Degradation of Cyclopentanol by *Trichosporon cutaneum* Strain KUY-6A

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*Trichosporon cutaneum* strain KUY-6A, a cyclohexanecarboxylic acid-utilizing yeast, was able to grow on cyclopentanol (CPOL) as a sole source of carbon and energy. Growth experiments revealed the strain, KUY-6A, could utilize up to 42 mM of CPOL with an optimum at 24 mM. Optimal growth was found between pH 4.0 to 9.0. The generation time under optimal growth conditions on CPOL was 3.0 h. Analysis indicated that cyclopentanone (CPON) and glutaric acid were intermediates of CPOL metabolism in strain KUY-6A. The results of growth and enzyme experiments are consistent with the degradation of CPOL via CPON, 5-valerolactone, 5-hydroxyvaleric acid, and glutaric acid.

Key words: cyclopentanol, cyclopentanone, biodegradation, *Trichosporon cutaneum*

*Trichosporon cutaneum* is a yeast used for the treatment of waste liquid, and is very often isolated from activated sludge. In addition, some strains utilize phenol or its derivatives.¹,²,¹²,¹³,¹⁴ Previously, we reported the isolation of *T. cutaneum* strain KUY-6A which grew on cyclohexanecarboxylic acid (CHCA) as a sole source of carbon, the degradation pathway of CHCA, and the utilization of aromatic compounds by the strain KUY-6A.⁷–⁹

*T. cutaneum* KUY-6A also grew on CPOL. Although the bacterial degradation of CPOL by *Comamonas* (formerly *Pseudomonas*) sp. NCIMB 9872 has been well established, the degradation of CPOL by yeast has not been reported. The present report describes the degradation of CPOL by *T. cutaneum* strain KUY-6A.

Strain KUY-6A was grown at 30°C in Basal salt medium (BSM), pH 7.0, containing 0.05 g/l of yeast extract, and 6–48 mM CPOL, 24 mM CPON, or 24 mM 5-valerolactone. BSM contains the following components per liter: 0.4 g (NH₄)₂SO₄, 1.0 g KH₂PO₄, 0.2 g MgSO₄·7H₂O, 0.05 g FeCl₃·6H₂O, 0.02 g CaCl₂·2H₂O. Microbial growth was measured as the increase in optical density (OD) at 660 nm. An effect of the concentration of CPOL on growth was investigated. KUY-6A grew well up to 42 mM of CPOL with the optimal concentration for growth after 48-hours incubation found to be 24 mM. At 48 mM CPOL, the growth was severely inhibited. Strain KUY-6A grew well in a broad pH range from 4.0 to 9.0 as compared to bacteria, with an optimum at pH 7.

No apparent growth was found at a pH of >10.0 or <3.0. Growth of *T. cutaneum* strain KUY-6A with 24 mM CPOL as a sole carbon source is shown in Fig. 1. Characteristically, the culture reached maximum cell density after 48 hours incubation in 100 ml of the growth medium with complete consumption of CPOL, and 100 mg of cell culture (dry weight) was obtained. The generation time of strain KUY-6A on CPOL was 3.0 h. Under the conditions, accumulation of CPON in the medium was observed in the logarithmic growth phase (Fig. 1). The observation suggested that CPOL was transformed into CPON by strain KUY-6A.

To elucidate the major route of CPOL degradation, we identified the metabolites that accumulated in the medium.
Ethyl ester extracts from the culture broth of strain KUY-6A grown on CPOL and CPON were analyzed by thin-layer chromatography (TLC) and TCD gas chromatography (GC). TLC analysis of organic acids was carried out on 0.25 mm-thick layers of Kieselgel 60 F$_{254}$ (Merck, Darmstadt, Germany) developed with solvent A (benzene:ethylacetate:formic acid, 25:25:2 by vol.) or with solvent B (benzene:dioxane:acetic acid, 90:45:4 by vol.). Organic acids were detected by spraying thoroughly dried plates with 0.1% (w/v) bromocresol green in aqueous 95% (v/v) ethanol adjusted to pH 6.0 with NaOH. For GC, methylated acid with diazomethane was used as a sample. GC was performed with a Yanaco G-80 TCD apparatus and a column (1.25 m by 3 mm) that was packed with 10% (wt/wt) SE-30 on Chromosorb W under the following conditions: carrier gas, H$_2$ at a flow rate of 30 ml/min; oven temperature, 150°C; and injection and detector temperatures, 170°C. Table 1 shows the $R_F$ value on TLC and the retention time on GC of the sample prepared from a 2-day culture broth. In both cases, only one metabolite corresponding to an $R_F$ value of 0.50 in solvent system A and 0.45 in solvent system B was detected and the $R_F$ values were in good agreement with the value of the authentic gultaric acid. Also, the peak with a retention time of 1.4 min was identified to be dimethyl gultarate by comparison to an authentic standard and accumulation of 2.1 mM gultaric acid in the medium was observed. Concerning the metabolic pathway of CPOL or cyclohexanol, Trudgill and his coworkers proposed the formation of lactone from cycloalkanone (3,5,11). In this situation, if the product of oxygenation of CPON is the

![Graph](image-url)
Degradation of CPOL by Trichosporon lactone, as occurs in the metabolism of CPON by bacteria, accumulation of the lactone is unlikely. The expected reaction product of 5-valerolactone, 5-hydroxyvaleric acid, was identified by TLC and GC. These results with the cell extracts of strain KUY-6A are consistent with Fig. 2.

To gain a better understanding of the degradation of CPOL by *T. cutaneum* strain KUY-6A, the biochemical and genetic characterization of the enzymes involved is in progress.

**References**


