**Additional file**

# Large-scale production of tauroursodeoxycholic acid products through fermentation optimization of engineered *Escherichia coli* cell factory

Yingpeng Xu, Li Yang, Shujuan Zhao*, Zhengtao Wang

The SATCM Key Laboratory for New Resources & Quality Evaluation of Chinese Medicine, The MOE Key Laboratory for Standardization of Chinese Medicines and Shanghai Key Laboratory of Compound Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China

*Corresponding author. Tel: +8621-51322576, Fax: +8621-51322495, E-mail: zhaoshujuan@126.com, zhaoshujuan@shutcm.edu.cn. ORCID: 0000-0002-2269-925X

**Table S1 Orthogonal experimental design of IPTG induction**

| Std | IPTG addition time points (h)[a] | Substrate addition time points (h)[b] |
|---|---|---|
| 1 | 4 | 3 |
| 2 | 4 | 4 |
| 3 | 4 | 5 |
| 4 | 5.5 | 3 |
| 5 | 5.5 | 4 |
| 6 | 5.5 | 5 |
| 7 | 7.5 | 3 |
| 8 | 7.5 | 4 |
| 9 | 7.5 | 5 |

**Note:** a. Number in this line means the bacteria were sub-cultured for 4 hours, 5.5 hours, and 7.5 hours, correspondingly. b. Number in this line represents that substrates were supplemented 3 hours, 4 hours, and 5 hours later by addition of IPTG inducer.

**Table S2 Analysis variance (ANOVA) results of the Box-Behnken Design for $OD_{600}$**

| Source | Sum of squares | df | Mean Square | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Model | 123.71 | 14 | 8.84 | 6.55 | **0.0006** |
| A-Glucose | 59.67 | 1 | 59.67 | 44.26 | **< 0.0001** |
| B-Glycerol | 0.53 | 1 | 0.53 | 0.39 | 0.5411 |
| C-Tryptone | 2.76 | 1 | 2.76 | 2.04 | 0.1748 |
| D-Yeast extract | 24.57 | 1 | 24.57 | 18.22 | **0.0008** |
| AB | 0.22 | 1 | 0.22 | 0.16 | 0.6918 |
| AC | 5.45 | 1 | 5.45 | 4.04 | 0.0640 |
| AD | 1.76 | 1 | 1.76 | 1.30 | 0.2730 |
| BC | 1.32 | 1 | 1.32 | 0.98 | 0.3388 |
| BD | 0.19 | 1 | 0.19 | 0.14 | 0.7104 |
| CD | 1.06 | 1 | 1.06 | 0.79 | 0.3901 |
| A^2 | 15.04 | 1 | 15.04 | 11.15 | **0.0049** |
| B^2 | 1.14 | 1 | 1.14 | 0.85 | 0.3726 |
| C^2 | 0.2 | 1 | 0.2 | 0.15 | 0.7049 |
| D^2 | 9.75 | 1 | 9.75 | 7.23 | **0.0176** |
| Residual | 18.88 | 14 | 1.35 | | |
| Lack of fit | 10.02 | 10 | 1 | 0.45 | 0.859 |
| Pure error | 8.86 | 4 | 2.21 | | |
| Total | 142.58 | 28 | | | |

$R^2$ = 0.8676; $R_{adj}^2$ = 0.7352; Adeq-Precision = 9.489

**Note:** The P values implied the significance of each coefficient. The smaller the value of P-value, the more significant the corresponding coefficient. P-value<0.05 means significant. The highest significant values were highlighted in bold.

**Table S3 ANOVA results of the Box-Behnken Design for conversion efficiency**

| Source | Sum of squares | df | Mean Square | *F*-Value | *P*-value |
|---|---|---|---|---|---|
| Model | 1040.08 | 14 | 74.29 | 8.72 | **0.0001** |
| A-Glucose | 599.44 | 1 | 599.44 | 70.32 | **< 0.0001** |
| B-Glycerole | 7.79 | 1 | 7.79 | 0.91 | 0.3554 |
| C-Tryptone | 1.2 | 1 | 1.2 | 0.14 | 0.7131 |
| D-Yeast extract | 13.07 | 1 | 13.07 | 1.53 | 0.2359 |
| AB | 27.31 | 1 | 27.31 | 3.2 | 0.0951 |
| AC | 11.67 | 1 | 11.67 | 1.37 | 0.2615 |
| AD | 24.94 | 1 | 24.94 | 2.93 | 0.1092 |
| BC | 0.022 | 1 | 0.022 | 2.62E-003 | 0.9599 |
| BD | 1.12 | 1 | 1.12 | 0.13 | 0.7229 |
| CD | 3.6 | 1 | 3.6 | 0.42 | 0.5264 |
| A^2 | 300.54 | 1 | 300.54 | 35.26 | **< 0.0001** |
| B^2 | 11.66 | 1 | 11.66 | 1.37 | 0.2617 |
| C^2 | 8.68 | 1 | 8.68 | 1.02 | 0.3299 |
| D^2 | 14.29 | 1 | 14.29 | 1.68 | 0.2163 |
| Residual | 119.34 | 14 | 8.52 | | |
| Lack of Fit | 119.34 | 10 | 11.93 | 5.71E+005 | **< 0.0001** |
| Pure Error | 8.36E-05 | 4 | 2.09E-05 | | |
| Cor Total | 1159.42 | 28 | | | |

$R^2$ = 0.8971; $R_{adj}^2$ = 0.7941; Adeq-Precision =10.624

**Note**: The *P* values implied the significance of each coefficient. The smaller the value of *P*-value, the more significant the corresponding coefficient. *P*-value<0.05 means significant. The highest significant values were highlighted in bold.

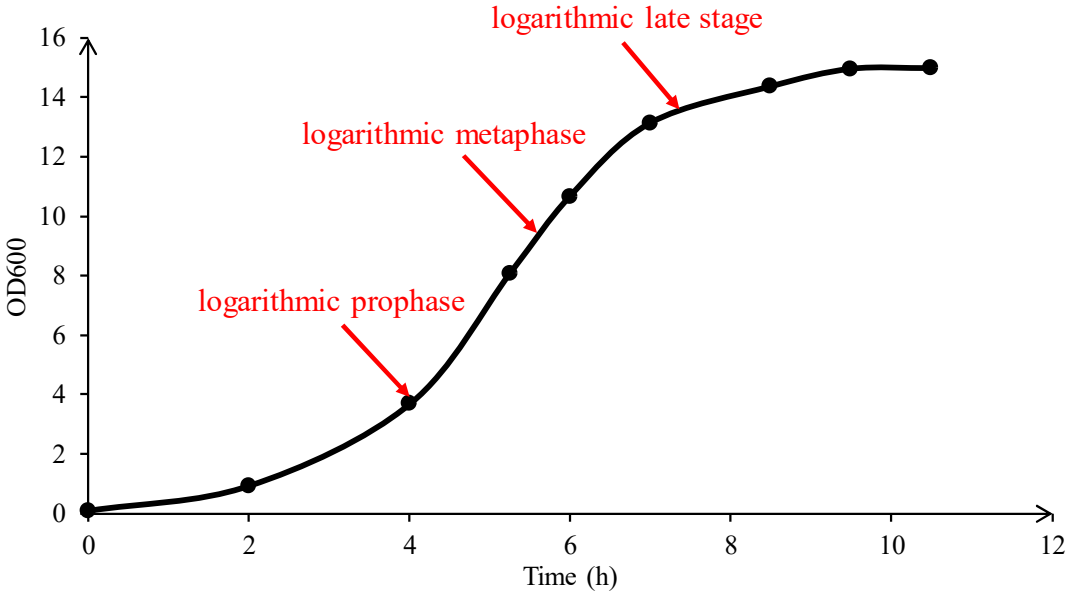


**Fig. S1 Growth curve of *E. coli* BL-pα1β2 strain in shake flask**

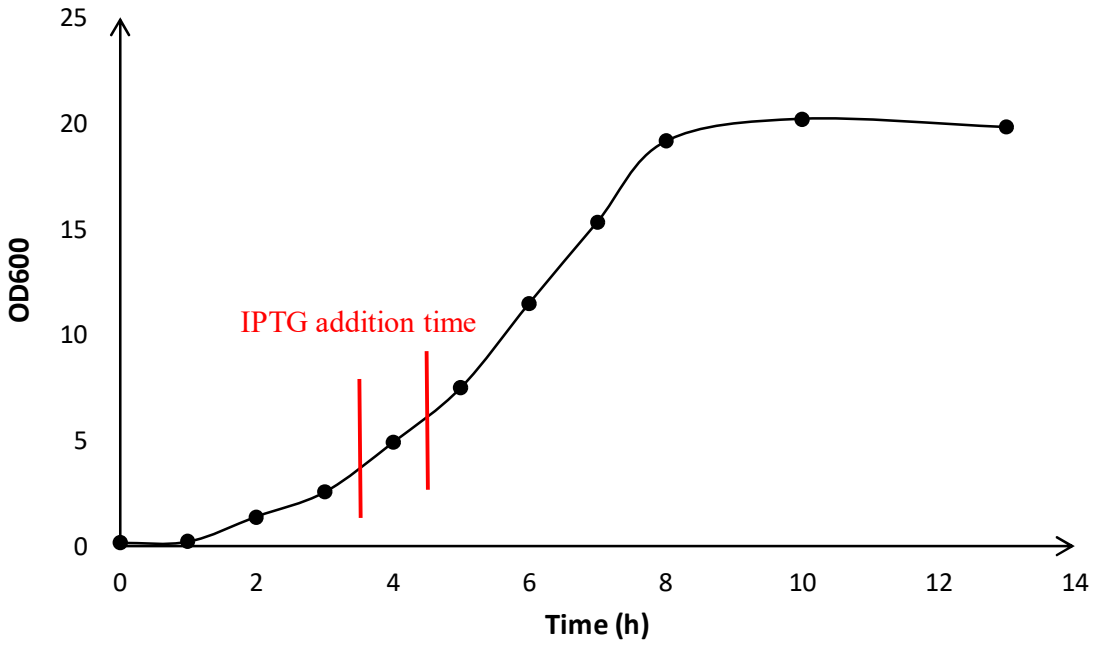


**Fig. S2 Growth curve of *E. coli* BL-pα1β2 strain in 5 L-stirred tank fermenter**

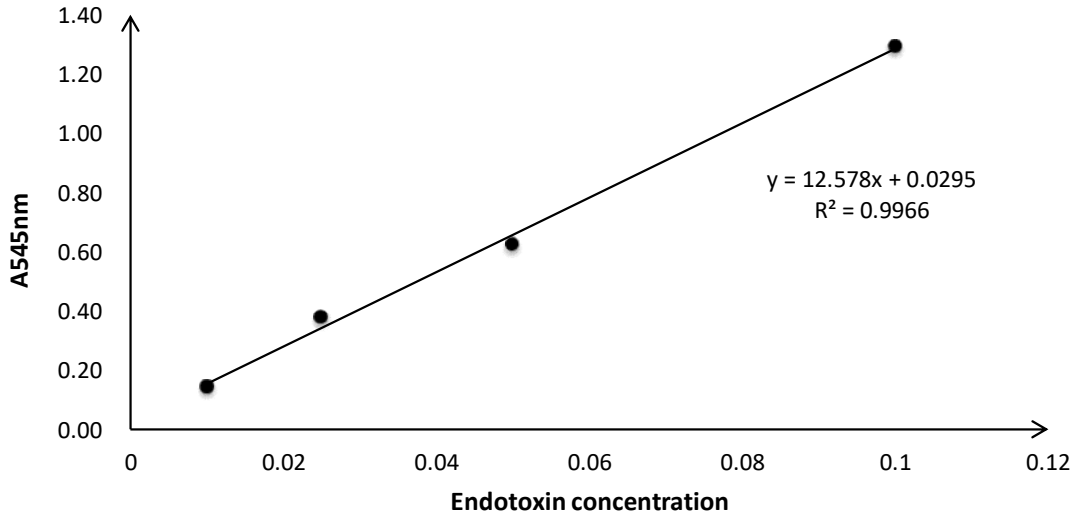


**Fig. S3 Standard curve of endotoxin**

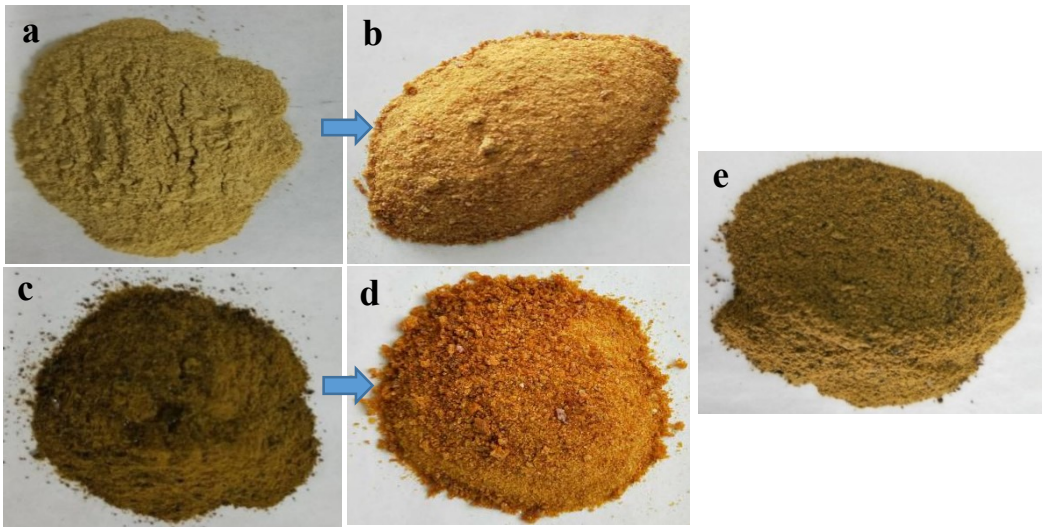


**Fig. S4 The morphology of chicken bile powder and the corresponding products**

a. Refined chicken bile powder (RCBP). b. Products from RCBP. c. Crude chicken bile powder. d. Products from crude chicken bile powder. e. Natural bear bile powder.

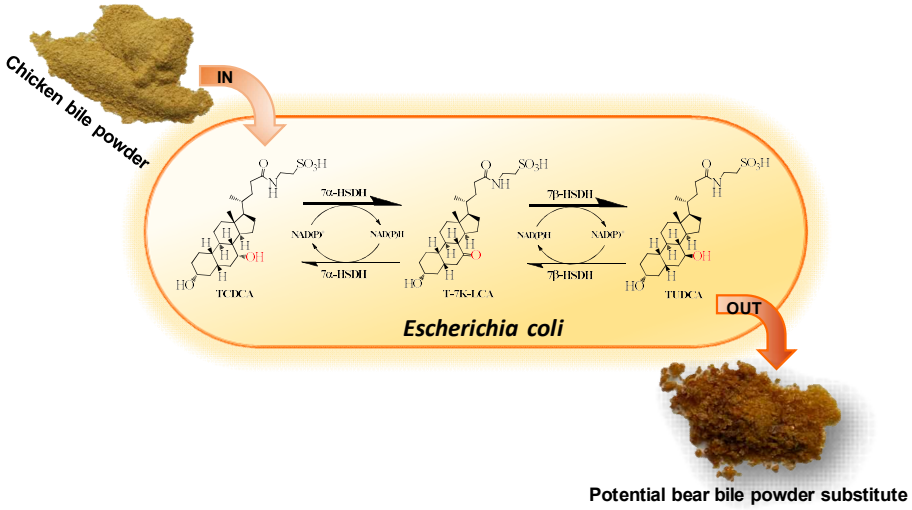


**Fig. S5 Engineered *E. coli* factory for potential substitute of bear bile powder**