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Microbial hydrolysis of 7-xylosyl-10-deacetyltaxol to 10-deacetyltaxol

Kang Wang[a,1], Tingting Wang[a,b,1], Jianhua Li[a], Jianhua Zou[a], Yongqin Chen[b,*], Jungui Dai[a,*]

[a] Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College (Key Laboratory of Biosynthesis of Natural Drugs, Ministry of Public Health; Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education), 1 Xian Nong Tan Street, Beijing 100050, People's Republic of China
[b] Faculty of Life Sciences, Hubei University, Wuhan 430062, People's Republic of China

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ABSTRACT

Enterobacter sp. CGMCC 2487, a bacterial strain isolated from the soil around a *Taxus cuspidata* Sieb. et Zucc. plant, was able to remove the xylosyl group from 7-xylosyltaxanes. The xylosidase of this strain was an inducible enzyme. In the bioconversion of 7-xylosyl-10-deacetyltaxol (7-XDT) to 10-deacetyltaxol (10-DT), for the purpose of enhancing the conversion efficiency, the effects of NH_4^+, oat xylan, temperature, pH value, cell density and substrate concentration on the bioconversion have been systematically investigated. 3.0 mM NH_4^+, 0.6% oat xylan in the media could enhance the yield of 10-DT; the optimum biocatalytic temperature was 26 °C and optimum pH value was 6.0. The highest conversion rate and yield of 10-DT from 7-XDT reached 92% and 764 mg/L, respectively. In addition, the biocatalytic capacity of the cell cultures remained 66.1% after continuous three batches. These results indicate that converting 7-XDT to 10-DT, a useful intermediate for the semisynthesis of paclitaxel or other taxane-based anticancer drugs by a novel bacterial strain, *Enterobacter* sp. CGMCC 2487, would be an alternative for the practical application in the future.

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1. Introduction

The diterpene paclitaxel (Taxol®, Fig. 1), first isolated by Wall and Wani from Pacific yew, *Taxus brevifolia* Nutt. [1], is an effective anticancer drug for treatment of a variety of human cancers [2]. Although the paclitaxel content in Pacific yew bark ranges from 0.01% to 0.08%, the average isolated yield is in the range of 0.014–0.017% [3]. About 16,000 pounds of bark is required to produce 1 kg paclitaxel [3]. This fact led to development programs for alternative sources of paclitaxel and a lot of important progress has been achieved [4–6]. Additionally, the report indicated that the content, 7-xylosyl-10-deacetyltaxol (7-XDT, Fig. 1), one of its analogues, is higher than that of paclitaxel in some *Taxus* plants [7]. The similar result has been achieved in our laboratory, which the content of 7-XDT in ethanol extract of twigs and leaves of *Taxus yunnanensis* was 2.98% (w/w), about five and three times higher than those of paclitaxel (0.50%) and 10-deacetyltaxol (10-DT, 0.74%), respectively (data not shown). Thus, converting this compound to the more valuable intermediate, 10-DT, for the semisynthesis of paclitaxel through a C-10 acetylation is of interest. The removal of the sugar moiety by chemical method has been reported [8], but the process was accompanied by side reactions, especially the epimerization of 7-OH group, as well as environmental pollution. Therefore, microbial/enzymatic hydrolysis is an alternative because of its high selectivity, mild condition and environmental benignancy. Concerning these points, Hanson et al. of Bristol–Myers Squibb reported their excellent work on the enzymatic hydrolysis of 7-xylosyltaxanes by xylosidase from *Moraxella* sp., a bacterial strain isolated from soil [5]. The maximum yield of 10-DT from 7-XDT was about 300 mg/L, not so high for the industrial process yet. Therefore, searching more efficient strain and optimizing bioconversion process to carry out this reaction is still necessary. Herein, we report a bacterial strain isolated from the soil around a *Taxus cuspidata* plant with the ability to remove the sugar moiety in 7-XDT molecule selectively, and the systematic investigations of some factors on the yield and conversion rate of 10-DT. After process optimization, the maximum conversion rate and yield of 10-DT reached 92% and 764 mg/L, respectively. It might be promising for the future practical use.

2. Materials and methods

2.1. General experimental procedures

The ^1H and ^{13}C NMR spectra were recorded on Bruker ARX-400 spectrometers using $CDCl_3$ as solvent and internal reference. ESIMS spectra were obtained using a VG ZabSpec mass spectrometer. Analytical HPLC was carried out on an Agilent 1200 with a

* Corresponding authors. Tel.: +86 10 63165195; fax: +86 10 63017757.
E-mail addresses: chenyongqin03@yahoo.com (Y. Chen), jgdai@imm.ac.cn (J. Dai).
[1] These two authors contributed this work equally.

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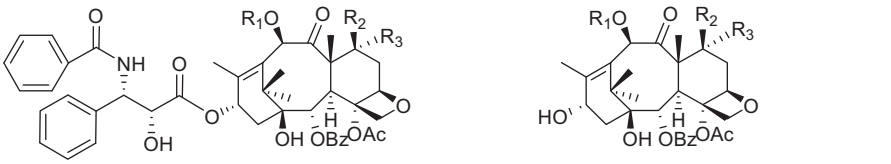

R_1=OAc, R_2=OH, R_3=H, paclitaxel
1 R_1=OH, R_2=O-β-D-xylose, R_3=H, 7-XDT
2 R_1=OH, R_2=OH, R_3=H, 10-DT
3 R_1=OH, R_2=H, R_3=OH, 7-*epi*-10-DT
4 R_1=OH, R_2=OH, R_3=H, 10-DAB
5 R_1=OH, R_2=O-β-D-xylose, R_3=H, 7-XDB

Fig. 1. The chemical structures of paclitaxel, 7-XDT (**1**) and its bioconverted products (**2–5**) by *Enterobacter* sp. CGMCC 2487.

BDS HYDERSIL column (C_{18}, 5 μm, 250 mm × 4.6 mm i.d., the flow-rate was 1 mL/min), the UV detector was set at 230 nm, and the column was operated at 30 °C. HPLC analysis was achieved with a two-pump gradient program for pump A (solution A: MeCN) and pump B (solution B: water) as follows: 30% solution A, ramped to 38% solution A within 12 min, then to 52% solution A until 30 min; held at 52% solution A for 2 min, then reset to 30% solution A until 40 min for equilibrating the column and stabilizing the baseline before the next injection. Semi-preparative reverse-phase HPLC was performed on a Shimadzu LC-6AD instrument with an YMC-Pack ODS-A (5 μm, 250 mm × 10 mm i.d., the flow-rate was 2 mL/min) and a Shimadzu RID-10A detector. Si gel (200–300 mesh) was used for flash column chromatography. Analytical TLC was carried out on Si gel GF254 plates (Qingdao Oceanic Chemicals, China), and the visualization of TLC plates was performed by spraying with 5% H_2SO_4 in EtOH followed by heating at 105 °C. A Finnigan LCQ Advantage ion trap mass spectrometer (Thermo Finnigan, USA) was connected to the Agilent 1200 HPLC instrument via an ESI interface. The LC effluent was introduced into the ESI source in a post-column splitting ratio of 10:1. Ultrahigh-purity helium (He) was used as the collision gas and high-purity nitrogen (N_2) as the nebulizing gas. The optimized parameters in the positive ion mode were as follows: ion spray voltage, 4.5 kV; capillary temperature, 325 °C; sheath gas (N_2), 6 vol./min. For full scan MS analysis, the spectra were recorded in the range of *m*/*z* 750–1050. HPLC grade acetonitrile (Fisher) and ultra-pure water were used for all analyses. AR grade methanol, ethyl acetate, dichloromethane were purchased from Beijing Chemical Corporation (Beijing, China). The substrates, 7-XDT, 7β-D-xylosyl-10-deacetylcephalomannine and 7β-D-xylosyl-10-deacetyltaxol C were gifted from Guilin Hui'ang Biochemistry Medicine Industry Co. Ltd., Guilin, China. The conversion rate of 10-DT was calculated by the molar ratio of obtained amount of 10-DT to the added amount of 7-XDT. All aliquots were treated in 3 replicates, and data were the means ± SD (standard deviation) and compared using Student's *t*-test.

2.2. Microorganism isolation and identification

Soil samples 5 cm-deep beyond ground were collected around a *T. cuspidata* plant in the Botanical Garden of Beijing, China. A 1.0 g of soil sample was added into an Erlenmeyer flask containing 99 mL of enriched medium with 10 g/L of oat xylan (Sigma), 1 g/L of peptone, 1 g/L of yeast extract and several glass beads. The flasks were shaken at 120 rpm in the dark at 24 °C. After 2 h of incubation followed by resting for 15 min, the cultured mixture was diluted in a 10-fold dilution series (10^{-4} to 10^{-6}) of concentrations successively by sterilized distilled water. 0.1 mL of each concentration of cultured mixture was plated on a medium containing 1.2 g Ca_2Cl_2, 0.5 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.2 g NaCl, 0.1 g K_2SO_4, 1.0 g K_2HPO_4, 10 g oat xylan and 20 g agar in 1000 mL distilled water. After 4–5 days' incubation at 24 °C, plates were overlaid with 0.8% agarose containing 1 mM 4-methylumbelliferyl-β-D-xyloside. Colonies those showed fluorescence under 360 nm UV lamp after 1–2 min, were transferred to slants containing wheat bran medium [50 g of wheat bran, 2.0 g of K_2HPO_4, 4.0 g of $(NH_4)_2SO_4$, 1000 mL of distilled water]. A loopful of inoculum from the plates was grown in a 250 mL shake flask containing 50 mL of wheat bran medium. After two days' cultivation at 25 °C, 2 mg of 7-XDT in 0.25 mL DMF was added. The incubation was continued for one week, then the broth was extracted with ethyl acetate (1× 100 mL) and analyzed by TLC [developing solution: under layer solution of the mixture of $CHCl_3$–MeOH–H_2O/7:2:1 (v/v/v)], HPLC and LC–MS. The strain with the highest yield among 4 strains possessed the ability to remove the sugar group from 7-XDT was selected for the next experiments and identified as *Enterobacter* sp. by CICC (China Center of Industrial Culture Collection, Beijing, China) using 16S rRNA sequence analysis and deposited at CGMCC (China General Microbiological Culture Collection Center) with the designated number of CGMCC 2487. The procedure of incubation, extraction and analyses of 7β-D-xylosyl-10-deacetylcephalomannine and 7β-D-xylosyl-10-deacetyltaxol C with this strain, was performed as above described.

2.3. Preparative biotransformation of 7-XDT

A two-stage fermentation procedure was used. A 1 mL sample of 2-day-old seed culture was added to one flask (350 mL of wheat bran medium per 1000-mL flask), and 500 mg substrate in 15 mL DMF was evenly distributed among 35 flasks after 2 days' cultivation. After incubating for additional 7 days, the cultures were pooled and filtered under reduced pressure. The filtrate was saturated with NaCl and extracted with ethyl acetate (4× 15 L), and the dried cell mass was extracted with MeOH (4× 100 mL) by sonication for 30 min each time. The extracts were combined and concentrated under vacuum at 40 °C to afford 4.78 g residue. Then this residue was subjected to Si gel column chromatography eluting with a gradient CH_2Cl_2/MeOH to give 5 fractions. Further Si gel chromatography of fractions 1–3 by eluting with *n*-hexane/ethyl acetate (30/70, v/v) led to the isolation of compounds **2** (10-DT, 125.6 mg, ∼29%), **3** (7-*epi*-10-DT, 30 mg, ∼6.9%), **4** (10-deacetylbaccatin III, 10-DAB, 4.8 mg, ∼1.7%). Fractions 4 and 5 were subjected to reverse-phase semi-preparative HPLC (mobile phase: MeOH–MeCN–H_2O/20:25:55, v/v/v), and 4.3 mg of compound **5** (7-xylosyl-10-deacetylbaccatin III, 7-XDB, ∼1.2%), 193.0 mg of **1** (substrate, 7-XDT, ∼38.6%) were obtained. The products were determined by ^{1}H NMR, ^{13}C NMR, and mass spectra. Their spectroscopic data were in good accordance with those in Refs. [9–12].

2.4. The kinetics of growth and pH value of Enterobacter sp. CGMCC 2487

Wheat bran medium was used for the cell growth. A 1 mL sample of seed culture was inoculated to a 250-mL flask with 50 mL medium and rotated at 120 rpm at 26 °C, 37 °C, and 50 °C. Every 12 h, the pH values of the sampled broth were measured. The cells

were collected by centrifuging at 20,000 × *g* for 10 min, dried at 60 °C and weighted.

2.5. Bioconversion with growing cells

The two-stage procedure was performed, the cultivation and bioconversion were carried out in 250-mL flask with 50 mL wheat bran medium as described previously. The final concentration of substrate was 2.0 mg/50 mL (final concentration: 40.0 mg/L), and the reaction was quenched by adding 50 mL ethyl acetate and extracted three times totally. The pooled extract was evaporated under reduced pressure, then dissolved in 10 mL MeOH and filtered through 0.25 μM-pore-sized membranes just prior to HPLC analysis.

2.5.1. Effects of NH_4^+ on the conversion

Into basic medium, were added 0, 3.0, 6.0, 13.0, and 20.0 mM $(NH_4)_2SO_4$, respectively. After the above procedure, the resulting samples were analyzed by HPLC.

2.5.2. Effects of oat xylan on the bioconversion

0.04%, 0.06%, 0.08%, and 0.16% (w/v) of oat xylan were added into basic medium, respectively. After the above procedure, the resulting samples were analyzed by HPLC.

2.6. Bioconversion with resting cells

The cell cultured procedures were carried out as previously outlined, and the cells were collected by centrifuging at 10,000 × *g* at 4 °C, and then re-suspended in PBS buffer (50 mM) prior to bioconversion use. The procedures of incubation, extraction and analysis were performed as described before.

2.6.1. Effects of PBS buffer pH values on the bioconversion

A 0.1 g (dry weight) of fresh cells were suspended in 100 mL flask containing 25 mL PBS buffer with different pH values (2.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0), and 1.25 mg of 7-XDT (final concentration: 50 mg/L) in 50 μL DMF was added into each flask. After 7 days' incubation, the reactions were quenched by adding 25 mL ethyl acetate, and extracted thrice, then analyzed by HPLC.

2.6.2. The kinetics of bioconversion with resting cells

The experiments were performed on a 25-mL scale (100-mL flask, 50 mM pH 6.0 PBS buffer solution), shaking at 120 rpm at 26 °C. Every 24 h, three flasks were sampled randomly. The process of extract, concentration and HPLC analysis was followed as outlined before.

2.6.3. Effects of cell density on the bioconversion

In this experiment, the different cell densities (2.0, 4.0, 10.0, 20.0, and 40 g/L dry weight) were used for bioconversion in 50 mM PBS buffer (pH 6.0), the final added 7-XDT concentration was 250 mg/L (6.25 mg/25 mL reaction solution). The processes of incubation, extraction and analysis were performed as described before.

2.6.4. Effects of substrate concentration on the bioconversion

In this experiment, the cell density used for bioconversion was 40 g/L cells (dry weight) in 50 mM PBS buffer (pH 6.0), the final added substrate concentrations were designated to 250 mg/L, 500 mg/L, 1000 mg/L, 2000 mg/L, 5000 mg/L, and 10,000 mg/L. The procedures of incubation, extraction and analysis were performed as described before.

2.7. Continuous use of cells for bioconversion

The first reaction was carried as follows: 0.5 g (dry weight) of fresh cell mass, 6.25 mg 7-XDT, 25 mL 50 mM PBS buffer (pH 6.0) in a 100-mL flask (7-XDT final concentration: 250 mg/L), 7 days' incubation at 120 rpm at 26 °C. After first run, the cell mass was collected by centrifuging at 10,000 × *g* at 4 °C, washed by the same buffer (3× 5 mL), and re-suspended in the same buffer for the next run. The pooled supernatant of each run was extracted, analyzed by HPLC. The same process was performed three times.

2.8. Bioconversion of ethanol extract of T. yunnanensis

The content of 7-XDT in ethanol extract of *T. yunnanensis* was 2.98% by HPLC analysis. The bioconversion were performed as follows: 0.5 g (dry weight) of fresh cell mass, 210 mg extract (7-XDT final concentration: 250 mg/L), 25 mL 50 mM PBS buffer (pH 6.0) in a 100-mL flask, 7 days' incubation at 120 rpm at 26 °C. The procedures of incubation, extraction and analysis were performed as described before.

3. Results and discussion

Plants release enormous amounts of chemicals through their roots, at a significant carbon cost, to combat pathogenic microorganisms and attract beneficial ones [13]. Considering these rhizosphere interactions, soil samples over 5 cm depth from ground surface around *T. cuspidata* plants were collected for the strain screening. Following the procedure described in Section 2.2, 34 strains with xylosidase activity as indicated by hydrolysis of 4-methyl-umbelliferyl-β-D-xyloside to the fluorescent 4-methyl-umbelliferone were isolated. Then, these strains were further subjected to identify their effectiveness with 7-XDT. By the combined analyses of TLC, HPLC, LC/UV, LC/MS, four strains of them have been determined to possess the ability to convert 7-XDT to 10-DT. Especially, in the LC–MS spectrum, the quasi molecular ion peak of the product at *m/z* 812 $[M+H]^+$, 834 $[M+Na]^+$ and 850 $[M+K]^+$ were observed, indicating the loss of xyloxyl moiety (mass 132 unit). The strain which gave the highest yield of 10-DT was identified as *Enterobacter* sp. by morphologic and molecular characteristics, and the designated number was CGMCC 2487. This strain was selected for the further investigation.

Moreover, the other two 7β-D-xylosyltaxanes, 7β-D-xylosyl-10-deacetyl-cephalomannine ($[M+H]^+$ *m/z* 922) and 7β-D-xylosyl-10-deacetyltaxol C ($[M+H]^+$ *m/z* 938) were used as substrates for examining the substrate specificity of this xylosidase. It was verified by the TLC, HPLC and LC–MS analyses that this xylosidase can remove the xyloxyl moiety from these two molecules. In the LC–ESIMS spectra, were observed the quasi ion peaks of $[M+H]^+$ at *m/z* 790 and 806 responsible for the products 10-deacetyl-cephalomannine and 10-deacetyltaxol C with the loss of xyloxyl moiety. Thus, the xylosidase of this strain may take various 7β-D-xylosyltaxanes as its substrates.

Based upon the above results, for the case of further identification of the desired product and by-products, the transformation of 7-XDT by *Enterobacter* sp. was scaled up to 12 L incubation by two-stage fermentation procedure outlined in Section 2.3. Finally, along with 10-DT, the other three by-products (7-*epi*-10-DT, 10-DAB, 7-XDB) have been obtained in the yields of about 29%, 6.9%, 1.7%, and 1.2%, respectively (Fig. 1). All the structures of products were determined by ^1H NMR, ^{13}C NMR and MS spectroscopic data. In this biotransformation process, the undesired reactions, 7-OH epimerization and 13-side chain hydrolysis were also observed. For the purpose of further examining substrate-spectrum of the xylosidase of this strain, one by-product, 7-XDB was used as a substrate,

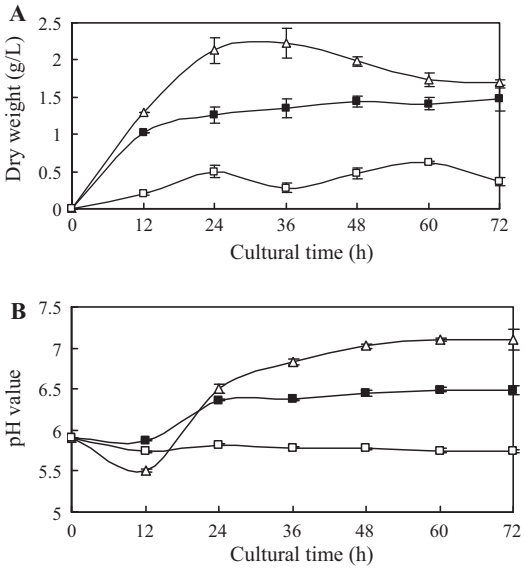

Fig. 2. The kinetics of the growth (A) and pH value (B) of *Enterobacter* sp. at different temperatures (△: 25 °C; ■: 37 °C; □: 50 °C).

the removal of sugar moiety from this molecule was also observed. These results further supported that the xylosidase of *Enterobacter* sp. CGMCC 2487 might hydrolyze the sugar moiety from other 7-xylosyltaxanes.

In order to investigate the effects of pH value and temperature on the growth of this bacterium, the kinetics of the growth and pH value of *Enterobacter* sp. at different temperatures were calculated. In this experiment, the cultural temperatures were designated at 26 °C, 37 °C, and 50 °C, the kinetics of the growth and pH value of *Enterobacter* sp. CGMCC 2487 at the three temperatures were shown in Fig. 2A and B. The growth of *Enterobacter* sp. was significantly influenced by cultural temperature, the optimum temperature was 26 °C with the maximum biomass of 2.23 g/L dry weight after 36 h of fermentation. During the growing period at 26 °C (Fig. 2B), the pH value decreased at the first 12 h, and increased sharply in the next 12 h, then increased slowly and maintained around 7.0. According to these results, the fermentation of this bacterium was performed at 26 °C in the next experiments.

In our early work, the bioconversions with growing cells were employed, and the effects of several factors on the bioconversion have been examined.

Knob and Carmona reported that the xylosidase of *Penicillium sclerotiorum* was remarkably stimulated by NH_4^+ at 10 mM, and indicated that NH_4^+ might be a cofactor for this enzyme [14]. Therefore, the effects of different concentrations of NH_4^+ on the enzyme activity were investigated. The results (Fig. 3) revealed that 3.0 mM NH_4^+ significantly increased the yield of 10-DT by more than two times.

Xylan often showed to be the best β-xylosidase inducer in different sources [15]. In our previous experiment, the xylosidase activity was only detected in the case of wheat bran as cultural medium, whereas not observed in the other two cultural media (1: LB medium; 2: PDA medium; data not shown). This suggested that xylosidase of this strain might be an inducible one. To confirm this deduction further, the different concentrations of oat xylan was added in the wheat bran medium. Through two-stage fermentation, the yield and conversion rate of 10-DT were examined by HPLC analysis. The results (Fig. 4) showed that xylan could enhance the yield and conversion rate of 10-DT. The conversion rate reached 73.7% when the concentration of oat xylan at 0.06%. Therefore, this xylosidase may be an inducible enzyme similar to other xylosidases, and xylan is an inducer.

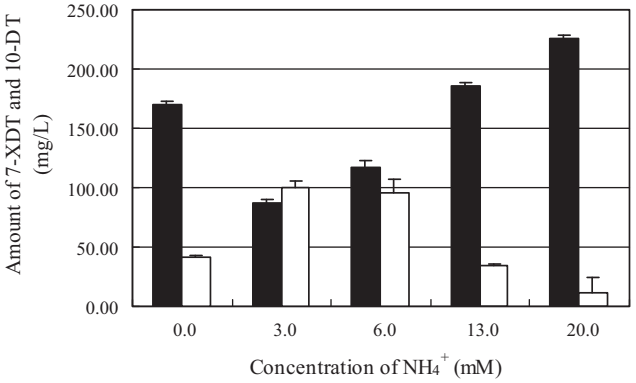

Fig. 3. Effects of NH_4^+ on the bioconversion (■: 7-XDT; □: 10-DT).

As described above, although a number of efforts to increase the yield of 10-DT have been attempted, the yield was still not so high for practical use. According to the above results, it was mainly resulted from two obstacles in the case of conversion by growing cells. One was that the enzyme amount (cell mass) cannot reach an enough level; the other was that the undesired side reactions (e.g. 7-OH epimerization, 13-side chain hydrolysis, etc.) occurred due to high pH value in the bioconversion system under no control. In order to overcome the obstacles and increase the yield of 10-DT, the strategy of biotransformation by resting cells has been carried out. In a two-step procedure, the first step is to collect cell mass by centrifuging after fermentation, the second step is to incubate substrate with resting cells under a controlled condition (e.g. a certain pH value and cell density).

Firstly, the effects of pH value on the conversion were investigated. The incubation of 7-XDT without/with cells in PBS buffer at eight different values have been performed. The influences of pH value on biotransformation are shown in Fig. 5. In the conversion system without cells, the side reaction, hydrolysis of C-13 side chain was observed when pH value above 6.0 (Fig. 5A). The enzyme activity was detected except at pH 2.0 and pH 10.0 in the presence of cells. The highest activity corresponding to the yield of 61% at pH 6.0 was observed, whereas the yields of by-products (7-*epi*-10-DT, 7-XDB) were very low. At higher pH value especially over pH 7.0, the yields of the two by-products increased, while the yield of 10-DT decreased. Thus, the favorable pH value for the xylosidase activity is at 6.0.

On the basis of the above results, the kinetics of bioconversion with resting cells was calculated. The kinetics of bioconversion with resting cells was illustrated in Fig. 6. As shown, the conversion continued throughout the 8 days' incubation. As a result, the yield and conversion rate of 10-DT increased with the incubation time. On the 8th day, the yield of 10-DT reached 35.2 mg/L, and the conversion

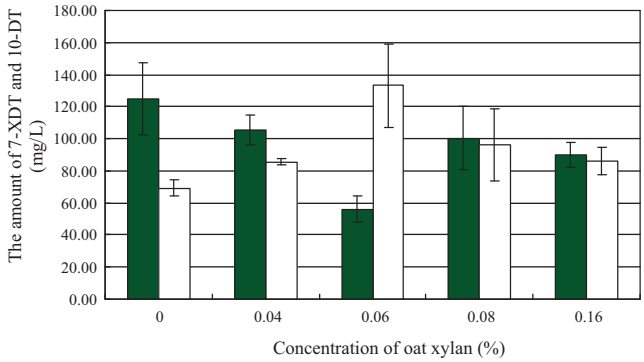

Fig. 4. Effects of oat xylan on the bioconversion (■: 7-XDT; □: 10-DT).

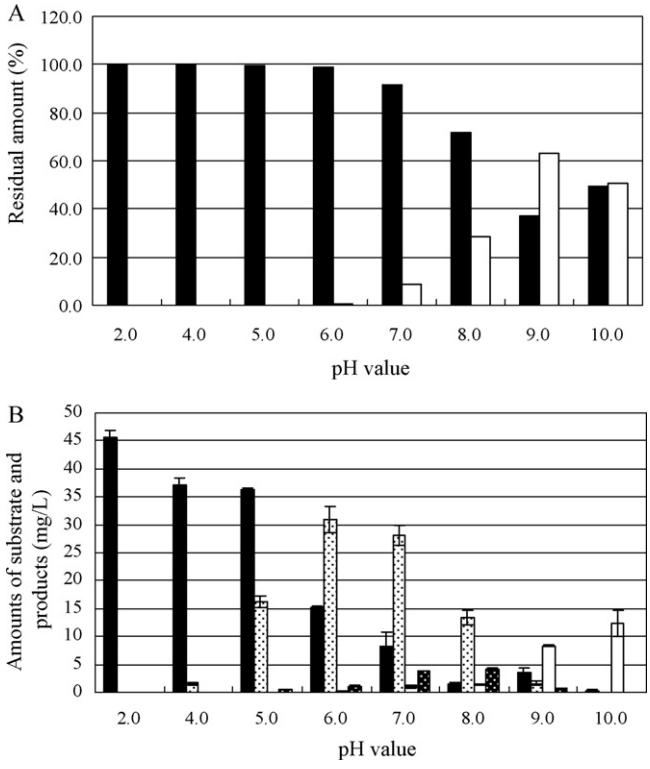

Fig. 5. Effects of pH value on the bioconversion (■: 7-XDT; □: 7-XDB; ⁙: 10-DT; ▩: 7-*epi*-10-DT; (A) incubation in PBS buffer without cells; (B) incubation in PBS buffer with cells).

rate 69.8%. However, the more by-products were detected from the 7th day. Therefore, regarding all these points, the optimal incubation time might be within six days. In addition, the fact that the conversion continued for 8 days even for a longer time suggested the insufficiency of enzyme and/or substrate. Thus, increasing the cell density and/or substrate concentration might enhance the yield and conversion rate of 10-DT.

As expected, the investigation of cell density on the conversion (Fig. 7) showed that the cell density significantly influenced both the yield and conversion rate of 10-DT. With the increasing of cell density from 2 g/L to 40 g/L (dry weight), the yield and conversion rate of 10-DT increased. A cell density of 40 g/L may afford the highest catalytic results both in the yield (190 mg/L) and conversion rate (90%) after 7 days' incubation in 50 mM PBS (pH 6.0) at 26 °C.

Additionally, the effects of substrate concentration on the bioconversion were examined. 0.25–10.0 g/L of 7-XDT in the reaction system (40 g/L of cell density, 50 mM pH 6.0 PBS, 6 days' incubation) was investigated for the effects on the catalytic efficiency of this strain. As Fig. 8 shows, a concentration of 1.0 g/L or less is well tolerated for the catalysis of the strain with a higher conversion rate of 63.3% and higher yield of 544 mg/L of 10-DT. 2.0 g/L of substrate, especially a higher concentration (5.0 g/L or 10.0 g/L) substantially inhibit its catalysis. However, the yield of 10-DT was 764 mg/L at the 2.0 g/L of substrate, higher than that at 1.0 g/L. It seems difficult to conclude the optimum substrate concentration. The conclusion might be drawn out after balancing the advantage and disadvantage of the yield, conversion rate and downstream work (extraction, purification, etc.).

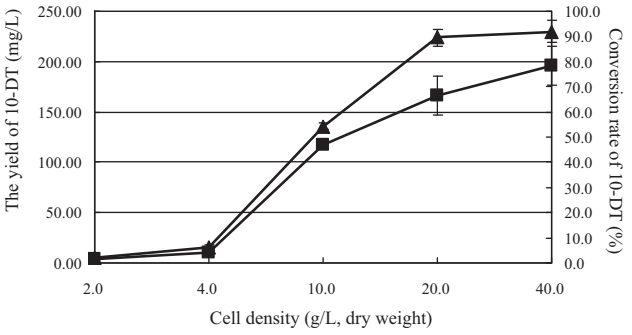

Fig. 7. Effects of cell density on the bioconversion (▲: the yield of 10-DT; ■: conversion rate of 10-DT).

In view of practical use, it is economic if the biocatalyst can be used continuously and its catalytic efficiency remains at an acceptable level. In this context, the collected cells were tested for the bioconversion three times, and the results showed that the enzyme activity remained 90.6% and 66.1% in the second and third bioconversion run, respectively, by taking the 10-DT yield as indicator. It suggests that the continuous bioconversion with resting cells would be feasible and of practical interest in the future.

As mentioned previously, in the ethanol extract of *T. yunnanensis*, the content of 7-XDT is about 5 and 3 times higher than those of paclitaxel and 10-DT, respectively. In the conventional industry process, the ethanol extract was subjected directly to the chromatography for the paclitaxel purification. The complex constituents, especially its analogues often make the purification difficult. If incubating the extract with cells before purification, 7-XDT and other 7β-D-xylosyl taxanes may be converted to 10-DT and corresponding xylosyl moiety hydrolyzed taxanes. This process might simplify the constituents and enhance the contents of the desired compounds, as a result, the purification can become easier. Thus, the ethanol extract of *T. yunnanensis* was used as "substrate" with the equivalent concentration of 250 mg/L 7-XDT for the bioconversion. After 6 days' reaction, the concentration of 7-XDT decreased to 96.7 mg/L from 250 mg/L, while that of 10-DT

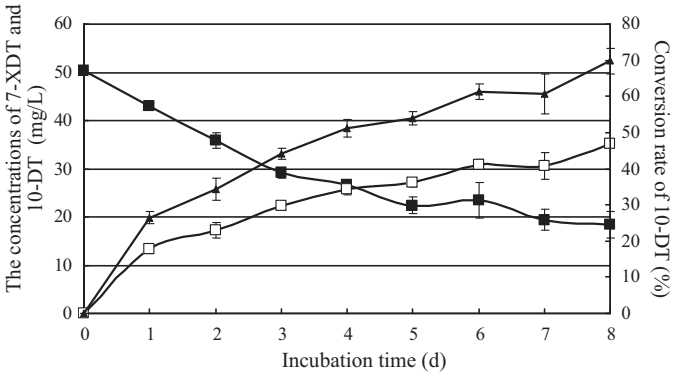

Fig. 6. The kinetics of bioconversion with resting cells (■: 7-XDT; □: 10-DT; △: conversion rate of 10-DT (%)).

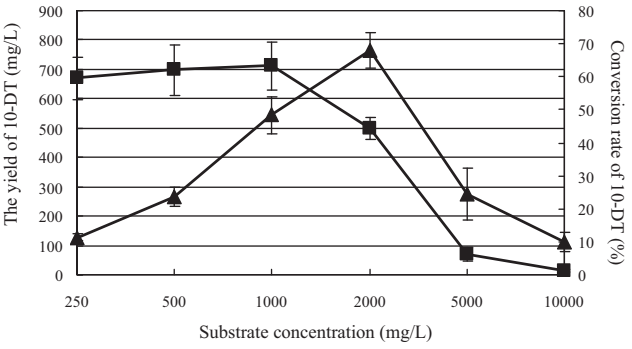

Fig. 8. Effects of substrate concentration on the bioconversion (▲: the yield of 10-DT; ■: conversion rate of 10-DT).

increased to 159.1 mg/L from 62.1 mg/L by about 1.56 times. This result may provide another strategy for the purification strategy of paclitaxel and its analogues.

In summary, this paper reports the successful high-throughput screening of a strain, *Enterobacter* sp. CGMCC 2487, with potential to remove the xylosyl moiety from 7-xylosyltaxanes, by combination fluorescent probe with TLC, HPLC, LC-UV and LC–MS analytic techniques. In the bioconversion of 7-XDT to 10-DT, for the purpose of enhancing the conversion efficiency of the strain, the effects of NH_4^+, oat xylan, pH, temperature (of growth and biocatalysis), cell density, and substrate concentration were systematically investigated, the highest yield and conversion rate can reach 764 mg/L and 92%, respectively. Bioconversion with resting cells is better than with growing cells because of controllable reaction conditions and cell density. In addition, the continuous use of resting cells and bioconversion of ethanol extract of *Taxus* plants were preliminarily examined, and some interesting results have been achieved. These results indicate that converting 7-XDT to 10-DT, a useful intermediate for the semisynthesis of paclitaxel by a novel bacterial strain, *Enterobacter* sp. CGMCC 2487, would be a promising alternative for the practical application in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2010.11.013.

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