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Lithocholic Acid Side-chain Cleavage to Produce 17-Keto or 22-Aldehyde Steroids by *Pseudomonas putida* strain ST-491 Grown in the Presence of an Organic Solvent, Diphenyl Ether

Yasumasa SUZUKI, Noriyuki DOUKYU, and Rikizo AONO†

Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta 4259, Yokohama 226-8501, Japan

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We devised a method to screen for microorganisms capable of growing on bile acids in the presence of organic solvents and producing organic solvent-soluble derivatives. *Pseudomonas putida* biovar A strain ST-491 isolated in this study produced decarboxylated derivatives from the bile acids. Strain ST-491 grown on 0.5% lithocholic acid catabolized approximately 30% of the substrate as a carbon source, and transiently accumulated in the medium androsta-1,4-diene-3,17-dione in an amount of corresponding to 5% of the substrate added. When 20% (v/v) diphenyl ether was added to the medium, 60% of the substrate was converted to 17-keto steroids (androst-4-ene-3,17-dione-like steroid, androsta-1,4-diene-3,17-dione) or a 22-aldehyde steroid (pregna-1,4-dien-3-on-20-al). Amounts of the products were responsible for 45, 10, and 5% of the substrate, respectively. In the presence of the surfactant Triton X-100 instead of diphenyl ether, 40% of the substrate was converted exclusively to androsta-1,4-diene-3,17-dione.

Key words: cleavage of steroid side-chain; lithocholic acid; *Pseudomonas putida*; two-phase persolvent culture system; organic solvent

Organic solvents are used widely in the chemical industry as resolvents for water-insoluble compounds. However, organic solvents have not been frequently used in the fermentation industry because of their toxicity for many microorganisms. In recent years, highly organic solvent-tolerant strains of microorganisms have been isolated.[1-6] Progressive usage of organic solvent-tolerant microorganisms would develop a new field in the fermentation industry.

Organic solvent-tolerant microorganisms have the potential advantage of possible applications in microbial conversion of water-insoluble compounds. For bioconversion of organic compounds with low solubility in water, large volumes of appropriate medium are required for solubilization of the compounds. This consumption of medium or water and the inevitable treatment of the waste-water constitute one of the major cost factors in bioconversion fermentation. If the water-insoluble compounds were suspended in a small volume of the medium, however, a long reaction period would be required to complete the conversion. Although such compounds can be dissolved in media by emulsifying with surfactants, conversion products must be separated from the media. These difficulties could be solved by the appropriate use of organic solvent-tolerant microbes.

There is an increasing range of applications in which water-insoluble organic compounds are used as substrates in bioreactors containing growing or resting bacterial cells.[7-10] We previously found that a cyclohexane-tolerant and cholesterol-converting microorganism, *Pseudomonas* sp. ST-200, effectively oxidized cholesterol dissolved in an organic solvent overlaying the medium.[11] Among the oxidized products, the predominant product was altered in response to a change in the organic solvent overlaying the medium.[12] Cholesterol, the substrate used in our previous investigation of bioconversion in a two-phase system is soluble in organic solvents. In this study, we were interested to examine the use of two-phase fermentation systems for bioconversion of a substrate with low solubility in aqueous media and organic solvents.

Microbial cleavage of the side chains of steroids has received much attention, since it allows the use of inexpensive steroids as raw materials for production of steroid hormones. Several reviews have been published on this bioconversion.[13] We planned to use a two-phase fermentation system for cleavage of the side chains of organic solvent-insoluble steroids. Deoxycholic acid and lithocholic acid have C-24 carboxyl groups on their side chains. These bile acids are insoluble in hydrophobic organic solvents due to the polar carboxyl groups. We devised a convenient method to screen for microorganisms capable of cleavage the side chains of bile acids and production of steroid hormone precursors. Effects of organic solvents on the cleavage of lithocholic acid were examined for a microorganism isolated by the method.

This report describes the isolation of *Pseudomonas putida*, a strain ST-491 tolerant of some organic solvents, and activated conversion of lithocholic acid to steroid hormone precursors upon the addition of diphenyl ether.

Materials and Methods

Organism. The bacterial strain used was *P. putida* strain ST-491, isolated from a sample of humus soil in this study.

† To whom correspondence should be addressed. Fax: +81-45-924-5819; E-mail: raono@bio.titech.ac.jp

Media. Screening for an organism tolerant of organic solvents and capable of converting deoxycholic acid to other products was done using DOC medium, consisting of 0.5% sodium deoxycholate, 0.1% $(NH_4)_2SO_4$, 0.05% Bacto Yeast Extract (Difco Laboratories, Detroit, MI), 25 mM Na_2HPO_4, and 25 mM KH_2PO_4. The initial pH of this medium was adjusted to 8.5 with NaOH. Strain ST-491 was grown in LIC medium, consisting of 0.5% lithocholic acid, 0.1% $(NH_4)_2SO_4$, 2% (vol/vol) Stanier's vitamin free mineral base solution,[14] 25 mM Na_2HPO_4, and 25 mM KH_2PO_4. The initial pH of this medium was adjusted to 8.0 with NaOH. LB agar medium, consisting of 1% Bacto Tryptone (Difco), 0.5% Bacto Yeast Extract, 1% NaCl, and 1.5% (wt/vol) agar, was used for single-colony isolation of microorganisms and for counting viable cell numbers. The organic solvent tolerance of ST-491 was examined on LBMg agar, which was LB agar containing 10 mM $MgSO_4$.

Isolation of the deoxycholate-converting microorganism. A small amount of soil sample was added to 5 ml of the screening medium overlaid with 1 ml of a hydrophobic organic solvent. The culture was incubated with shaking (160 rpm) at 30°C for 7 days. Samples (3 μl) were withdrawn separately from the solvent and medium layers of the culture that had become turbid, and were spotted onto a 0.2 mm-thick silica gel thin layer plate (E. Merck AG; no. 60F254). The sample was developed by ascending chromatography in chloroform-methanol-water (65:25:4, vol/vol/vol) at room temperature. Deoxycholic acid derivatives were detected by charring after spraying the plate with 35% (wt/vol) sulfuric acid.

From the medium of a culture in which deoxycholic acid derivatives were detected, microorganisms were isolated by repeated single-colony isolation on LB agar medium.

Characterization of the deoxycholate-converting microorganism. The morphological properties and taxonomic characteristics of the bacterial isolate were examined by the methods described in references.[14-18]

Purification of derivatives produced from lithocholic acid. Strain ST-491 was grown at 30°C in 100 ml of LIC medium with 20 ml of diphenyl ether by rotation at 160 rpm for 15 days. The solvent was filtered through phase separation filter paper (2S-filter; Toyo Roshi International Inc., Tokyo, Japan). The filtrate (5 ml) was put on a column (4.5 by 1.5 cm) of dry silica gel (E. Merck; 90–230 mesh), the column was washed with 60 ml of *n*-hexane and then eluted with 120 ml of diethyl ether. The diethyl ether elute was concentrated in a rotary evaporator. The residue was put on a 2-mm thick silica gel plate (E. Merck; no. 60F254) then developed zonally in diethyl ether twice. After chromatography, each compound was located on the silica plate by UV illumination. The compounds were recovered with chloroform from the silica powder scraped from central regions of the UV-absorption-positive zones.

Measurement of lithocholic acid and its derivatives. A sample obtained from the organic solvent layer was diluted 10-fold with isopropanol, and analyzed by normal-phase chromatography on a column (4.6 by 250 mm) of silica gel (Cosmosil-5SL; Nacalai Tesque, Tokyo, Japan) attached to an HPLC apparatus. The column was eluted with *n*-hexane-isopropanol (4:1, vol/vol) at a flow rate of 1.2 ml/min. Elution was monitored by measuring A_{254}. To measure each derivative, the relevant compound obtained commercially or purified as described below was used as the standard.

To measure lithocholic acid, a sample (0.2 ml) was taken from the medium layer and dried *in vacuo* over solid NaOH at 25°C. The residual matter was dissolved in methanol. This solution was analyzed by reverse-phase chromatography on a column (4.6 by 250 mm) of octadecylsilica gel (ODS-1251-H; Senshu Science Co., Tokyo, Japan) attached to an HPLC apparatus. The column was eluted with acetonitrile-methanol (1:1, vol/vol) at a flow rate of 0.4 ml/min. elution was monitored by measuring A_{220}.

Measurement of the molecular weights of the derivatives. The molecular weights of the derivatives were measured by low-resolution electron impact mass spectrometry (Shimadzu QP-5000). The samples were ionized at 230°C and at 70 eV.

UV absorption spectra of the derivatives. The samples were dissolved in 95% (vol/vol) ethanol. UV absorption spectra of the derivatives were recorded with a Hitachi 220A spectrophotometer.

NMR spectra of the derivatives. The sample was dissolved in $CDCl_3$ at a concentration of about 5–20 mg/ml. ^1H nuclear magnetic resonance (^1H-NMR) spectra were recorded at 20°C using a 200-MHz NMR spectrometer (Varian; model Gemini-200). ^{13}C-NMR spectra were taken using a 50-MHz NMR spectrometer (Varian; model Gemini-200) with complete decoupling. Tetramethylsilane was used as the internal standard for the spectra.

Materials. Deoxycholic acid and androsta-1,4-diene-3,17-dione were purchased from Nacalai Tesque, Kyoto, Japan. Lithocholic acid was a product of Wako Pure Chemical Industries, Osaka, Japan.

Results and Discussion

Isolation of a microorganism tolerant of organic solvents and capable of production of organic solvent-soluble derivative from deoxycholic acid, a water-soluble bile acid

The main purpose of this study was assessment of the effects of organic solvents on microbial metabolism of a substrate with low solubility in both aqueous medium and organic solvents. Lithocholic acid was singled out as such a substrate. Media containing lithocholic acid were turbid despite the presence of an organic solvent layer. This insolubility is unfavorable for following the growth of a bacterium assimilating lithocholic acid. In

particular, it was impossible to evaluate microbial growth turbidometrically on lithocholic acid in the presence of a harmful organic solvent. This situation is unfavorable for screening of many samples to find lithocholic acid-assimilating bacteria.

Deoxycholic acid, a hydrophilic analogue of lithocholic acid, is soluble in water at alkaline pH. We screened soil samples for the presence of organic solvent-tolerant and steroid side-chain cleaving bacteria using a medium containing deoxycholic acid and an organic solvent. About 500 soil samples were put into two-phase culture systems consisting of slightly alkaline DOC medium and various organic solvents. Conversion of deoxycholic acid was found in only one culture overlaid with cyclooctane (Fig. 1). Most of the derivatives were recovered from the medium layer rather than from the cyclooctane layer, and only one compound (Rf 0.5) was partitioned in the cyclooctane layer. We assumed that this cyclooctane-soluble compound was generated by removal of the C-24 carboxyl group of deoxycholic acid.

Microorganisms transferred from the conversion-positive culture produced colonies with a uniform appearance on LB agar. It was likely that few kinds of microorganisms grew in the two-phase screening medium containing deoxycholic acid and cyclooctane. A deoxycholic acid-converting bacterium (ST-491) was isolated after single-colony isolation on LB agar.

Identification of the organic solvent-tolerant and deoxycholic acid-converting bacterium

Table 1 summarizes the microbiological characteristics of the deoxycholic acid-converting bacterium ST-491. The isolate was strictly aerobic, Gram negative, and motile. The cells were short rod-shaped, with two or three polar flagella. ST-491 formed cream-yellow colonies on LB medium at 30°C after incubation for 1 day. The colonies were circular, convex, entire, and smooth. Other microbiological characteristics of ST-491 are shown in Table 1. It was concluded that strain ST-491 belongs to *P. putida* according to Bergey's Manual of Determinative Bacteriology (ninth ed.).[18]

This bacterium grew on deoxycholic acid or lithocholic acid as the sole carbon source but not on organic sol-

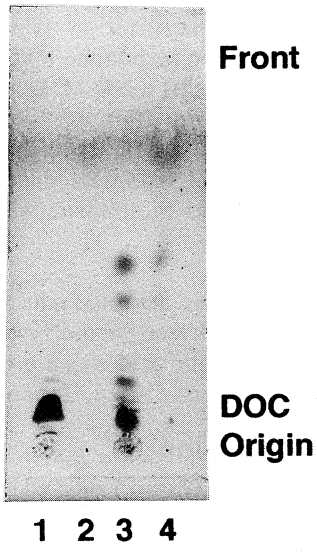

Fig. 1. Bioconversion of Deoxycholic Acid by Cyclooctane-Tolerant Bacteria.

Soil samples were incubated at 30°C for 7 days with shaking at 160 rpm in DOC medium overlaid with cyclooctane. Samples obtained from the medium and cyclooctane layers were spotted onto a silica gel thin-layer plate and developed in chloroform-methanol (15:1, vol/vol). Compounds were located by charring with sulfuric acid. DOC, deoxycholic acid. 1, medium layer before incubation; 2, cyclooctane layer before incubation; 3, medium layer after 7-days incubation; 4, cyclooctane layer after 7-days incubation.

Table 1. Microbiological Characteristics of the Side-chain Cleaving Microorganism, Strain ST-491

Characteristic	Finding for ST-491
Morphological[a]	
Form	Short rods
Cell size	0.8 to 1.2 by 1.5 to 2.5 μm
Gram stain	−
Motility	Motile
Flagella	Polar, 2–3
Sporulation	−
Cultural features[b]	
Reaction to oxygen	Strictly aerobic
Oxidation/fermentation test	Oxidative
Growth at:	
4°C	−
41°C	−
Production[c] of:	
Fluorescent pigment	+
Pyocyanine	−
Levan from sucrose	−
Reduction[d] of:	
Nitrate	−
Nitrite	−
Hydrolysis[c] of:	
Gelatin	−
Growth[c] on:	
Glucose	+
Mannose	+
Arabinose	+
Trehalose	−
Mannitol	+
Erythritol	−
Sorbitol	−
Adipate	−
Malate	+
Citraconate	−
Gluconate	+
2-Ketogluconate	+
β-Alanine	+
Hippurate	−
Caprate	+
Deoxycholic acid	+
Lithocholic acid	+
Cyclooctane	−
Diphenyl ether	−
Biochemical features[d]	
Oxidase activity	+
Catalase activity	+
Urease	+
Lecithinase	−
Arginine dihydrolase	+
GC content of DNA[e]	62.3%

Experiments were done according to the references: a,[15] b,[16] c,[14] d[17] and e.[22]

vents, such as cyclooctane or diphenyl ether. ST-491 grew in the presence of various organic solvents, such as cyclooctane, *n*-hexane, or *p*-xylene, when grown on LBMg agar medium.

Identification of the major derivatives produced from lithocholic acid, a water-insoluble bile acid, in the presence of an organic solvent

When ST-491 was grown in the two-phase system consisting of LIC medium and diphenyl ether, several UV-absorbing derivatives were produced from lithocholic acid not adsorbing UV and accumulated in the organic solvent layer (Fig. 2). None was found in the aqueous medium layer. Lithocholic acid contained in the medium is not observed as a UV-absorbing spot. These hydrophobic derivatives are designated LIC-A, -B, and -C in this paper.

The molecular weights of derivatives LIC-A, -B, and -C were 326, 286, and 284, respectively, that is 50, 90, or 92 lower than that of the substrate, lithocholic acid (M_r 376). On the basis of these molecular weights, it was concluded that the derivatives were generated by degradation of lithocholic acid. The λ_{max} values measured in 95% (vol/vol) ethanol were as follows: 244 nm, LIC-A and -C; 238 nm, LIC-B; and 220 nm, lithocholic acid. The values were 236 nm for authentic androst-4-ene-3,17-dione (4AD) and 244 nm for androsta-1,4-diene-3,17-dione (ADD). These λ_{max} values suggested that the A-ring structures of the derivatives were 1,4-dien-3-one in LIC-A and -C, and 4-en-3-one in LIC-B.

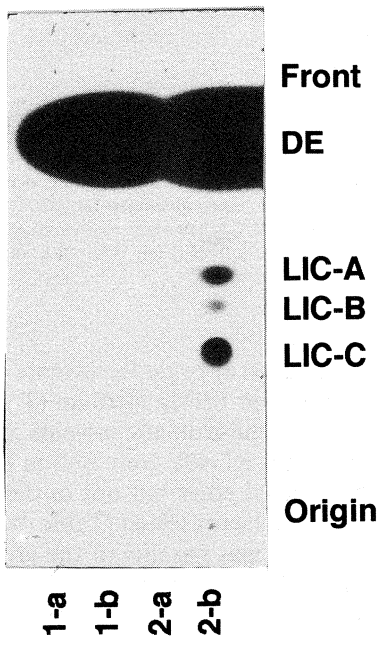

Fig. 2. Bioconversion of Lithocholic Acid by *P. putida* Strain ST-491.

Strain ST-491 was grown in a two-phase system consisting of LIC medium and diphenyl ether with shaking at 160 rpm for 15 days. Before (1) and after (2) cultivation, samples were taken from the medium (a) and diphenyl ether (b) phases. The samples were developed on a fluorescent silica gel thin-layer plate in diethyl ether. Lithocholic acid derivatives were observed on a fluorescent background under UV illumination. DE, diphenyl ether; Derivatives LIC-A, -B, and -C were named in order of Rf.

Table 2. Assignments of ^{13}C-NMR Chemical-shift Values for the Derivatives[a]

Position	LIC-A	LIC-C	ADD
1	128.03	127.66	127.66
2	156.24	155.23	155.23
3	186.88	186.15	186.17
4	124.37	124.09	124.09
5	169.58	168.22	168.22
6	34.00	32.49	32.48
7	36.93	31.12	31.12
8	35.98	35.03	35.03
9	55.23	52.20	52.20
10	43.71	43.36	43.35
11	23.24	21.86	21.86
12	33.30	32.24	32.48
13	49.88	47.63	47.62
14	52.73	50.34	50.34
15	25.20	22.03	22.02
16	39.62	35.58	35.57
17	51.33	219.91	219.90
18	13.88	13.75	13.75
19	19.18	18.67	18.67
20	44.02		
21	12.92		
22	205.26		

[a] Spectra were taken at 50 MHz in $CDCl_3$. Chemical shift values are expressed in ppm downfield from an internal standard, tetramethylsilane.

Table 3. Assignments of ^{1}H-NMR Chemical-shift Values for the Derivatives[a]

Position	H (ppm)	Multiplicity	J_{HH} (Hz)
LIC-A			
1-H	7.05	d	10
2-H	6.24	d, d	2, 10
4-H	6.08	s	
18-Me	0.79	s	
19-Me	1.23	s	
22-H	9.53	d, d	4, 10
LIC-C			
1-H	7.05	d	10
2-H	6.25	d, d	2, 10
4-H	6.10	s	
18-Me	0.95	s	
19-Me	1.27	s	
ADD			
1-H	7.06	d	10
2-H	6.25	d, d	2, 10
4-H	6.09	s	
18-Me	0.95	s	
19-Me	1.26	s	

[a] Spectra were taken at 200 MHz in $CDCl_3$. Chemical shift values are expressed in ppm downfield from an internal standard, tetramethylsilane.

^{13}C-NMR spectra of the derivatives were measured in $CDCl_3$ (Table 2). Derivatives A and C showed 22 and 19 signals, respectively. Chemical shift values of LIC-C were the same as those of authentic ADD. The ^{1}H-NMR spectrum of LIC-C was also identical with that of ADD (Table 3). Thus, LIC-C was identified as ADD.

In the ^{13}C-NMR spectrum of LIC-A, the chemical shift of the C-22 signal was 205.3 ppm, indicating that this carbon was in a carbonyl group. Multiplicity analy-

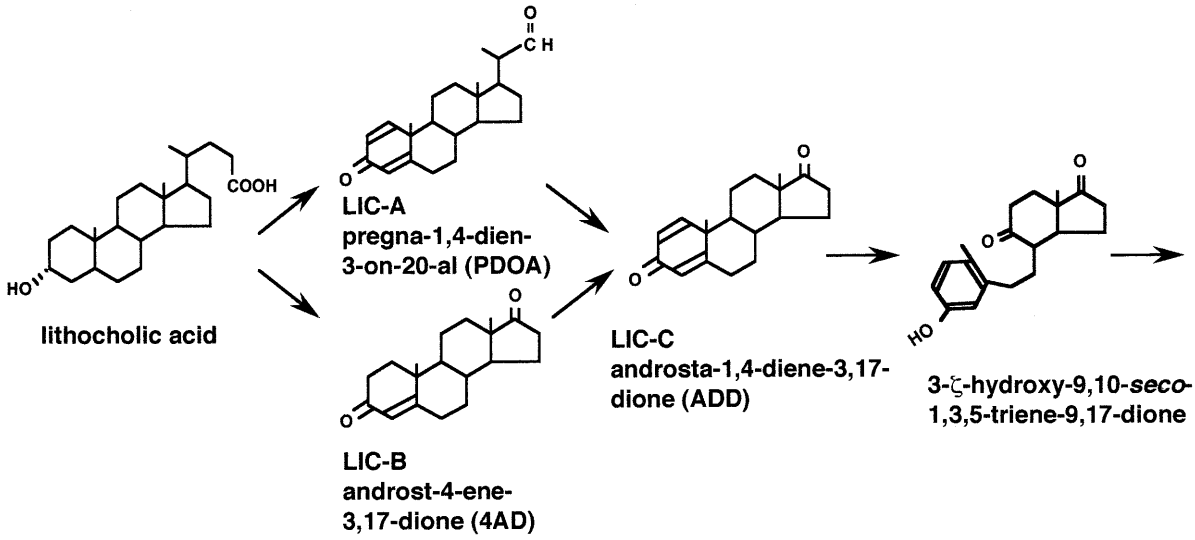

Fig. 3. Proposed Scheme of Conversion of Lithocholic Acid by *P. putida* Strain ST-491.

sis of 22-H indicated that C-22 was an aldehyde group, not a ketone or carboxyl group (Table 3). Therefore, LIC-A was concluded to be pregna-1,4-dien-3-on-20-al (PDOA).

The ^{13}C-NMR spectrum of LIC-B was not measured because its recovery was too low to measure the spectrum. On silica gel thin-layer chromatography, Rf values of LIC-B and 4AD were the same in chloroform-methanol-water (65:24:4, vol/vol/vol; Rf 0.71), *n*-hexane-isopropanol (4:1, vol/vol; Rf 0.34), and diethyl ether (Rf 0.28). Therefore, LIC-B was thought to be 4AD. Although a further analysis may be required, this derivative is referred as 4AD-like steroid in this article.

The results described here show that the screening method enabled us to isolate microorganisms capable of cleavage of the side chains of the bile acids. Degradation pathways of steroids have been reported.[13,19-21] The C-24 carboxylic acid side chain has been proposed to be degraded via C-22 carboxylic acid to C-17 keto steroid. We found C-22 aldehyde but not C-22 carboxylic acid among the major products recovered from the diphenyl ether layer. The putative C-22 carboxylic acid was probably not extractable with this solvent. Among the deoxycholic acid derivatives (Fig. 1), we found 12ζ-hydroxyandrosta-1,4-diene-3,17-dione and 3,12-ζ-dihydroxy-9,10-*seco*-1,3,5-triene-9,17-dione (results not shown). As suggested by previous reports and our results, it seems likely that ST-491 cleaves the side chain of lithocholic acid as shown in Fig. 3. This cleavage reaction is similar to β-oxidation catabolism of fatty acids.

Selection of the organic solvent to overlay on LIC medium to recover lithocholic acid derivatives

In this study, ST-491 was grown in LIC-medium overlaid with an organic solvent layer to recover the lithocholic acid derivatives produced by the organism. An organic solvent in which the products are highly soluble is probably preferable for effective extraction of the products from the medium layer. We examined the solubility of ADD in several organic solvents tolerated by ST-491 when grown on LBMg medium (Table 4). ADD was highly soluble in the aromatic solvents among the organic solvents tested. ST-491 grew well in LIC medium overlaid with diphenyl ether but not in the presence of the other aromatic solvents tested (Table 4). Production of ADD and PDOA was parallel to the growth (results not shown). Therefore, we overlaid LIC-medium with diphenyl ether to extract the derivatives.

Table 4. Selection of Organic Solvent

Solvents	ADD solubility[a] (mg/ml)	growth of ST-491[b]
aromatic solvents		
p-xylene	38.5	−
mesitylene	41.9	±
diphenyl ether	33.4	+
alicyclic solvents		
cyclohexane	0.2	−
cyclooctane	0.3	+
aliphatic solvents		
n-hexane	0.1	−
n-octane	0.1	+

[a] A large amount of ADD was added to a two-phase system of water (2 ml) and each organic solvent (400 μl). This mixture was shaken at 30°C overnight. The organic solvent layer was assayed for ADD.

[b] ST-491 was incubated at 30°C in a two-phase system consisting of LIC medium (5 ml) and the organic solvent (1 ml). After 24 h, the viable cells were counted.

Effects of diphenyl ether on conversion of lithocholic acid by P. putida ST-491

ST-491 was grown in 100 ml of LIC medium containing 500 mg (1.33 mmol) of lithocholic acid together with 20 ml of diphenyl ether (Fig. 4A). Lithocholic acid suspended in the medium layer was consumed and the conversion products accumulated gradually in the diphenyl ether layer. After 13 days, the products recovered from the diphenyl ether layer were as follows: ADD, 170 mg

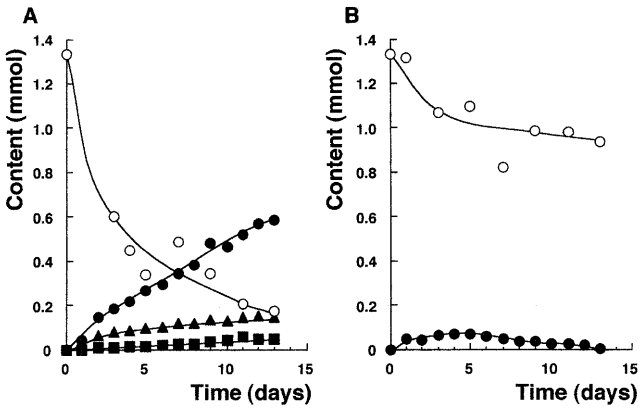

Fig. 4. Conversion of Lithocholic Acid by Strain ST-491 in the Presence of Diphenyl Ether.

Strain ST-491 was grown at 30°C with shaking at 160 rpm in 100 ml of LIC medium containing 1.33 mmol of lithocholic acid. (A) To the medium, 20 ml of diphenyl ether was added. (B) No solvent was added. Periodically, samples were taken from the medium and solvent layers, and assayed for lithocholic acid and derivatives. Symbols: ○, lithocholic acid; ●, ADD; ▲, PDOA; and ■, 4AD-like steroid.

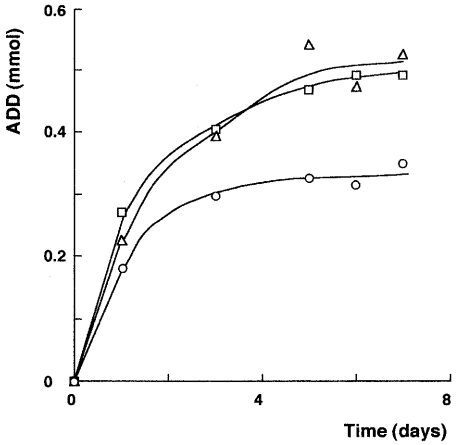

Fig. 5. Effects of Triton X-100 on Conversion of Lithocholic Acid.

Strain ST-491 was grown at 30°C with shaking at 160 rpm in 100 ml of LIC medium containing 1.33 mmol lithocholic acid and 0.5% (○), 1.0% (△), or 1.5% (□) Triton X-100. ADD was measured using samples taken periodically from the medium.

(0.60 mmol); PDOA, 48 mg (0.15 mmol); and 4AD-like steroid, 14 mg (0.05 mmol). The total amounts of these products corresponded to 60% (mol/mol) of the lithocholic acid added. At the time, 0.19 mmol lithocholic acid remained in the medium.

When diphenyl ether was not added to the medium, approximately 350 mg (0.94 mmol) of the lithocholic acid remained in the medium (Fig. 4B). This measurement might contain some error because samples were taken from a non-homogeneous lithocholic acid suspension. However, it was clear that most of the lithocholic acid added was not metabolized by ST-491. PDOA and 4AD-like steroid were not detected during the culture period. Only 19 mg (0.07 mmol) of ADD was produced in the medium by 5 days. This amount corresponded to only 5% (mol/mol) of the lithocholic acid added. Thereafter, ADD was gradually consumed.

These results suggested that ST-491 metabolized approximately 150 mg (0.4 mmol) of lithocholic acid as a carbon source in the presence or absence of the organic solvent. In the presence of diphenyl ether, about 300 mg (0.8 mmol) of the lithocholic acid was converted to 17-keto steroids or 22-aldehyde steroid. ST-491 seems to catabolize lithocholic acid via ADD, as shown in Fig. 3. It is likely that diphenyl ether facilitates catabolism from lithocholic acid to ADD rather than suppressing the assimilation of ADD.

Conversion of lithocholic acid to ADD by ST-491 in the presence of Triton X-100

Surfactants are often used to facilitate microbial conversion by emulsifying water-insoluble substrates. ST-491 was grown in LIC medium containing Triton X-100 without any organic solvent (Fig. 5). The effect of Triton X-100 on lithocholic acid conversion by ST-491 was different from that of diphenyl ether. PDOA and 4AD-like steroid were not produced in this monophasic culture system. ST-491 converted lithocholic acid exclusively to ADD in the presence of Triton X-100. The conversion rate and level of ADD production were maximum in the presence of 1.5% Triton X-100. At this concentration of surfactant, ADD production was more rapid than that found in the presence of diphenyl ether, although the final yield of ADD in each case was similar.

Therefore, one of the most advantageous merits to convert lithocholic acid by ST-491 in the presence of diphenyl ether is readiness to recover the conversion products rather than effectiveness to produce ADD, compared with the conversion in the medium emulsified with the surfactant. At present, it is not clear how diphenyl ether facilitates the side-chain cleaving reaction. Diphenyl ether might contribute to extraction of conversion intermediates, such as 4AD and PDOA, from the ST-491 cells. The conversion system is likely to be appropriate for PDOA production.

The results described here intimate that latent capabilities of microorganisms can be revealed by the addition of appropriate organic solvents. Previously, we showed that cholesterol, an organic solvent-soluble substrate, was effectively oxidized in the presence of appropriate organic solvents.[11] The results described here indicate that a two-phase fermentation system can be used also for bioconversion of a compound with low solubility in organic solvents.

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