Article

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Regio-selective Reduction of Oxysophoridine by Microorganism Isolated from Soil

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Oxysophoridine (OSR), an alkaloid extracted from a traditional Chinese medicine, attracted more and more attention because of remarkable biological activities. Biocatalysis of OSR by 86 stains including soil microbes and endophytic fungi were investigated. Incubation of OSR with soil bacteria T003 identified as *Escherichia coli* could lead to only one reductive product with highly regio-selectivity. The structural elucidations of transformation product were achieved mainly by the NMR spectroscopic methods and references. Effect of pH, substrate concentration, conversion time and strain concentration was studied, the optimization of reaction was analyzed by LC-MS. The production of sophoridine (SR) from OSR was highest at pH 7.0, 36 h of incubation and in presence of 625 μg/mL substrate concentration, 50 μL/mL strain concentration respectively. Biocatalysis was considered the alternatives to preparation of sophoridine with green and sustainable synthetic processes except for less time-consuming and more environmentally friendly.

Keywords: Biocatalysis; Region-stereoselectivity; Soil microbes; Natural product; Condition optimization.

INTRODUCTION

Oxysophoridine (Sophoridine N-oxide,OSR) was isolated a natural alkaloid extracted from a traditional Chinese medicine *Sophora lopecuroides* L. It is reported that OSR displayed significant activities, such as anti-tumor,[9] anti-arrhythmia,[12] anti-inflammation,[7] central inhibitory,[4,10,11,13,15] antinociception.[3,8] Besides, Wang *et al.* found that OSR had a neuroprotective effect against cerebral ischemia/reperfusion injury in mice.[6] OSR may reduce streptozotocin-induced diabetic rat aorta lesions and have a protective effect on the ultrastructure of the aorta.[10]

Derivatives of natural product were synthysied by chemical methods while biotransformation because of its mild conditions, environment-friendly, sustainable attracted more and more attention in recent years. Microorganisms with multi-enzyme systems were known to have the ability to perform the regio- and stereo-selective biocatalysis to organic compounds. Thus, microbial catalysis technique is increasingly being used as one of the feasible approaches by far for structurally modifying natural and synthetic compounds.[2] A good number of microorganisms have demonstrated their catalytic capacities at non-activated positions of the skeleton that were difficult to achieve by classical chemical means.[5] However, there was no report on the structural modifications of OSR by microbes. Herein, the present was to study the structural modification of OSR by microbial transformation to obtain OSR derivatives.

RESULTS AND DISCUSSION

Screening of OSR by biocatalysis

86 strains isolated were used to screening of OSR and 18 strains belong to two kinds of reactions showed activity of transforming OSR into other structures while the endophytic fungi had not such capacity. Two types of reactions were found, one reaction can produce two products with smaller polarity and the other with the only product was detected. T003 which was identified as *Escherichia coli* on 16S rDNA was selected to prepare the transformation product because of its high region-selectivity.[1]

Preparation of transform product of OSR

Strain T003 with only one product was selected to

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prepare the only derivative (Figure 1). The transformation product was obtained by silica gel column chromatography, Sephadex LH-20 and recrystallization.Product: White crystalline solid (methanol). ^{1}H-NMR ($CDCl_3$) (Figure 2): 3.21~3.45 (3H, m, 11-H, 17-2H), 2.78 (2H, m, 10,2-H), 2.32 (3H, m, 14-2H, 6-H), 2.12 (2H, m, 10, 2-H), 2.07 (2H, m, 3-Ha, 5-H), 1.92 (2H, m, 12,4-H), 1.85~1.01 (10H, m). ^{13}C-NMR ($CDCl_3$) (Figure 3): 170.1 (C-15), 63.4 (C-6), 56.0 (C-2), 55.8 (C-11), 50.4 (C-10), 47.7 (C-17), 41.0 (C-7), 32.6 (C-14), 30.9 (C-5), 30.3 (C-12), 28.2 (C-4), 23.8 (C-3), 21.9 (C-8), 21.6 (C-9), 19.0 (C-13). Compared with ^{1}H-NMR and ^{13}C-NMR, spectral data with those reported in the literature,[14] its structure was confirmed as SR.

Optimization of reaction conditions

It could be found that pH 7, 8 and 9 were suitable for both the growth of the strain and catalytic activity of enzyme, and the highest SR production occurred when pH value was at 7 (Figure 4). Apparently, OSR of 625 μg/ml was optimum for SR production; above this concentration, production of SR decreased, SR production was considered unnormal which suddenly dropped to 0, however, from the OSR reduction, the optimal substrate concentration could also be inferred (Figure 5). Every test tube was harvested

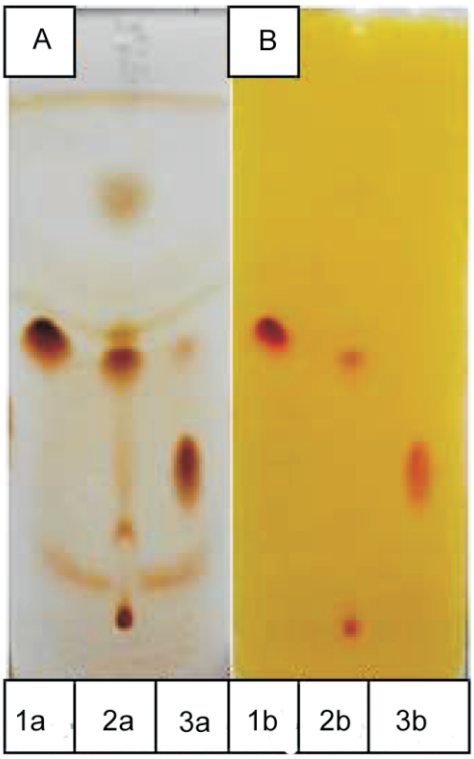

Fig. 1. Selectively transformation of OSR by strain T003.

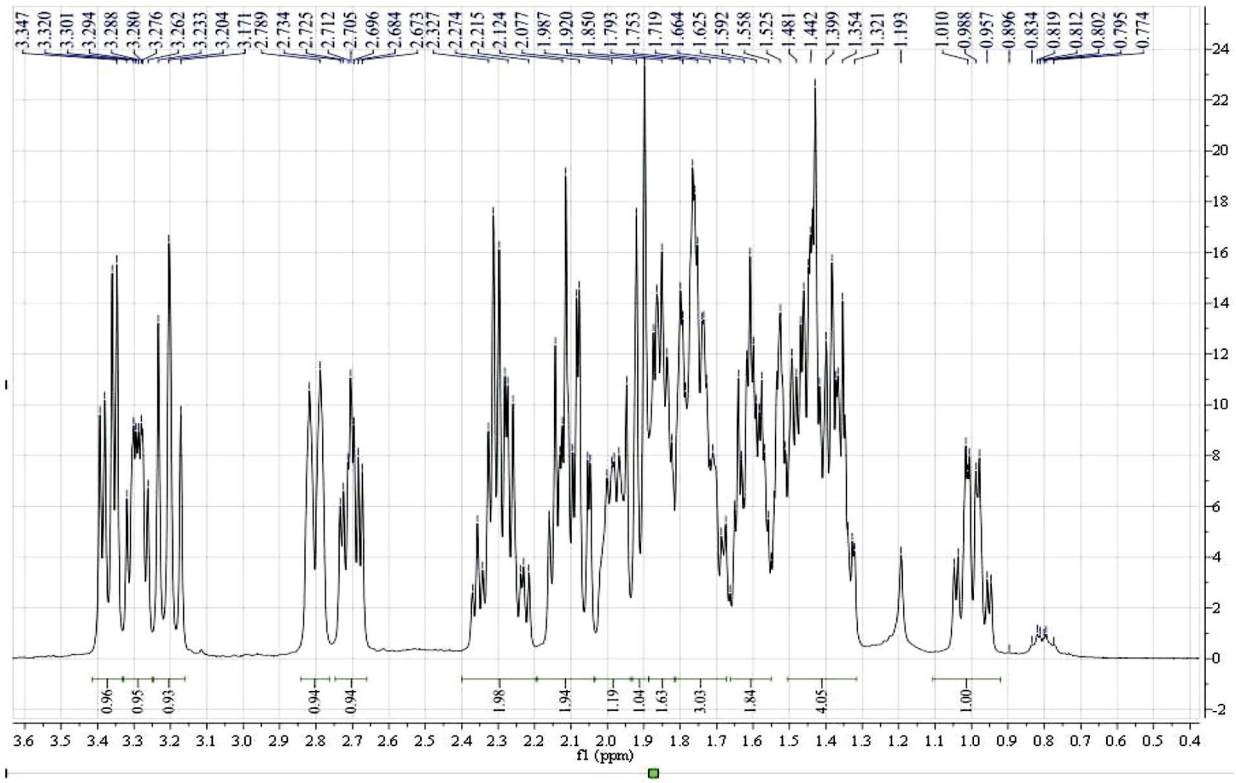

Fig. 2. ^{1}H-NMR of transformation product of T003.

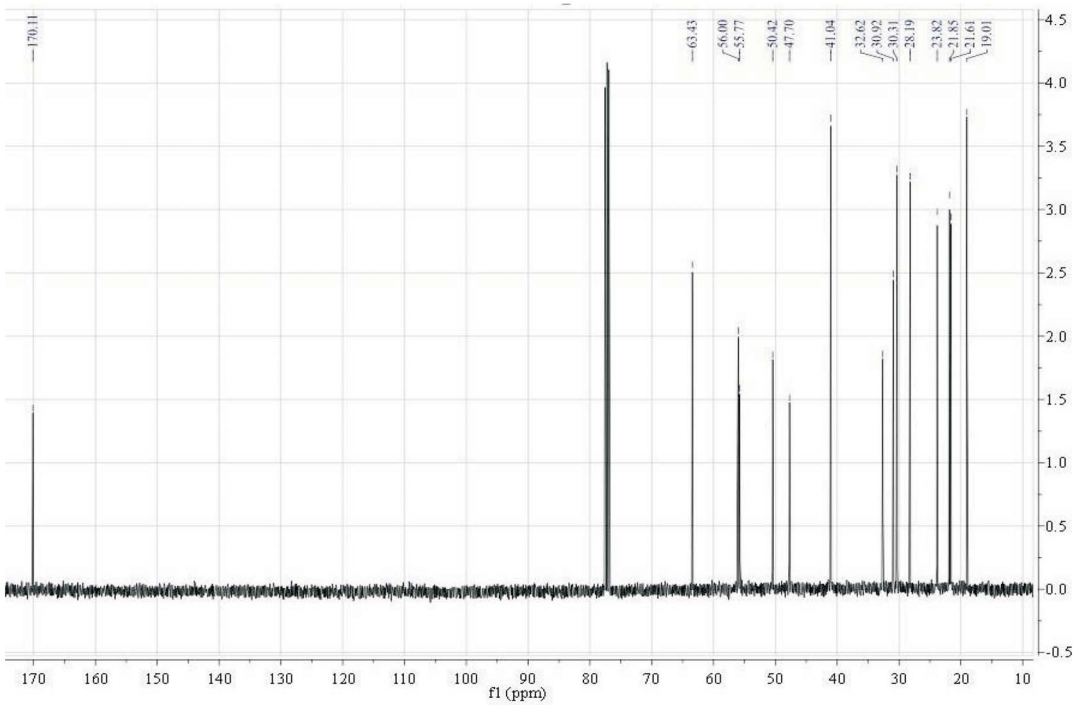

Fig. 3. ^{13}C-NMR of transformation product of T003.

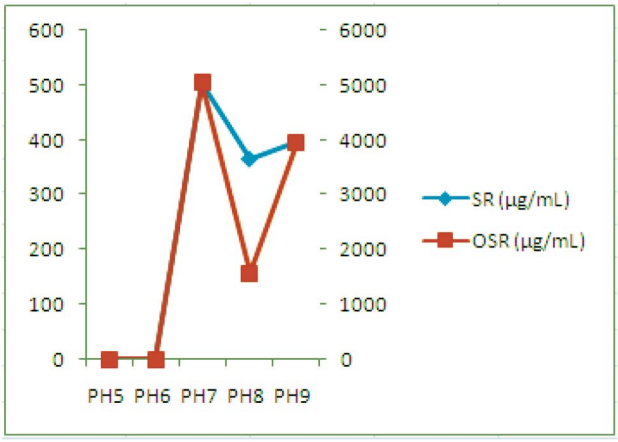

Fig. 4. Effect of pH on transformation.

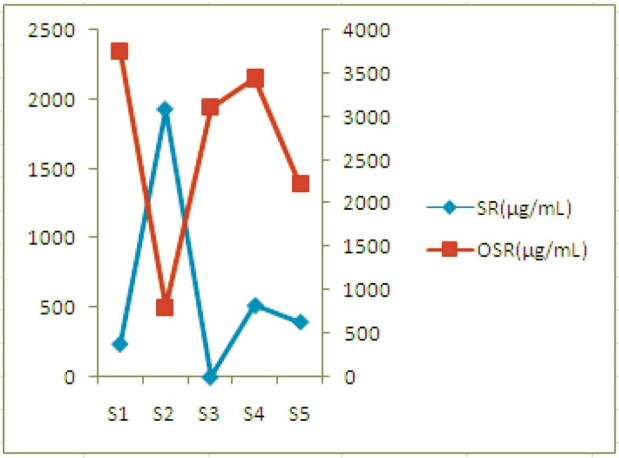

Fig. 5. Effect of substrate concentration on transformation.

ever 12 h. The results demonstrated that SR reached to maximum at 36 h (Figure 6). The production of H reached to the maximal levelof 856 μg/mL. Strain concentration could also affect the SR production from OSR. According to Figure 7, with the increase of strain concentration, the conversion activity gradually increased. SR reached the maximum production (50 μL/mL) and then decreased.

EXPERIMENTAL

General experimental procedure: NMR spectra were recorded on an Agilent NMR Spectrometer operating at 400 MHz in $CDCl_3$ with TMS as an internal standard. TLC analyses were carried out on pre-coated silica gel GF_{254} plates (0.25 mm thick, Qingdao Oceanic Chemicals, China). LC-MS (AB SCIEX) was carried out on C-18 column with methanol – water = 85:15 and with the condition of CUR: 40.00, CAD; Medium; IS:-4500.00; TEM: 550.00; GS1: 55.00; GS2: 55.00; DP: -140.00; EP: -10.00; CE: -40.00; CXP: -15.00. Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Oceanic Chemicals, China).

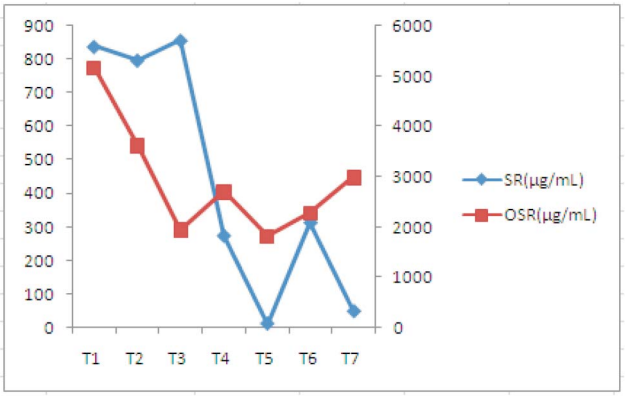

Fig. 6. Effect of conversion time on transformation.

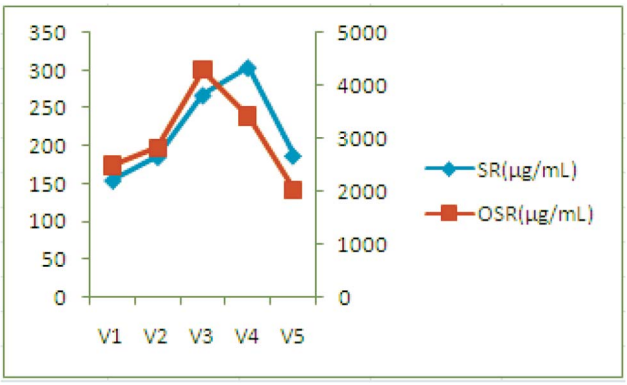

Fig. 7. Effect of strain concentration on transformation.

Visualization of the TLC plates was performed by Bismuth potassium iodide spray reagent that is specific chromogenic agent to alkloids. General solvents and reagents were purchased from Chengdu kelong Chemical Industry Company, Chengdu, China.

Substrate: Substrate OSR (with purity > 98%) was purchased from Beijing Gold- moutain Chromatography Science and Technology Co. Ltd.

Microorganisms: Microbial strains consist of soil microorganisms isolated from soil in the campus of Zunyi Medical University and endophytes isolated from plant in Guizhou province. Soil microbes were isolated by soil dilution method.[1]

Screening procedures: The preliminary screening experiment of soil microbes was carried out in LB media and endophytes in PDA media. Fermentations were carried out according to a standard two-stage protocol. The cultures were grown in 100 mL Erlenmeyer flasks containing 40 mL of medium. The flasks were incubated at 37 °C for soil microbes and 28 °C for endophytes and 140 rpm on a shaker. And then 2 mg substrate (in ethanol) was added after 6 hours for soil microbes and the cultures were incubated for another 3 days. For endophytic fungi, the same quantity of substrate was added after incubation for 48 hours and the cultures were incubated for another 5 days. Then the cultures were then extracted three times with EtOAc. The extracts were evaporated under reduced pressure and were analyzed by TLC. Culture with microorganisms but without substrate and culture without microorganism but with substrate were both designated as the controls and were incubated in the same conditions as described above.

Biocatalysis of OSR by T003 and purification of the product: The preparative scale biocatalysis of OSR by soil microbes was carried out in 8.2 L medium in sixteen 1 L flask and 900 mg substrate in total was added. Incubation conditions were the same as mentioned above. But after being extracted with EtOAc, the product can be detected in the fermentation liquid. The aqueous phase of the fermentation liquid was vaporated under reduced pressure again. About 4.04 g crude extract was obtained.

The crude extracts were subjected to silica gel column chromatography that was adjusted pH > 7 with diethylamine and eluted with mixtures of chloroform/methol from 40:1 to 0:1. Fraction A, B, C and D were obtained. Fraction B was further purified by Sephadex LH-20 with chloroform /methol, gel column chromatography with chloroform/methol several times and recrystallization, and then the only transformation product (96.8 mg) were obtained.

Optimization of reaction conditions: Optimization of pH (from 5 to 9), substrate concentration (1250 μg/ml, 625 μg/ml, 312.5 μg/ml, 156.25 μg/ml and 78.125 μg/ml), transformation time (12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h) and strain concentration (12.5 μl/ml, 25 μl/ml, 37.5 μl/ml, 50 μl/ml, 65.5, 62.5 μl/ml) was investigated, and the optimization of reaction was analyzed by LC-MS.

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REFERENCES

1. Fu, S. B.; Wen, F.; Yan, S.; Zhang, Y.; Li, Y.; Yue, C. *J. Zunyi*

Med. Uni. **2015**, *38*, 190-192. (In Chinese)
2. Fu, S. B. *Fitoterapia* **2013**, *86*, 123-128.
3. Gao, J.; Tao, L.; Yu, J.; Jin, S.; Yang, G.; Xu, Y.; Jiang, Y. *Chin. Pharm. Bull.* **2009**, *25*, 1453-1456.
4. Jian-Qiang, Y. U.; Jiang, Y. X.; Wang, L. Y. *Ningxia Med. J.* **2002**, 24. (In Chinese)
5. Parra, A.; Rivas, F.; Garcia-Granados, A.; Martinez, A. *Mini-Rev. Org. Chem.* **2009**, *6*, 307-320.
6. Wang, H.; Li, Y.; Jiang, N.; Chen, X.; Zhang, Y.; Zhang, K.; Wang, T.; Hao, Y.; Ma, L.; Zhao, C. *Neural Regen. Res.* **2013**, *8*, 1349-1359.
7. Xia, L. Q.; Zhao, B. G.; Yun-Wei, J. U.; Dai, L. *J. Nanjing Forestry Univ.* **2001**, *25*, 81-84. (In Chinese)
8. Yang, G.; Gao, J.; Jia, Y.; Yan, L.; Yu, J.; Jiang, Y. *Planta Med.* **2012**, *78*, 874-880.
9. Yao, X. Q.; Zhang, Y. H.; Long, W.; Liu, P. X. *Chin. J. Integr. Med.* **2012**, *18*, 209-213.
10. Yu, J.; Li, Y.; Yuan, J. *Shizhen Guoyi Guoyao* **2010**, *21*, 31-32. (In Chinese)
11. Yu, J.; Li, Y.; Zhao, C.; Gong, X.; Liu, J.; Wang, F.; Jiang, Y. *Zhongguo Zhong Yao Za Zhi* **2010**, *35*, 1170-1172. (In Chinese)
12. Zhang, H. M.; Li, H. Q. *Zhongguo Yao Li Xue Bao* **1999**, *20*, 517-520.(In Chinese)
13. Zhang, K.; Zhao, J.; Liu, H.-Y.; Wang, H.-B.; Zhao, C.-J.; Jiang, Y.-X.; Yu, J.-Q. *Pharmacol. Clin. Chin. Mater. Med.* **2011**, *27*, 26-28. (In Chinese)
14. Zhang, L.; Li, J.; Haowfton, P.; Jekson, X.; Temman, T. *Chin. J. Chin. Mater. Med.* **1997**, *22*, 740-743. (In Chinese)
15. Zhao, J.; Wu, Y.; Sun, M.; Wang, J.; Li, Y.; Zhang, K.; Yu, J. *Zhongguo Zhong Yao Za Zhi* **2012**, *37*, 94-98. (In Chinese)

