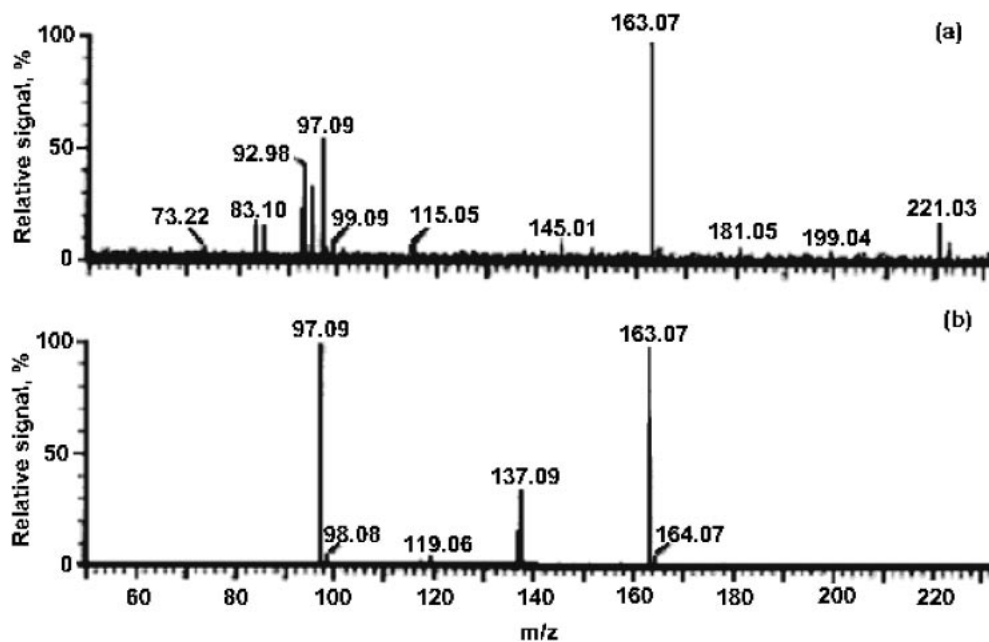
Fig. 6. Electrospray Ionization Mass Analysis (ESI) of the Product of Catechol Catalyzed by Crude Enzyme Extract.

The sample containing monosodium muconic acid (mw.164), the anticipated product of catechol-1,2-dioxygenase-catalyzed reaction, was analyzed by ESI/MS (a) and confirmed by ESI/MS/MS (b). The feature at m/z 163 corresponds to monosodium muconate (M^-). The pattern of fragments shown in (b) is identical to that of standard monosodium muconate.
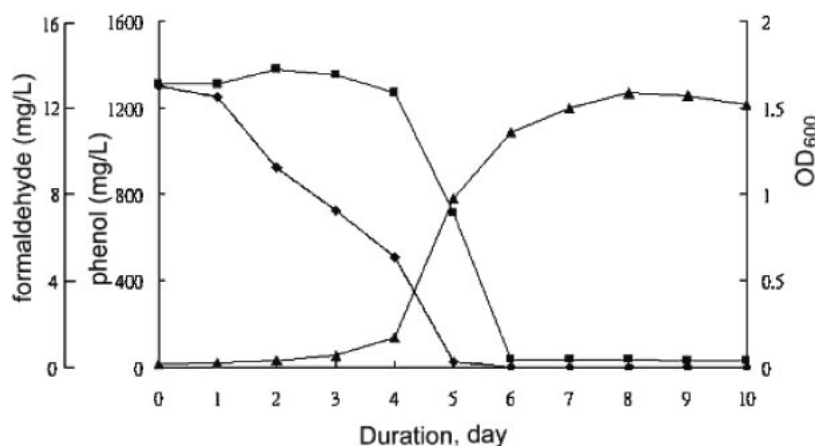
Fig. 7. Growth of *C. albicans* TL3 (–▲–) and the Remaining Phenol (mg/l) (–■–) and Formaldehyde (mg/l) (–◆–) in Waste Water as a Function of Time.

At the beginning, *C. albicans* TL3 culture (0.3 ml, $OD_{600} = 1.5$) at a stationary phase was added to waste water (49.7 ml) containing phenol (14.5 mM) and formaldehyde (0.44 mM). Each data point represents the mean of triplicate independent measurements. Data shown are the mean of triplicate experiments with standard deviation within 10%.

only catechol as a nutrient, unlike *Acinetobacter* sp. CNU961[37] which can grow in the medium with either intermediate as a carbon source. Perhaps *C. albicans* TL3 lacks a transport system for *cis,cis*-muconic acid so that no nutrient can be appropriately uptaken to support cell growth.

Application to the treatment of industrial effluent

To evaluate the prospective application of *C. albicans* TL3, we obtained a sample of waste water containing formaldehyde (65 mg/l; *i.e.* 2.17 mM) and phenol (6750 mg/l; *i.e.* 71.8 mM) from a local factory producing phenolic resin as a carbon source. *C. albicans* TL3 grew in the waste water diluted 5-fold. The cell growth and the residual concentrations of phenol and formaldehyde at various intervals are shown in Fig. 7. After incubation for six days with *C. albicans* TL3 at 30 °C, phenol and formaldehyde were nearly completely degraded. The degradation of formaldehyde and phenol correlated well with the growth of *Candida albicans* TL3. Although some strains were reported to function as degraded of phenol and of trichloroethene,[4,31,41] a microbe with a biodegrading power toward phenol and formaldehyde concurrently has been scarcely reported. *Candida*

albicans TL3 is the first documented microbe that has a bio-degrading function for both phenol and formaldehyde. Being highly tolerant of phenol and having a large rate of degradation of phenol and a capacity to degrade phenol and formaldehyde directly in waste water are features that make *C. albicans* TL3 particularly useful for treatment of waste water containing phenolic resin from its industrial sources. Chen *et al.* reported that the power to degrade phenol by *Candida tropicalis* was greatly improved with a simple cell-immobilization process.[42] How to enhance the efficiency of degrading phenol by *Candida albicans* TL3 using immobilization technology will therefore be the subject of future work.

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