Electronic Supplementary Materials
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Isolation of *Penicillium expansum* WH-3 for the production of L(+)-tartaric acid*#

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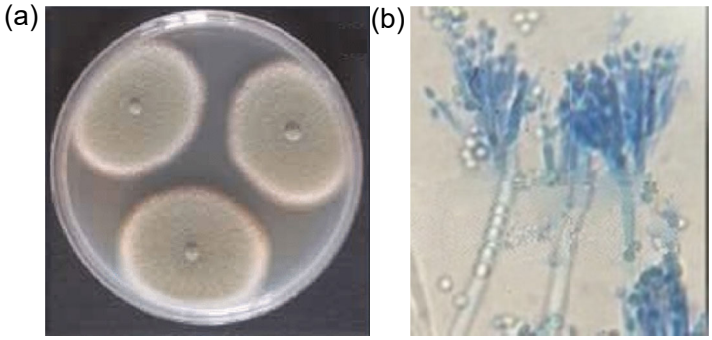

Fig. S1 Morphology of *Penicillium expansum* WH-3

(a) *Penicillium expansum* WH-3 colony morphology figure. (b) Photomicrograph of conidiophore of *Penicillium expansum* WH-3 (40×)

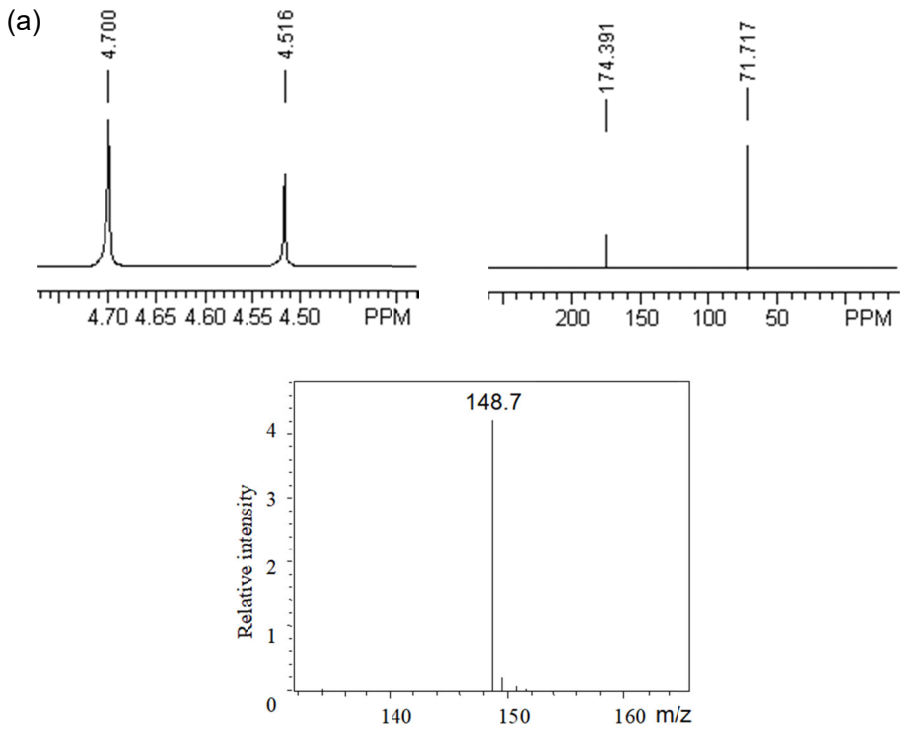

Fig. S2 ^{1}H-NMR (a), ^{13}C-NMR (b), and mass spectrum (c) of the biotransformated product

Materials and methods

Isolation of strains

Two hundred milliliter of disodium oxiranedicarboxylate (0.2 M, pH 7.0) in a 500 mL jar without bottle cap was kept at the room temperature for two months. After two months, flocculent substances were formed above the solution and the fungal strain was isolated from these flocs. The flocculent sample was collected by inoculating loop, mixed with 10 mL of physiological saline, diluted from 10^{-1} to 10^{-6}, inoculated in Potato Dextrose Agar (PDA) media with ampicillin 100 mg/mL and cultivated at 25 °C for 7 d. Then pure cultures of all colonies were obtained in PDA media and inoculated into Potato Dextrose Broth (PDB) for another 5 d. Finally disodium oxiranedicarboxylate was added to transform at 30 °C for 3 d and the content of DA was detected (Xue et al., 2018).

Identification of strain WH-3

Identification was carried out by observation its morphological properties (observed by microscope) and analyzation the ITS sequence (determined by TaKaRa Biotechnology (Dalian) Co., Ltd). The ITS sequence of strain WH-3 was aligned with those of related strains and the phylogenetic tree was constructed (Wu et al., 2007).

Identification of the biotransformation product

The biotransfromation product was purified and identified by nuclear magnetic resonance (NMR), mass spectrum (MS) and optical rotation analysis according to the methods by Pan et al. (2008).

Optimization of fermentation conditions

The spores in PDA medium were collected with distilled water, inoculated into PDB medium, and cultivated at 25 °C for 24 h. Then the seed culture with 10% incubating volume (v/v) was transferred to new PDB medium, cultured at 25 °C for another 3 days, centrifuged at 5000 g for 10 min, and the mycelial pellet and supernatant were collected. The effects of fermentation conditions on enzyme activity were investigated according to Xu et al. (2018).

Batch fermentation experiment

Batch culture was performed in a 5 L fermentor (Jiangsu Kehai Bio-engineering Equipment Co., Ltd., Yangzhong, China). The seed culture with 10% incubating volume (v/v) was inoculated into 3 L fermentation medium after optimization on flask, cultivated at 25 °C, 200 rpm with 0.8 vvm and monitored its biomass and enzyme activity (Bao et al., 2020).

Characterization of ORCH and biotransformation

After fermentation, the mycelial pellet and supernatant were obtained by centrifugation from fermentation broth. The equal amount of disodium oxiranedicarboxylate (10 mM, pH 7.0) was added to the fermentation broth, mycelial pellet and fermentation supernatant respectively, and transformed at 30 °C by monitoring the conversion rate based on the amount of L(+)-DA increment. Then the effects of temperature and pH on the activity of ORCH from mycelial pellet were detected, and the relative activity of the mycelial pellet transformed in 1 M disodium oxiranedicarboxylate (pH 7.0) at 30 °C for 1 h was defined as 100%. Repeated batch conversions were carried out by mycelial pellet and each batch was transformed for 7 d, following by calculation its conversion rate and enantiomeric excess (EE) value.

Activity and enantioselectivity assay

The activities of mycelial pellet, fermentation supernatant and fermentation broth were performed and the activity units were U/g, U/L and U/L according to Bao et al. (2019). Mycelial pellet was filtered by gauze, centrifuged to remove fermentation supernatant, washed by physiological saline, re-suspended in 10 mL of 1 M disodium oxiranedicarboxylate solution (pH 7.0), and transformed at 30 °C for 1 h. The activity of mycelial pellet was calculated according to the definition as the amount of enzyme capable of generating 1 μmoL of DA per hour per gram (dry cell weight) under the experimental conditions described above (U/g). The dry cell weight was determined by holding the biomass at 105 °C for 12 h. The activity of fermentation supernatant was determined as that of mycelial pellet and calculated as the amount of enzyme capable of generating 1 μmoL of DA per hour per liter under the experimental conditions described above (U/L). The content of enantiomeric DA and EE value were determined by HPLC (Bao et al, 2019).

Nucleotide sequence accession number

The ITS sequence of *Penicillium expansum* WH-3 was submitted to the GenBank database under the accession number MN587988.