Glycosylation of antitubercular agent chlorflavonin for combating *Mycobacterium tuberculosis*

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1. Research Background

- Around 1.5 million people died from the bacterial pathogen *Mycobacterium tuberculosis* causing tuberculosis (TB) in 2020.
- Current first-line (isoniazid and rifampicin) and second-line (capreomycin, streptomycin, and cycloserine) therapies cannot tackle the emerging challenge of multi-drug resistance (MDR) and extensively drug resistant (XDR) of *M. tuberculosis*.
- Applications of new anti-TB drugs, including bedaquiline, delamanid, and pretomanid, are still limited due to potential side effects and long treatment periods.

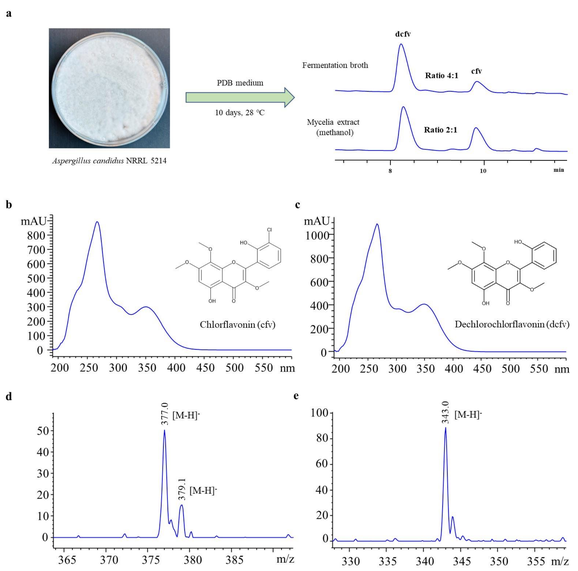


Fig. 1. Flavonoids produced by *Aspergillus candidus* NRRL 5214.

- Flavonoids dechlorochlorflavonin (dcfv) & chlorflavonin (cfv) were produced by *Aspergillus candidus* NRRL 5214 (**Fig. 1**).
- Chlorflavonin exhibited significant *in vitro* inhibitory activity against *M. tuberculosis.*
- Chlorflavonin showed synergistic effects with the first-line antibiotic isoniazid.
- Chlorflavonin has stronger antifungal activity against *Candida albicans* and *Aspergillus fumigatus* than the well-known antifungal agent amphotericin B.

2. Research Objectives

- Low water-solubility often hinders the bioavailability of flavonoids which exhibit various biological functions. This research aimed to improve the water solubility of chlorflavonin through microbial glycosylation.
- The long-term goal of this research is to discover new drug candidates of chlorflavonin to combat drug-resistant TB and contribute to the World Health Organization's goal of ending the global TB pandemic by 2035.

3. Results and Discussion

3.1 Enhanced production of chlorflavonin from *Aspergillus candidus* NRRL 5214

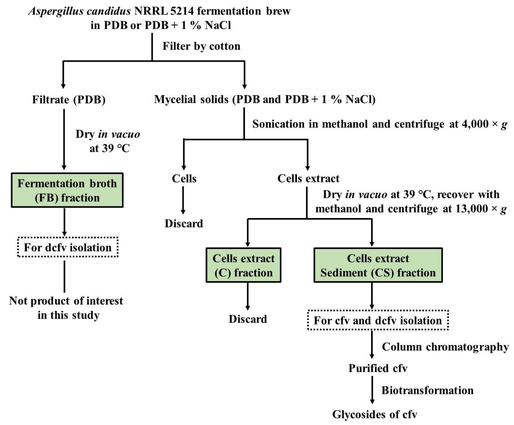


Fig. 2. Flow chart for isolation of dcfv and cfv.

We isolated cfv from the CS fraction (**Fig. 2**) in PDB after 14 days of fermentation supplemented with 2.5 % NaCl. The optimized yield of cfv in CS fraction reached 5.71 ± 0.06 mg/L (**Fig. 3**).

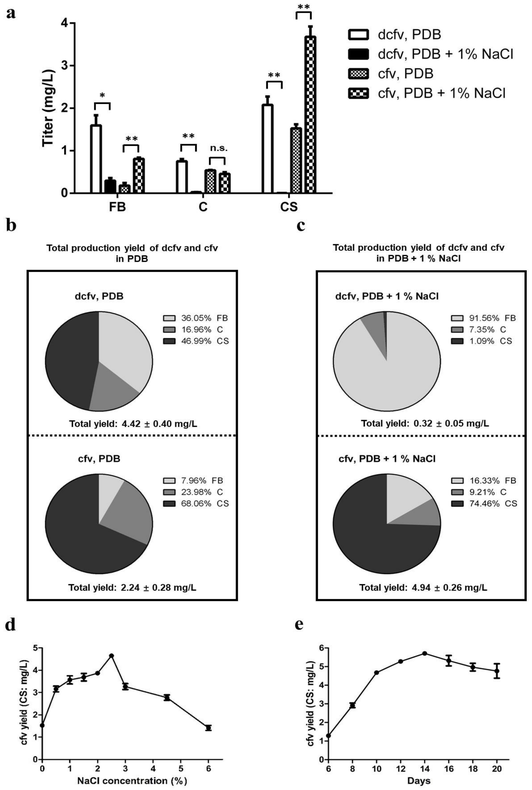


Fig. 3. The production yield of dcfv and cfv from *A. candidus* NRRL 5214.

3.2 Screening of five microorganisms for the ability to glycosylate chlorflavonin

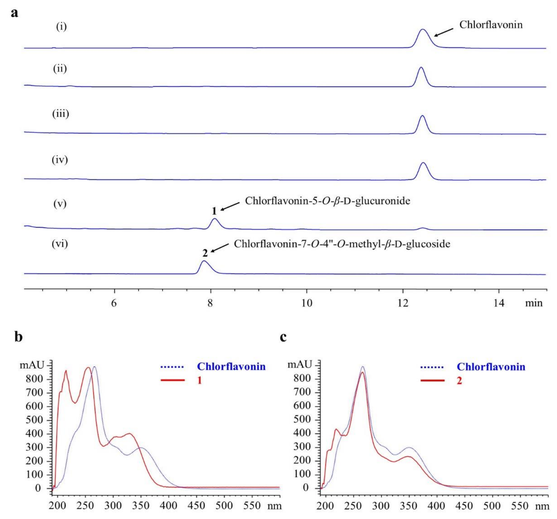


Fig. 4. Screening of five microorganisms for the ability to biotransform cfv.

Two more polar metabolites, at 8.2 min for product **1** and 7.8 min for product **2**, were biosynthesized from chlorflavonin by *Streptomyces chromofuscus* ATCC 49982 and *Beauveria bassiana* ATCC 7159, respectively (**Fig. 4**). They were characterized as chlorflavonin-5-*O*-*β*-D-glucuronide (**1**) and chlorflavonin-7-*O*-4"-*O*-methyl-*β*-D-glucoside (**2**) (**Fig. 5**) .

3.3 Structural characterization of biotransformed products of chlorflavonin

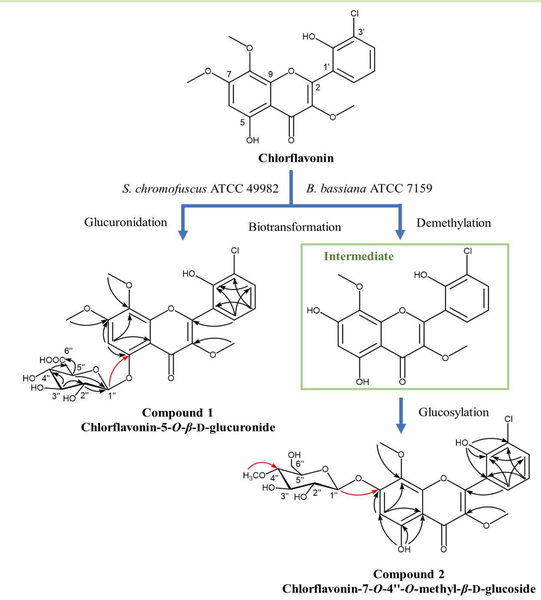


Fig. 5. The chemical structures of compounds **1** and **2**.

3.4 Further structural confirmation of compound 2

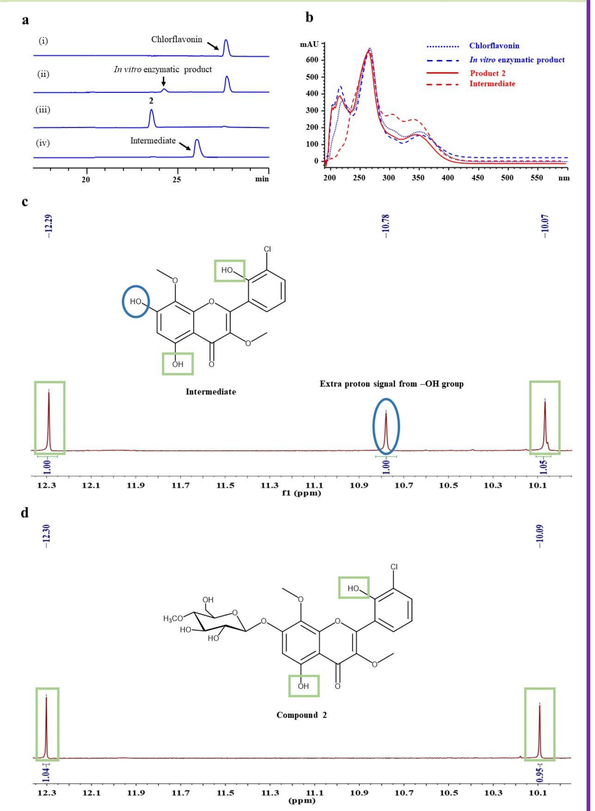
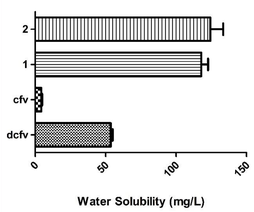


Fig. 6. Further chemical structure confirmation of compound **2**.



The intermediate was first formed by demethylation of chlorflavonin at 7-OCH_3, and then 4"-*O*-methyl-*β*-D-glucose moiety was attached to 7-OH group of chlorflavonin by BbGT (**Fig. 6**). The water solubility of chlorflavonin glycosides **1** and **2** are both around 27 times higher than the substrate chlorflavonin (**Fig. 7**) .

Fig. 7. Water solubility of dcfv, cfv, compound **1**, and compound **2**.

4. Conclusions and Future Works

- ✓ Enhanced production of chlorflavonin was achieved.
- ✓ The chemical structures of two biotransformed chlorflavonin products were characterized as novel glycosides.
- ✓ The comparison of anti-TB activity between chlorflavonin and its glycosides is ongoing and the results will be analyzed.

5. Achknowledgment

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