APPLIED MICROBIOLOGY, Aug. 1973, p. 217-218

Vol. 26, No. 2
Printed in U.S.A.

Pseudomonas oleovorans Hydroxylation-Epoxidation System: Additional Strain Improvements

ROBERT D. SCHWARTZ AND C. J. McCOY

Corporate Research Laboratories, Esso Research and Engineering Company, Linden, New Jersey 07036

Received for publication 13 April 1973

The isolation and characterization of a strain of *Pseudomonas oleovorans* which epoxidates 1-octene at a rate nine times that of the original strain are described. In addition, it has been confirmed that a greater amount of the mono-epoxide product is formed from 1,7-octadiene than is formed from 1-octene. 1,7-octadiene will not support growth, but will induce the enzymes required for epoxidation.

A previous report described the isolation and characterization of a superior alkane-oxidizing, octene-epoxidizing strain of *Pseudomonas oleovorans* (5). This communication describes additional strain improvements and compares the new and old strains relative to 1-octene and 1,7-octadiene epoxidation and enzyme induction.

P. oleovorans 1-RAM, *P. oleovorans* TF4-1, media, growth conditions, resting cell preparation, and epoxide assays have been described (5). All cultures were grown on octane unless otherwise stated.

When minimal agar was required, 15 g of agar (Difco) was added to 1 liter of P_1 minimal salts medium. Substrate was supplied as a vapor by adding several drops of hydrocarbon to a piece of Whatman no. 1 filter paper placed in the lid of the inverted petri dish.

The gas chromatographic procedures of May and Abbott (3, 4) were used to quantitate the amounts of epoxide produced.

During the previous experiments with TF4-1, it was noticed that after 3 days of incubation on nutrient agar plates, about 2% of the colonies were considerably larger than the majority. The larger colonies were 6.0 to 6.5 mm in diameter compared to the 2 to 3 mm diameter smaller colonies. Several large and small clones were picked, the strains derived from them were designated *P. oleovorans* TF4-1L and *P. oleovorans* TF4-1S, respectively, and they were analyzed for their capacity to grow on and epoxidate 1-octene and 1,7-octadiene. It is noted that the size of the small colonies present in the mixed parent culture, TF4-1, are the same as those in the derivative culture, TF4-1S.

Table 1 summarizes 1-octene and 1,7-octadiene epoxidation by the four strains examined. It is of interest that TF4-1 could not use 1-octene as a sole source of carbon and energy unless it was supplied as a vapor, whereas TF4-1L and TF4-1S grew on 1-octene in liquid media. The reason for this strain difference is not understood. As the method of growth on octene was different, TF4-1 is not directly comparable to the others, relative to octene epoxidation by octene-grown cells.

Octane is superior to 1-octene for growth and enzyme induction. TF4-1L is the most active strain for enzymatic epoxidation, having about nine times the activity of the original strain, 1-RAM.

None of the strains was found capable of using 1,7-octadiene as a sole source of carbon and energy, although 1,7-octadiene was epoxi-

TABLE 1. *Epoxidation of 1-octene and 1,7-octadiene by resting cell suspensions of 1-RAM, TF4-1, TF4-1L, and TF4-1S*

Strain	Epoxide produced after 1 h (μg/ml)		
	1,2-Epoxyoctane		7,8-Epoxy-octene
	Octane-grown cells	Octene-grown cells	Octane-grown cells
1-RAM	20	ND[a]	ND
TF4-1	96	57[b]	ND
TF4-1L	183	152	450
TF4-1S	150	110	400

[a] Not determined.
[b] Cells harvested from minimal agar.

dized to 7,8-epoxyoctene. Resting cell preparations of octane-grown TF4-1L and TF4-1S produced 7,8-epoxyoctene at concentrations of 450 and 400 μg/ml, respectively, after 1 h. With 1-octene as substrate, TF4-1L and TF4-1S produced 1,2-epoxyoctane at concentrations of 183 and 150 μg/ml, respectively, after 1 h. Although 1,7-octadiene gives a greater amount of monoepoxide product under the conditions employed, it should be noted that other products are also formed from these substrates (2–4, 6).

It was of interest to ascertain whether 1,7-octadiene could induce the synthesis of the enzymes required for its oxidation. TF4-1L was grown on acetate (non-inducer) in the presence and absence of 1,7-octadiene; resting cell suspensions were prepared and assayed for the epoxidation of octadiene. The results are shown in Fig. 1. Cells grown in the absence of 1,7-octadiene show a considerable lag in the epoxidation of 1,7-octadiene, while those grown in its presence do not, indicating that 1,7-octadiene does induce the epoxidation enzymes. Optical density measurements indicated that the presence of 1,7-octadiene (or 7,8-epoxyoctene?) in the growth medium inhibited, but did not prevent, growth.

TF-1L growing on substrates which support growth and enzyme induction (1-octene, C_5-C_{12} *n*-alkanes) or on non-inducing substrates (acetate, glucose) in the presence of 1,7-octadiene, carries out the enzymatic conversion of the latter to 7,8-epoxyoctene, which accumulates in the growth medium. Substantial amounts of the product remain bound to the cells but can be removed by extraction with hexane (Table 2). Concentrating the cells by centrifugation prior to extraction was found to be a unique method for concentrating and purifying the product. Increased product concentration at the cell surface during hydrocarbon oxidation was reported previously (1).

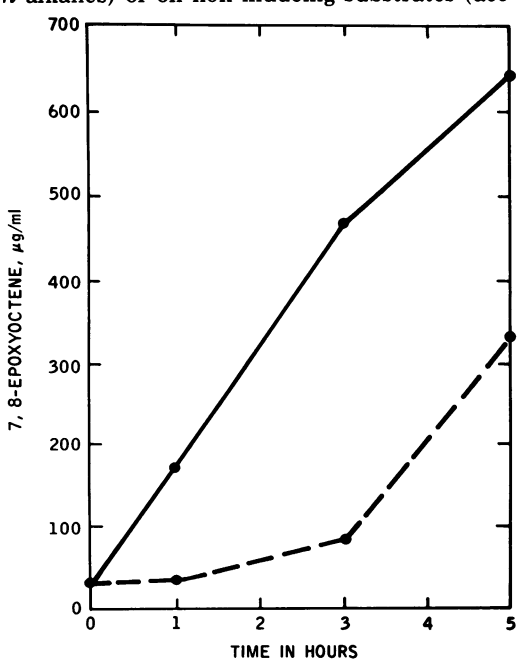

FIG. 1. *Induction by octadiene of the enzymes required for octadiene epoxidation. 7,8-epoxyoctene synthesis by resting cell suspensions of TF4-1L grown on acetate in the presence (——) or absence (- - -) of octadiene.*

TABLE 2. *Epoxyoctene acumulation by growing cells of TF4-1L in the presence of 1,7-octadiene*

Growth[a] substrate	7,8-Epoxyoctene present after 20 h of growth (μg/ml)		
	Broth + cells[b]	Broth[c]	Resuspended cells[c]
Octane, 1%	1,084	233	ND[d]
Glucose, 1%	776	356	359
Acetate, 0.5%	232	100	83

[a] Concn of octadiene was 1% vol/vol.
[b] Cells were not separated from the broth prior to hexane extraction.
[c] Cells were separated from the broth plus cells before the broth or the cell pellet (resuspended in buffer) was extracted with hexane.
[d] Not determined.

Preliminary experiments indicate that, in the presence of 1-octene, 1,2-epoxyoctane accumulates in a similar manner, although the product may be further metabolized.

Knowledge of the metabolic fate of the epoxides, their ability to support growth or induction, or both, and details of the kinetics of epoxide accumulation by growing cells await further experimentation.

LITERATURE CITED

1. Abbott, B. J., and W. E. Gledhill. 1971. The extracellular accumulation of metabolic products by hydrocarbon-degrading microorganisms. Advan. Appl. Microbiol. 14:249–388.
2. Huybregtse, R., and A. C. van der Linden. 1964. The oxidation of α-olefins by a Pseudomonas. Reactions involving the double bond. Antonie van Leeuwenhoek J. Microbiol. Serol. 30:185–196.
3. May, S. W., and B. J. Abbott. 1972. Enzymatic epoxidation I. Alkene epoxidation by the ω-hydroxylation system of *Pseudomonas oleovorans*. Biochem. Biophys. Res. Commun. 48:1230–1234.
4. May, S. W., and B. J. Abbott. 1973. Enzymatic epoxidation. II. Comparison between the epoxidation and hydroxylation reactions catalyzed by the ω-hdroxylation system of *Pseudomonas oleovorans*. J. Biol. Chem. 248:1725–1730.
5. Schwartz, R. D. 1973. Octene epoxidation by a cold-stable, alkane-oxidizing isolate of *Pseudomonas oleovorans*. Appl. Microbiol. 25:574–577.
6. van der Linden, A. C. 1963. Epoxidation of α-olefins by heptane-grown *Pseudomonas* cells. Biochim. Biophys. Acta 77:157–159.