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## Using *Pichia pastoris* as a cell factory for producing $\beta$ -xylosidase from *Lentinula edodes* and removing the xylosyl group from 7- $\beta$ -xylosyl-10-deacetyltaxol

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In the previous study, we cloned a  $\beta$ -xylosidase LXYL-P1-2 from *Lentinula edodes* for efficiently removing the  $\beta$ -xylosyl group from 7- $\beta$ -xylosyl-10-deacetyltaxol (XDT) and constructed the recombinant *Pichia pastoris*. In the present report, we introduce the optimization and scale-up testing of high-cell-density fermentation (HCDF) of the recombinant yeast from 10-L to 1,000-L. Using the biomass-stat methanol feeding, 1% induction of dissolved oxygen and  $\sim 0.10$  MPa, the volumetric and biomass enzyme activities (not peak values) of the 1,000-L scale culture reached  $4.5 \times 10^7$  U/L and  $4.7 \times 10^5$  U/g dry cells, respectively at 112 h after induction. Second, we present a biocatalytic test and scale-up bioconversion from 2 mL to 10 L reactions with the dry cells from 200-L scale HCDF (biomass activity:  $2.4 \times 10^4$  U/g dry cells) as the biocatalyst and XDT or XDT<sub>ex</sub> (containing 71% XDT) as the substrates. In the 0.1 M sodium acetate buffer (pH4.0) and at 45 °C for 24 h, the conversion rates of XDT were up to 95% (10 g/L substrate plus 80 g/L dry cells) and 83% (15 g/L substrate plus 128 g/L dry cells) in 10 L reaction, respectively, the same as those in 2 mL reaction. Finally, we introduce a lab-scale project for improving the catalytic fitness of 10-deacetylbaccatin III-10- $\beta$ -O-acetyltransferase (DBAT) of *Taxus* towards the unnatural substrate 10-deacetyltaxol (DT) using protein engineering techniques and realizing the production of Taxol from the abundant analogue XDT through DT by enzymatic one-pot reaction of DBAT and LXYL-P1-2, yielding 0.64 mg Taxol/mL in 50 mL reaction at 15 h.

### Biography

Ping Zhu completed his MSc degree in 1988 from Peking Union Medical College. Then he has been working at IMM (research field: medicinal biotechnology). He got the post-doctoral fellowship from the EEC and participated in the Sino-EEC Joint Program in University of Nottingham from 1991 to 1993. He was a visiting professor of University of Hawaii at Manoa from April to November of 2004. He is the head of the Department of Biosynthesis of Natural Products of IMM. He has published more than 100 papers in peer-reviewed journals, including Nature Communications and contributed seven book chapters.

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