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Transformation of Chlororesorcinol by the Hydrocarbonoclastic Yeasts *Candida maltosa*, *Candida tropicalis*, and *Trichosporon oivide*

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Abstract. The inhibitory effects of chlorinated monoaromatic compounds on three hydrocarbonoclastic yeasts grown on glucose or resorcinol were examined. At concentrations of 1.0 M, all of the monoaromatic compounds were inhibitory. When the concentration of chlororesorcinol was significantly reduced (0.0005 M), the inhibition to each yeast was minimized. Extracts of the cultures of yeasts growing on resorcinol plus chlororesorcinol were analyzed for residual resorcinol and chlororesorcinol with high pressure liquid chromatography. Neither compound was detected in the culture broth of *Candida maltosa*, but several unidentified compounds were present. Only chlororesorcinol was detected in the culture broth of *Trichosporon oivide*, and both resorcinol and chlororesorcinol were present in extract from cultures of *C. tropicalis*. Cultures of *C. maltosa* grown on resorcinol-yeast nitrogen base, washed and suspended in phosphate buffer and subsequently incubated with chlororesorcinol, turned the culture broth a distinct pink color. The data indicate that *C. maltosa* has the potential to co-metabolize chlororesorcinol.

Numerous fungi are known to oxidize a variety of monoaromatic and polycyclic aromatic compounds, but few fungi have been reported to metabolize chlorinated aromatic compounds [2, 5, 6]. White rot fungi, particularly *Phanerochaete* spp., seem to be exceptions because these lignin-degrading basidiomycetes degrade chlorinated benzenes and polychlorinated biphenyls [1, 10, 12]. A few species of yeasts are known to grow on or metabolize at low concentrations certain monoaromatic compounds (e.g., catechol, resorcinol, phenol) as sole sources of carbon, and it is not uncommon for yeasts to co-oxidize aromatic compounds [2, 6, 8]. Sampaio (1994) found that basidiomycetous yeasts, in general, were more active metabolically with aromatic compounds than were ascomycetous yeasts [9]. Chlorinated aromatic compounds were not examined in their studies. A single report suggested that *Rhodotorula glutinis*, when phenol adapted, is capable of transforming chlorophenol [11]. This study reports the co-metabolism of chlororesorcinol by the ascomycetous yeast *Candida maltosa*.

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Materials and Methods

Cultures and media. Cultures of *C. maltosa* (R-42), *C. tropicalis* (ATCC 32113), and *Trichosporon oivide* (WO698) were obtained from the lyophilized culture collection at Georgia State University. *Candida maltosa* (R-42), initially isolated from an asphalt plant waste retention pond, is known to metabolize aromatics [2]. The cultures were reconstituted in Sabouraud's dextrose broth (SAB), incubated for 72 h, and streaked onto SAB agar. Isolated colonies were streaked to Mycological agar (Difco). A loop of cells from a 48-h culture was transferred to 0.67% yeast nitrogen base (YNB, Difco) broth supplemented with substrates and incubated for 48 h at 25°C. This cell suspension was adjusted with fresh medium to an optical density at 595 nm of 0.075.

Toxicity of monoaromatic compounds. Monoaromatic compounds were prepared in 1.0 M concentrations. All chemicals were purchased from Sigma (Sigma Chemical Co., St. Louis, MO) or Fisher (Fisher Scientific Co., Fair Lawn, NJ) and were at least 95% pure and solubilized in either N,N-dimethyl-formamide (DMF) or deionized water. Solutions of compounds were filter sterilized through 0.45 μM polycarbonate filters. A sterile filter disk (11 mm) was placed in the center of the solidified agar pour plates, and 50 μl of test compound was delivered carefully to the center of the disk. DMF or deionized water was employed as a control. The plates were incubated for 48 h at 25°C, and the diameters of the zones of inhibition were measured.

Table 1. Inhibition of the growth of *Candida* and *Trichosporon* species by monoaromatic compounds

Monoaromatic compound[a]	*C. maltosa*	*C. tropicalis*	*T. oivide*
Phenol (10%)	35[b]	32	38.5
Chlorophenol	NG	85	72.5
4-Chlororesorcinol[c]	32	30.5	54.5
2,4-Dinitrophenol	60	38	52.5
Pentachlorophenol	NG	42.5	54

NG, no growth on entire assay plate.
[a,c] All compounds were at concentrations of 1.0 M (equivalent to 10% phenol) in DMF or water,[c] 50 µl/disk.
[b] Diameter in millimeters of zones of inhibition on cells grown in YNB 1% glucose agar for 48 h.

Table 2. Growth and color production of *Candida* and *Trichosporon* species on various growth substrates

Substrates	*C. maltosa* growth/ color	*C. tropicalis* growth/ color	*T. oivide* growth/ color
Glucose[a]	+/−	+/−	+/−
Glucose + Chlororesorcinol[a]	+/−	+/−	+/−
Resorcinol[a]	+/−	+/−	+/−
Chlororesorcinol[a]	−/−	−/−	−/−
Resorcinol + Chlororesorcinol[a]	+/−	+/−	+/−
Chlororesorcinol PBS[b]	−/+	−/+	−/−

Glucose: 0.03 M; Resorcinol: 0.5 M; Chlororesorcinol: 0.0005 M.
[a] Cells (inoculum containing 10^5 cells) grown in YNB plus substrates with agitation at room temperature for 72 h.
[b] Cells (inoculum containing 10^5 cells) grown in YNB plus resorcinol, washed, starved and placed in PBS plus chlororesorcinol with agitation at room temperature for 72 h.

Monoaromatics as primary carbon and energy sources. Cells were grown, with agitation, in 100 ml YNB supplemented with 0.5% glucose. Cells were harvested, washed twice in 50 ml deionized water, and suspended in 20 ml deionized water.

YNB fortified with 100 µl 1.0 M concentrations of selected monoaromatic compound solutions was inoculated with 200 µl inoculum. Cultures were incubated at 25°C on a model TC-7 roller drum (New Brunswick Scientific Co., Inc., Edison, NJ) for 72 h and examined for growth. Turbid cultures were inoculated onto Mycological agar (Difco) and inspected for characteristic growth. All tests were performed in triplicate.

Dry weight comparison. Cells were grown in YNB supplemented with catechol, glucose, or resorcinol to a final concentration of 5 mM. After 48–96 h, an inoculum containing 10^5 cells was transferred to a nephelometer flask with 100 ml YNB supplemented with the same growth substrate. Chlororesorcinol was added at a final concentration of 0.5 mM. The absorbance was read hourly with a Klett photometer. When the culture reached the exponential phase of growth, 15 ml was removed from the flask and filtered through a preweighed 47-mm Metricel membrane filter (0.45 µm, GN-6; Gelman Sciences, Ann Arbor, MI), which was placed in an 85°C oven overnight and weighed.

Biotransformation/metabolism of monoaromatic compounds. The yeasts were grown in 100 ml YNB supplemented with catechol, glucose, or resorcinol at final concentrations of 5 mM. After 48–72 h, 10^5 cells were transferred to a flask with 100 ml YNB supplemented with the same growth substrate. Chlororesorcinol was added at a final concentration of 0.5 mM. After 72 h, ethyl acetate was added to a 250-ml separatory funnel, the culture in equal volume was added, and the mixture was shaken for 1 min. The phases were allowed to separate for 5 min, and the ethyl acetate fraction (top layer) was placed in a boiling flask and concentrated in vacuo with a water bath set at 55°C. The residue was suspended in acetonitrile (4 ml). The extracts were then analyzed, with concurrent analysis of standard solutions, with a Perkin Elmer LC Diode Array Detector with a C8 reverse phase column, monitored at 250–255 nm (spectra recorded) with an Advanced Sample Processor ISS 200 and a gradient of 50% acetonitrile for 1.9 min, decreasing to 8% acetonitrile in 1.5 min, and continuing at 8% acetonitrile for 6.6 min.

The yeasts were also grown in 100 ml YNB supplemented with

Table 3. Recovery of monoaromatic metabolites from culture extracts of hydrocarbonoclastic *Candida* and *Trichosporon* yeasts grown on resorcinol and chlororesorcinol[a]

	Peak 1		Peak 2		Peak 3	
Culture	Retention time (min)	Peak area[b] (µV · s)	Retention time (min)	Peak area (µV · s)	Retention time (min)	Peak area (µV · s)
Control[c]	1.418	3.4	1.784	0.13		
C. maltosa	1.881	5.5	2.177	1.7		
C. tropicalis	1.158	1.4	1.656	0.13	1.322	25.2
T. oivide	0.338	54.0	0.904	0.12		

[a] Extracts were analyzed with a diode array detector with a C8 reverse phase column, monitored at 250–255 nm (spectra recorded) with an Advanced Sample Processor ISS 200. The gradient used was 50% acetonitrile for 1.9 min, decreasing to 8% acetonitrile in 1.5 min, and continuing at 8% acetonitrile for 6.6 min; representative data of three experiments.
[b] Multiply by 10^5.
[c] YNB with resorcinol and chlororesorcinol.

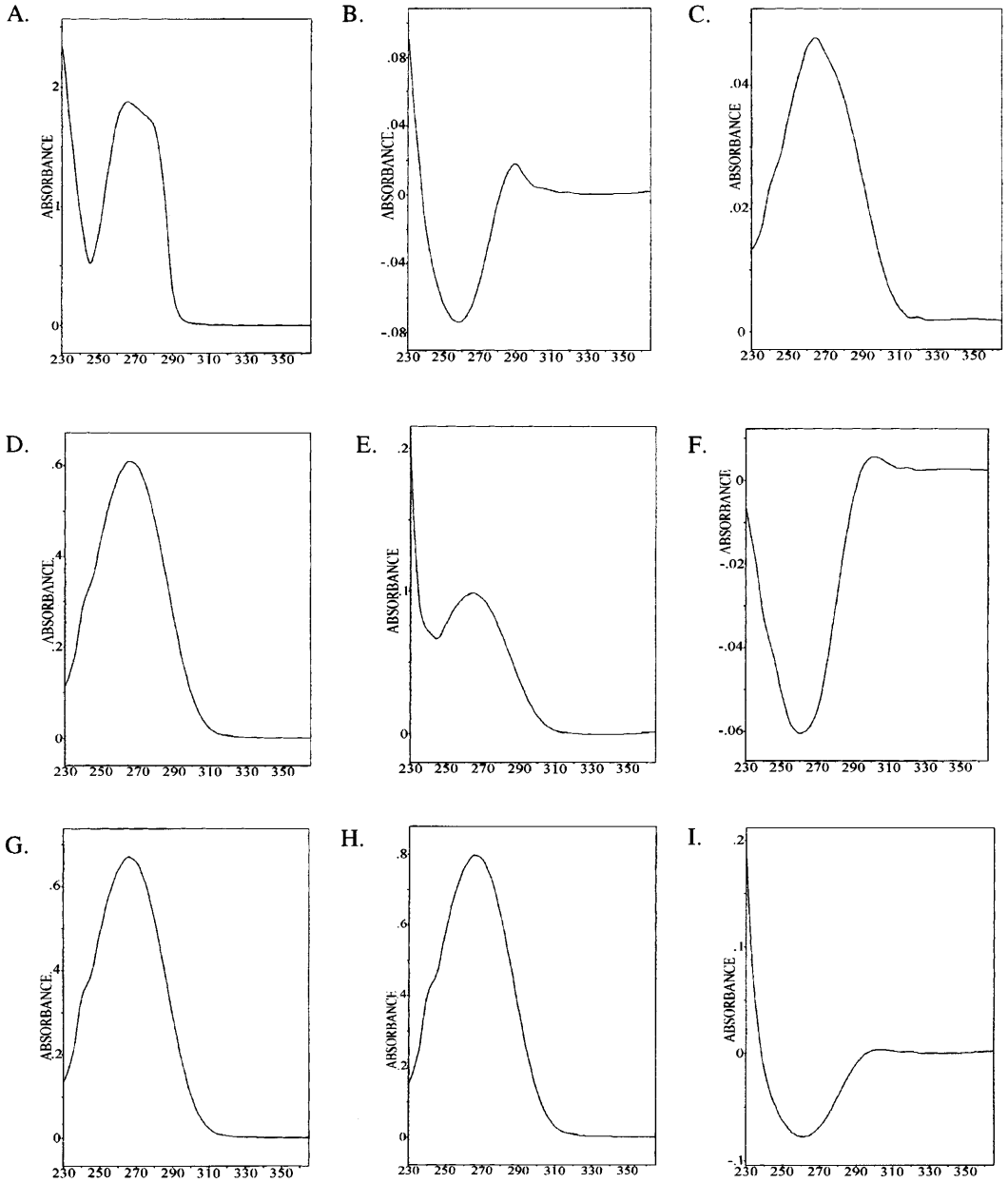

Fig. 1. Representative U.V. spectra of aromatics in culture broths of *Candida* spp. and *Trichosporon* sp.: resorcinol control (A); chlororesorcinol control (B); *C. maltosa* culture extract (C,D); *C. tropicalis* culture extract (E,F,G); and *T. oivide* culture extract (H,I). Spectra are plotted as absorbance vs. wavelength (nm).

glucose or resorcinol at a final concentration of 5 mM. After 72 h, the cultures were centrifuged for 20 min at 2500 *g*, and the pellets were washed three times with 20 ml sterile phosphate-buffered saline (PBS). The pellets were suspended in 10 ml sterile PBS in sterile centrifuge tubes and placed on a roller drum for 48 h at room temperature. The cells were poured into sterile 250-ml flasks, and 90 ml sterile PBS was added. Chlororesorcinol was added to the flasks at a final concentration of 5 mM. The flasks were incubated on a shaker table at room temperature for 48 h and monitored for conversion of chlororesorcinol. All experiments were performed in triplicate.

Results and Discussion

Growth of the hydrocarbonoclastic yeasts, *C. maltosa*, *C. tropicalis*, and *T. oivide* in YNB supplemented with glucose, was inhibited in the presence of the chlorinated aromatic compounds (Table 1).

To determine whether cells grown on resorcinol could be adapted to growth on chlororesorcinol, the least inhibitory compound for *C. maltosa*, we transferred cells

from 48-h cultures on resorcinol-YNB to YNB medium fortified with chlororesorcinol and to YNB with resorcinol plus chlororesorcinol. Nearly equivalent cell growth occurred on resorcinol and the resorcinol plus chlororesorcinol combination, but YNB plus chlororesorcinol did not support growth (Table 2). When chlororesorcinol in PBS was inoculated with cells from YNB-resorcinol, *C. maltosa* and *C. tropicalis* turned the broth pink (Table 2).

HPLC analyses and concurrent UV spectral analyses of culture extracts of the yeasts grown on YNB supplemented with 0.5 M resorcinol and 0.5 mM chlororesorcinol demonstrated that both compounds persisted in the cultures of *C. tropicalis* (Table 3; Figures 1E, F, G). In three repeat tests, only chlororesorcinol was recovered from the cultures of *T. oivide* (Table 3; Figure 1H,I), and neither compound was recovered from cultures of *C. maltosa* (Table 3; Figure 1C,D).

This study indicated that the ascomycetous yeasts *C. maltosa* and *C. tropicalis* can transform chlororesorcinol to an unknown pink-colored intermediate(s). The mechanisms by which this color is produced may be similar to the color production in the metabolism of tyrosine. In that metabolic pathway, tyrosine is degraded to dopachrome (red), 5,6-indolequinone (yellow), melanochrome (purple) and melanin (black) [7]. Yeasts usually have been found to have a restricted spectrum for hydrocarbon metabolism, with activities mostly limited to alkanes and alkenes. Recently a new basidiomycetous yeast species, *Rhodotorula vanillica*, was reported to use low-molecular-weight lignin-related aromatic compounds as sole carbon and energy sources [9]. Ascomycetous-type yeasts (i.e., those with multilayered cell walls that fail to stain with diazonium blue B [4]) have not previously been reported to metabolize chlorinated aromatics. However, *C. maltosa* has been reported to oxidize certain polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene [3], while *C. lipolytica* has been reported to oxidize PAHs including naphthalene and biphenyl [2]. Our data suggest *C. maltosa* may have an extended role in the degradation of recalcitrant molecules in the environment, i.e., the co-metabolism of chlororesorcinol.

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