High Utility of *Saccharomyces cerevisiae* Harboring Rat Liver Cytochrome P450 1A2 cDNA in Haloethanes Dehalogenations

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Yeast harboring rat liver P450 1A2 efficiently degraded trichloroethylene, pentachloroethane and hexachloroethane. Since liver P450s catalyze degradation of thousands of chemicals, this method is promising for chemical-directed degradation of environmental pollutants.

Environmental pollution with chemicals has been one of the recent major issues.[1] Degradation of the chemicals is needed because certain chemicals can accumulate in the environment to levels that threaten human health. Haloethanes such as trichloroethylene and other chlorinated hydrocarbons are major chemical pollutants.[1] They are toxic, carcinogenic and extremely persist in the environment. Bioremediation, the use of living organisms (primarily microorganisms) to degrade environmental pollutant or to prevent pollution through waste treatment, is emerging as one of several alternative technologies for removing pollutants from the environment.[1]

Liver microsomal cytochrome P450s catalyze the degradation of thousands of chemical compounds, pesticides, herbicides, halomethanes, that often threaten human health, but are extremely persistent in the environment and are recalcitrant to biodegradation by naturally occurring microorganisms.[2] The heterologous P450 expression system seems therefore appropriate to take advantage of the P450 genetic code for biodegradation of chemical pollutants. In the present study, we first successfully applied the genetic code of rat liver P450 in the yeast heterologous expression system to degrade haloethanes.

Expression of the wild-type P450 1A2 in *Saccharomyces cerevisiae* was carried out as previously described.[3] Experiments were carried out with 500 μL microsomes containing 1.0 μM P450 1A2 and 1.11 mg protein or 500 μL yeast containing 5.0 μM P450 1A2 and 8.05 mg protein in reaction buffer (pH 7.2) consisting of 50 mM potassium phosphate and 5 mM $MgCl_2$ at 25 °C on a rotary shaker at 100 rpm. Initial concentrations of halogenated substrates were 5.0 mM. Reactions were stopped by extracting the reaction mixtures three times with equal volumes of diethylether. 2-μL aliquots of the 1.5-mL organic phases were analyzed by gas-chromatography-mass-spectrometer. It should be emphasized that blank runs with control yeast not expressing P450 1A2 did not consume the halogenated substrates.

Qualitative and quantitative experiments were carried out on a Shimadzu QP-5000 gas-chromatography-mass-spectrometer equipped with an electron-capture detector and a DB-64 capillary column (60 m x 0.32 mm) (J & W Scientific, Folsom, CA). The He carrier gas flow rate was 30 mL/min. The column temperature was held isothermally at 40 °C for 2 min and then programmed to 240 °C at the rate of 5 °C/min. Detector gain was 1.5 kV. Substrate and product concentrations were quantitated by comparison with authentic standards.

Figure 1 shows the time-dependent increase of trichloroacetaldehyde and 2,2,2-trichloroethanol concentrations when trichloroethylene was mixed with microsomal (left) and

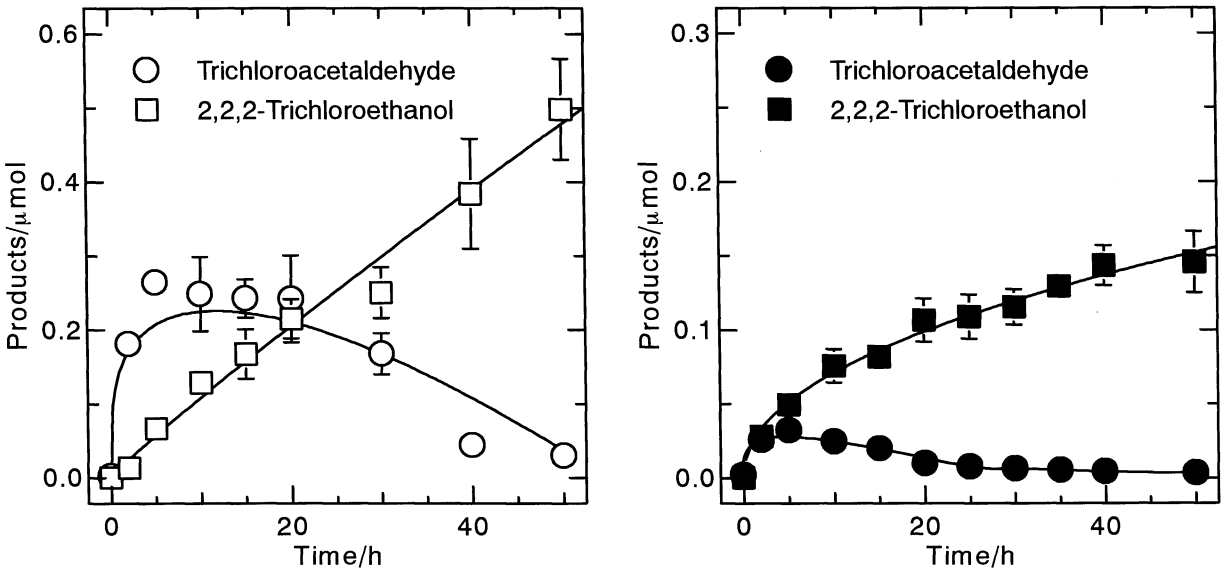

Figure 1. Time dependent changes of the product concentrations in P450 1A2-containing microsomes (left) and yeast (right) for trichloroethylene. P450 1A2 concentrations in microsomes and yeast were 1.0 μM and 5.0 μM, respectively. Initial substrate concentrations were 5.0 mM.

Table 1. Comparison of relative substrates and products turnover numbers between microsomal and yeast P450 1A2s

Substrates	Products	Turnover numbers[a] Microsomes	Yeast (N_2)
Trichloroethylene		2450 ± 183	191 ± 23.3
			(62.9 ± 11.7)
	Trichloroacetaldehyde	1370 ± 93.5	87.8 ± 6.79
	2,2,2-Trichloroethanol	938 ± 91.1	101 ± 17.5
Pentachloroethane		592 ± 84.6	96.9 ± 8.16
			(172 ± 28.0)
	Trichloroethylene	582 ± 68.4	96.0 ± 18.3
	Trichloroacetaldehyde	3.62 ± 0.42	0.3 ± 0.08
	2,2,2-Trichloroethanol	0.12 ± 0.03	< 0.01
Hexachloroethane		19.0 ± 2.17	21.4 ±2.11
			(33.3 ± 4.18)
	Trichloroethylene	0.05 ± 0.01	0.03 ± 0.01
	Tetrachloroethylene	12.7 ± 1.27	17.2 ± 0.93
	Pentachloroethane	5.31 ± 0.82	4.34 ± 0.59

[a] Turnover numbers are expressed by pmol/nmolP450/min for the substrates decrease and products formation and were evaluated by initial phase between 0 h and 5 h of the catalytic reaction. Values in parentheses are substrates decrease rates obtained under anaerobic conditions. Averaged values of two or three experiments are described here.

yeast (right) P450 1A2s under an air atmosphere. Trichloroacetaldehyde was formed faster than 2,2,2-trichloroethanol during the initial stage with microsomal P450 1A2, whereas with yeast P450 1A2 both products were formed at similar/identical rates. The catalytic mechanism of trichloroethylene formation may be different in the two systems. Table 1 shows the turnover numbers of microsomal and yeast P450 1A2s for formation of trichloroethylene, pentachloroethane and hexachloroethane. The substrate consumption rates correspond to the sum of the products' formation rates. The turnover numbers for the formation of trichloroethylene and pentachloroethane were higher with microsomes than with yeast. Turnover numbers obtained in the present study are comparable to these previously reported with rat liver microsomal P450s[4] and bacterial P450[5].

We examined the substrate consumption rates and product formation rates under a nitrogen gas atmosphere. As shown in the parenthesis of Table 1, the trichloroethylene degradation rate under anaerobic conditions was lower than that under aerobic conditions. In contrast, penta- and hexachloroethane degradation rates under anaerobic conditions were higher than those under aerobic conditions. Product formation patterns for all substrates under anaerobic conditions were essentially the same as those under aerobic conditions. Perhaps trace amount of O_2 in the cells, not eliminated by N_2 purging, may have contributed to the monooxygenation of trichloroethylene.

Glucose could affect the reactions by both increasing the size of the NADPH pools and by increasing the metabolism that leads to oxygen depletion. As shown in Table 2, the catalytic activity toward trichloroethylene was decreased by increasing the glucose concentration. In contrast, activities toward penta- and hexachloroethane were increased by adding 5% glucose.

Table 2. Effects of glucose on the turnover number of the P450 1A2-yeast system

Substrates	Glucose concentration 0 %	1%	5%	10%
Trichloroethylene	100	89	66	30
	(100)	(88)	(61)	(27)
Pentachloroethane	100	98	112	99
	(100)	(99)	(107)	(89)
Hexachloroethane	100	136	161	134
	(100)	(127)	(152)	(126)

Numbers in parentheses are those obtained from sum of product formation rates.

In conclusion, for the first time we have demonstrated the great potential of the P450 heterologous expression system for the degradation of environmental chemicals. Changes in oxygen tension and glucose concentration markedly improved the catalytic activities toward those substrates. Since liver P450s have broad substrate specificities,[2] this method may be of crucial importance as an alternative technology for the degradation of chemical pollutants.

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References

1 a) M. Alexander, "Biodegradation and Bioremediation," Academic Press, San Diego (1994); b) R. M. Atlas, *Chem. Eng. News,* **73(14)**, 32 (1995); c) E. K. Wilson, *Chem. Eng. News,* **73(27)**, 19 (1995); d) L. P. Wacket, in "Microbial Transformation and Degradation of Toxic Organic Chemicals," ed by L. Y. Young and C. E. Cerniglia, Wiley-Liss, New York (1995), p 217.

2 a) F. P. Guengerich, *J. Biol. Chem.* **266**, 10019 (1991); b) P. R. Ortiz de Montellano, ed "Cytochrome P450, Structure, Mechanism and Biochemistry", 2nd Ed., Plenum Press, New York (1995); c) T. D. Porter and M. J. Coon, *J. Biol. Chem.,* **266**, 13469 (1991).

3 a) T. Shimizu, Y. Murakami, and M. Hatano, *J. Biol. Chem.,* **269**, 13296 (1994); b) H. Sato, T. Shimizu, Y. Murakami, and M. Hatano, *Chem. Lett.*, **1994**, 311; c) H. Sato, and T. Shimizu, *Arch. Biochem. Biophys.*, **322**, 277 (1995); d) R. Nakano, H. Sato, A. Watanabe, O. Ito, and T. Shimizu, *J. Biol. Chem.*, **271**, 8570 (1996).

4 R. E. Miller and F. P. Guengerich, *Biochemistry*, **21**, 1090 (1982).

5 S. Li and L. P. Wachett, *Biochemistry*, **32**, 9355 (1993).