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Rapid Transformation of Ferulic Acid to 4-Vinyl Guaiacol by *Bacillus pumilus* S-1

DONG-LIANG HUA[1,*], XIAO-HUI LIANG[1], XIAO-DONG ZHANG[1,*], JIE ZHANG[1], HAI-PENG XU[1], YAN LI[1] and PING XU[2]

[1]Key Laboratory for Biomass Gasification Technology of Shandong Province, Energy Research Institute of Shandong Academy of Sciences, Jinan 250014, P.R. China
[2]School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, P.R. China

*Corresponding author: Fax: +86 531 82961954; Tel: +86 531 85599031; E-mail: hua016@foxmail.com

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A *Bacillus pumilus* strain S-1, earlier reported to efficient in converting of isoeugenol to vanillin, was found to have the biotransformation ability of ferulic acid. This strain rapidly dissimilated 1 g L^{-1} ferulic acid within 3 h and 4-vinyl guaiacol was identified as the main product. The highest concentration of 4-vinyl guaiacol, 0.72 g L^{-1}, was obtained with a molar yield of 93.1 %. Different from former reports, 4-vinyl guaiacol was not further metabolized and no other vanilla flavour compounds were found.

Key Words: *Bacillus pumilus*, Ferulic acid, 4-Vinyl guaiacol, Biotransformation.

INTRODUCTION

Ferulic acid (3-methoxy-4-hydroxycinnamic acid) is a highly abundant cinnamic derivative found in plant cell walls. It constitutes about 0.5-3.0 % in agricultural residues such as maize bran and sugar-beet pulp, which is linked to different positions on arabinose residues in the arabinoxylans through ester linkages[1]. Free ferulic acid can be released by a combination of physical and enzymatic processing. Due to its chemical similarity with vanillin, ferulic acid is considered to be a suitable precursor for vanilla flavour production by biotechnology[2,3]. Therefore, many microorganisms have been isolated to produce vanillin from ferulic acid and the metabolic pathway has been fully investigated[2,4-7].

The decarboxylation of ferulic acid in C-C side chain to 4-vinyl guaiacol (4-VG) has been reported in many bacteria and yeasts[2,5,6]. 4-Vinyl guaiacol possesses a spicy clove-like aroma and is widely used in the fragrance and perfume industry, the commercial cost of which is nearly 30 times more than that of ferulic acid[8]. 4-Vinyl guaiacol can also be transformed to other important compounds such as acetovanillone and ethyl guaiacol. Attempts have been made to produce this high valued aromatic compound from ferulic acid through microbial catalysis[9-13]. However, 4-vinyl guaiacol is found to be quickly converted to other vanilla flavour compounds such as vanillin, vanillic acid or guaiacol. Therefore, the biotransformation process is difficult to control and little 4-vinyl guaiacol accumulates in the culture broth.

In previous study, a *Bacillus pumilus* strain S-1 was isolated based on its ability of efficient transforming isoeugenol to vanillin[14]. We further found that this strain also had the ability of degrading ferulic acid. In this paper, biotransformation of ferulic acid to the high valued flavour compound, 4-vinyl guaiacol demonstrated.

EXPERIMENTAL

Ferulic acid (*trans*-, 99 %) was purchased from Sigma-Aldrich. 4-vinyl guaiacol (99 %) was obtained from Shanghai Apple Flavour & Fragrance Co. Ltd. (Shanghai, China). Solvents used for high-performance liquid chromatography (HPLC) were of the HPLC grade. All other materials were of the highest purity commercially available.

Strain, medium, culture condition and biotransformation: *B. pumilus* S-1 was preliminarily isolated from soil based on its ability of efficient transforming isoeugenol to vanillin, was used in this study[14]. This strain was deposited at the China Center for Type Culture Collection (CCTCC M 205165).

Cultures were first incubated in seed medium (200 rpm, 30 ºC and 30 mL in 300 mL flask), which contained 10 g L^{-1} glucose, 5 g L^{-1} yeast extract, 10 g L^{-1} peptone and 10 g L^{-1} NaCl (pH 7.2). The mid-logarithmic-stage preculture was then inoculated (6 %, v/v) into the biotransformation medium. The biotransformation medium was composed of 20 g L^{-1} glucose, 5 g L^{-1} yeast extract, 10 g L^{-1} peptone and 10 g L^{-1} NaCl (pH 7.2). After incubated for 12 h (200 rpm, 30 ºC and 50 mL in 500 mL flask) the cultures for the biotransformation were prepared. The biomass concentration was 3.6 g L^{-1} dry cell weight (DCW).

Ferulic acid was dissolved in 1 M NaOH solution (1:10, w/v) and the pH value was adjusted to 8.5 with 6 M HCl. Ferulic acid solution was directly added to the mature cultures described above. Control experiments were performed in phosphate buffer without the addition of cells (pH 7.0, 0.06 M). The biotransformation was conducted at 200 rpm, 30 ºC and 50 mL medium in 500 mL flask. Triplicate experiments were performed under the same condition. At regular time intervals, samples were removed from flasks to determine the concentrations of ferulic acid and products.

Isolation and identification of biotransformation product: Biotransformation product was isolated by preparative TLC and identified by gas chromatography-high resolution mass spectrometry (GC-HRMS)[4]. The product was further confirmed by nuclear magnetic resonance, which was analyzed on an Avance 600 spectrometer (Bruker) operating at 600 MHz.

Analytical methods: Cell growth was measured spectrophotometrically at 620 nm. Dry cell weight was calculated from the optical density ($OD_{620\ nm}$) with a linear correlation factor ($OD_{620\ nm}$ of 1 = 0.4 g L^{-1} dry cell weight).

Concentrations of ferulic acid and 4-vinyl guaiacol were estimated by HPLC (Agilent 1100 series, Hewlett-Packard). Before HPLC analysis samples were centrifuged at 12,000 rpm for 10 min. The supernatant was diluted with distilled water and filtered with aqueous phase filter film of 0.45 μm. The HPLC system was equipped with a KR100-5 C_{18} column (150 mm × 4.6 mm × 5 μm, Kromasil, Sweden) and a UV detector operating at 254 nm. The column was eluted with a mixture of methanol and water (90:10, v/v) at a flow rate of 0.5 mL min-1 and 30 ºC. Quantitative data were obtained by comparing the peak areas of the query compounds with those of standards of known concentrations.

RESULTS AND DISCUSSION

Four major pathways of ferulic acid metabolism have been found in different strains, which are nonoxidative decarboxylation, side chain reduction, coenzyme-A-independent deacetylation and coenzyme-A-dependent deacetylation[2,6]. Biotransformation of ferulic acid by *B. pumilus* S-1 was investigated and the main metabolite was identified by GC-HRMS and NMR. The mass spectrum from GC-HRMS (m/z 150.0683, calculated mass 150.0681), with a formula of $C_9H_{10}O_2$, was consistent with that of 4-vinyl guaiacol according to the standard library (WILEY275). 4-Vinyl guaiacol was further approved by ^{1}H NMR spectrum (Fig. 1). Therefore, ferulic acid was metabolized through 4-vinyl guaiacol in *B. pumilus* S-1.

Time course of ferulic acid biotransformation and 4-vinyl guaiacol production is shown in Fig. 2. The highest concentration of 4-vinyl guaiacol obtained was 0.72 g L^{-1} and the molar yield was 93.1 %. All of the ferulic acid added was dissimulated within 3 h, which was faster than many of the reports to date. Before this study Karmakar *et al.*[10] reported the fastest degradation speed of ferulic acid by organisms, in which 0.95 g L^{-1} ferulic acid was dissimilated in 7 h by a *B. coagulans* strain. The result suggested that the biotransformation ability of *B. pumilus* S-1 was better than the report.

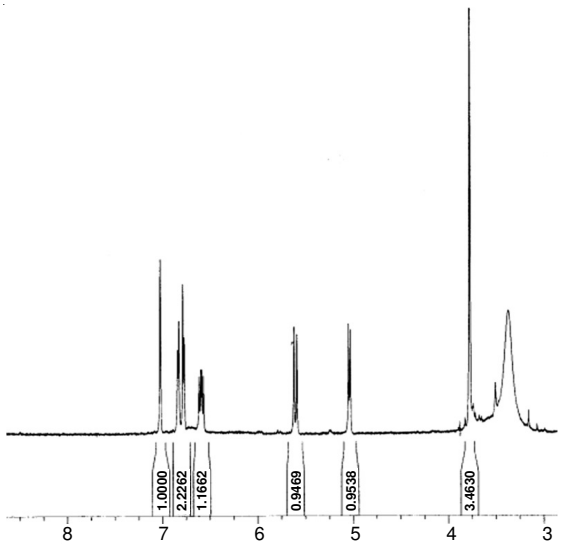

Fig. 1. ^{1}H NMR spectrum of 4-vinyl guaiacol

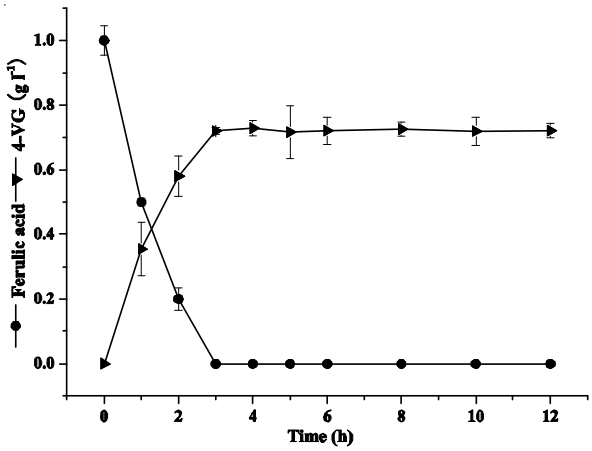

Fig. 2. Biotransformation of ferulic acid to 4-vinyl guaiacol (4-VG) by *Bacillus pumilus* S-1. The biotransformation medium was composed of 20 g L^{-1} glucose, 5 g L^{-1} yeast extract, 10 g L^{-1} peptone and 10 g L^{-1} NaCl (pH 7.2). The biotransformation was carried out on a rotary shaker with 180 rpm at 30 ºC and 50 mL medium in 500 mL flask. The values were means of three replicates, and the error bars indicated standard deviations. Symbols represent: ●, ferulic acid; ▶, 4-vinyl guaiacol

Fig. 3 shows the metabolic pathways of ferulic acid in different strains. Some strains (*Streptomyces setonii*, *Pseudomonas fluorescens*, *Amycolatopsis* sp.) convert ferulic acid directly to vanillin and vanillic acid through deacetylation, which was further metabolized through guaiacol or protocatechuic acid[2,6]. While in some other strains (*Debaryomyces hansenii*, *Candida versatilis*, *Schizophyllum commune*, *Lactobacillus plantarum*, *Enterobacter* sp., *Aspergillus niger*, *Sporotrichum thermophile*, *B. coagulans*), 4-vinyl guaiacol was found as an intermediate in the ferulic acid metabolism, which was further converted to vanillin or 4-ethylguaiaco[l5,6,9-13]. Though the fate of 4-vinyl guaiacol was different, it was quickly transformed to other vanilla flavour compounds. Therefore, most of the produced 4-vinyl guaiacol was not accumulated.

Different from former reports, 4-vinyl guaiacol was not further transformed and maintained unchanged in the following 9 h during the biotransformation. The result showed that *B. pumilus* S-1 had a weak biotransformation ability for 4-vinyl

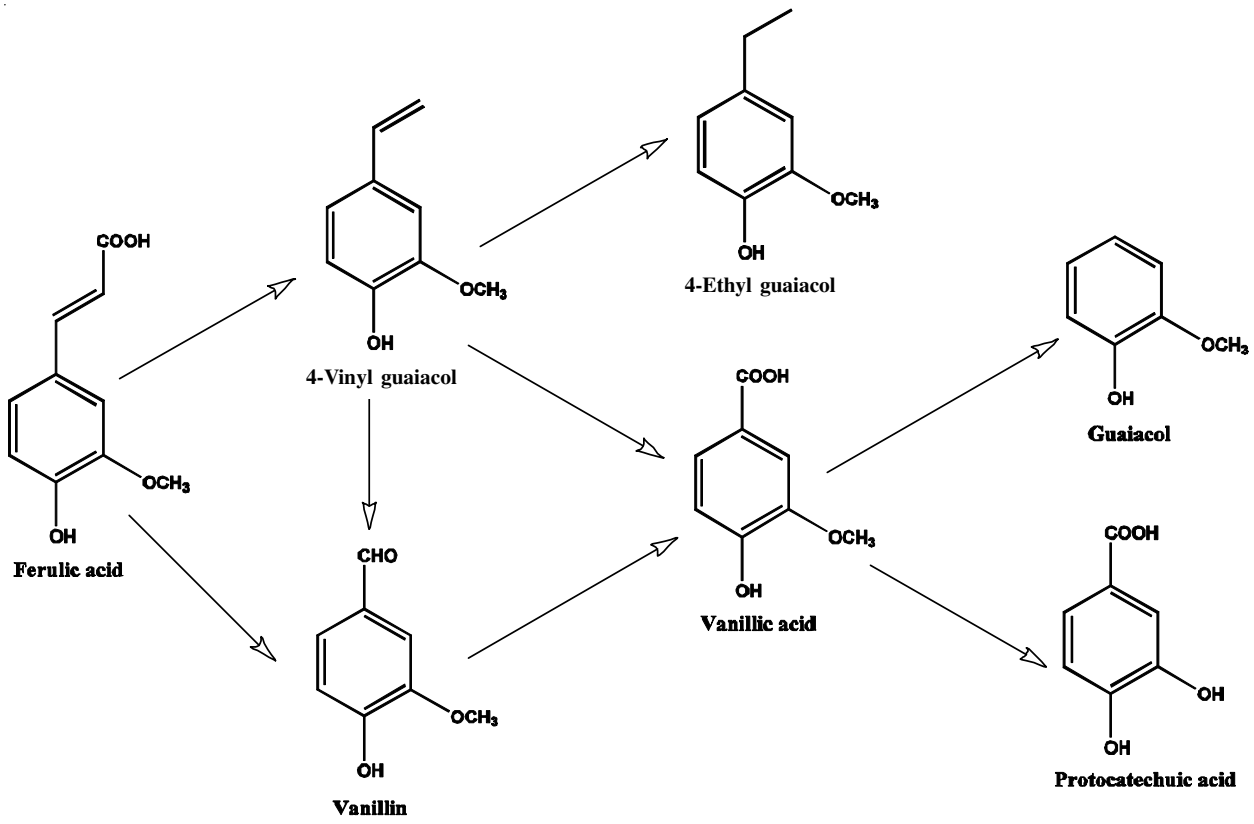

Fig. 3. Metabolic pathways of ferulic acid in different strains

guaiacol. No other vanilla flavour compounds, such as vanillin, vanillyl alcohol, vanillic acid or guaiacol, were detected. As a result, it becomes easier for product isolation, process optimization and scaling-up in 4-vinyl guaiacol production from ferulic acid. Therefore, the biotransformation process described in this paper suggests *B. pumilus* S-1 might have the potential in bioproduction of 4-vinyl guaiacol.

Conclusion

In this paper, a *Bacillus pumilus* strain S-1, earlier reported to efficient convert isoeugenol to vanillin, was found to rapidly dissimilated ferulic acid within 3 h. 4-Vinyl guaiacol was identified as the main product and the highest concentration reached 0.72 g L^{-1}, with a molar yield of 93.1 %. No other vanilla flavour compounds were found in this biotransformation process.

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