Research article

Direct biodegradation of eugenol to coniferyl aldehyde and other higher value-added products by *Gibberella fujikuroi* ZH-34

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ABSTRACT

Background: Eugenol is an economically favorable substrate for the microbial biotransformation of aromatic compounds. Coniferyl aldehyde is one kind of aromatic compound that is widely used in condiment and medical industries; it is also an important raw material for producing other valuable products such as vanillin and protocatechuic acid. However, in most eugenol biotransformation processes, only a trace amount of coniferyl aldehyde is detected, thus making these processes economically unattractive. As a result, an investigation of new strains with the capability of producing more coniferyl aldehyde from eugenol is required.
Results: We screened a novel strain of *Gibberella fujikuroi*, labeled as ZH-34, which was capable of transforming eugenol to coniferyl aldehyde. The metabolic pathway was analyzed by high-performance liquid chromatography–mass spectrometry and transformation kinetics. The culture medium and biotransformation conditions were optimized. At a 6 h time interval of eugenol fed-batch strategy, 3.76 ± 0.22 g/L coniferyl aldehyde was obtained, with the corresponding yield of 57.3%.
Conclusions: This work improves the yield of coniferyl aldehyde with a biotechnological approach. Moreover, the fed-batch strategy offers possibility for controlling the target product and accumulating different metabolites.
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1. Introduction

The use of microorganisms to degrade aromatic compounds has been the focus of investigation for decades. This is due to the fact that microorganisms are used for not only the remediation of water or soil polluted by aromatic hydrocarbons from the chemical industry but also their application in the biotechnological production of valuable aromatic compounds such as vanillin, coniferyl aldehyde, and associated metabolites [1,2,3,4]. Moreover, generating these compounds by microbial transformation is attractive because the process is considered "natural" by the US and European legislation [5,6]. Coniferyl aldehyde, also known as 4-hydroxy-3-methoxycinnamyl aldehyde, is a kind of phenylpropanoid that is used for flavor and in pharmaceutical and medical industries [7,8]. It is also an important raw material for producing other valuable products such as vanillin and protocatechuic acid [9]. Moreover, coniferyl aldehyde possesses fivefold higher anti-inflammatory activity than that of aspirin, as well

as potent antiplatelet aggregation activity [10,11]. Coniferyl aldehyde can be used as a potent inducer of heat shock factor 1, which upregulates heat shock proteins, thereby protecting cells from various stimuli including oxidative stress, heat, and radiation [12,13]. In nature, coniferyl aldehyde is isolated from the insecticidally active hot dichloromethane extract of heartwood of *Gliricidia sepium* and barks of *Cinnamomum cebuense* [14]. Although chemically produced coniferyl aldehyde occupies a majority of the total market share, it cannot be regarded as a natural aromatic chemical, and its use is restricted in food and fragrance industries. As a result, methods of microbial transformation for coniferyl aldehyde production are sought for.

Some plant-derived phenylpropanoids such as isoeugenol, eugenol, and ferulic acid have attracted attention as natural renewable resources for the synthesis of fine chemicals [15,16,17,18]. To date, a great variety of studies on microbial biotransformation for chemical production using isoeugenol and ferulic acid as substrates have been reported, while few of them have directly used eugenol as the substrate. Eugenol is the main component of the essential oil of the clove tree *Syzygium aromaticum*, and it is inexpensive. Several bacteria and fungi such as *Corynebacterium*, *Byssochlamys*, *Pseudomonas*, *Rhodococcus,* and *Penicillium* were reported to degrade eugenol [19,20,

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21,22,23,24,25]. *Pseudomonas* sp. was most intensively studied at the genetic and biochemical levels for its ability to metabolize eugenol to vanillin and related methoxyphenols [26]. The epoxidation of eugenol to form eugenol oxide was suggested as the initial step in the degradation of eugenol by *Pseudomonas* sp. In this work, coniferyl alcohol and coniferyl aldehyde were detected before being further metabolized to ferulic acid [27]. *Pseudomonas* sp. HR199 was reported to degrade eugenol to coniferyl alcohol, and the yield of coniferyl alcohol was 43.5% [28]. Other strains such as *P. fluorescens* E118, a clove oil-tolerant strain, can accumulate ferulic acid with the intermittent addition of eugenol, and *Bacillus cereus* PN24 degraded eugenol to protocatechuic acid, which was further metabolized by a β-ketoadipate pathway [29,30].

However, in most eugenol biotransformation processes, only a trace amount of coniferyl aldehyde was detected, thus making these processes economically unattractive. This requires an investigation of new strains with the capability of producing more coniferyl aldehyde from eugenol. In this work, we found a novel strain of *Gibberella fujikuroi*, labeled as ZH-34, capable of transforming eugenol to coniferyl aldehyde and related phenolic aromatic products. The major metabolites were detected, and a pathway for the biotransformation of eugenol was proposed. Moreover, the culture medium and biotransformation condition for coniferyl aldehyde production were optimized to achieve a high product yield. This work is thought to offer possibility of improving the yield of high value-added products such as coniferyl aldehyde with a biotechnological approach.

2. Materials and methods

2.1. Materials

Eugenol, coniferyl aldehyde, coniferyl alcohol, ferulic acid, and vanillin were all chromatographically pure and obtained from Aladdin Co., Ltd. (Shanghai, China). Acetonitrile (99.9%) was purchased from J&k Scientific Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and used without further purification.

2.2. Strain screening

A total number of 64 filamentous fungi stored in our laboratory were screened for eugenol-degrading strains. Typically, the strains were cultured aerobically in sterile seed medium containing 200 g/L potato, 20 g/L glucose, and 3 g/L yeast extract and incubated in an orbital shaker at 110 r/min and 30°C for 2 days. Then, 20 g/L eugenol emulsion containing eugenol and Tween 80 was added to the medium at a final eugenol concentration of 1 g/L. Biotransformation was conducted in the shaker at 110 r/min and 30°C. A control experiment was carried out by adding 1 g/L eugenol into the same medium without mold inoculation. Samples were taken at 48 h after addition of eugenol and were acidified to pH 2.0 with HCl and extracted with equal volumes of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, concentrated, and analyzed by thin-layer chromatography (TLC) to test the degradation of eugenol and the accumulation of reaction metabolites. The developing agent was a mixture of hexane, chloroform, anhydrous ether, ethyl acetate, and acetic acid with a volume ratio of 4:3:2:2:0.1. These metabolites were quantified by high-performance liquid chromatography (HPLC). The strain with the highest yield of coniferyl aldehyde was designated as ZH34 and chosen for further investigation.

2.3. Strain characterization

Phenotypic characterization of the strain ZH34 was carried out [31]. Genomic DNA of the strain was extracted using a Fungal Genomic DNA Isolation Kit (B518229; Sangon Biotech) by following the recommended procedure of the manufacturer. ITS-rDNA was amplified with the

universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The PCR program used was as follows: 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 90 s, and a final extension step at 72°C for 10 min. The sequencing of the gel-purified PCR products was performed by Sangon Biotech Co., Ltd. (Shanghai, China). The resulting sequence was aligned using the CLUSTALW program and compared with those in the GenBank database using the BLASTN (http://www.ncbi.nlm.nih.gov/BLAST) program. Phylogenetic and molecular evolutionary analyses were performed with Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06 (http://www.megasoftware.net/) [32].

2.4. Identification of reaction metabolites

HPLC–mass spectrometry (HPLC-MS) was used to identify reaction metabolites. The biotransformation mixtures were centrifuged at 1000 r/min for 10 min to remove cells and then filtered through a 0.45 μm filter before quantification by HPLC-MS with a gradient elution method. A Waters Acquity UPLC system (Waters, Massachusetts, USA) equipped with a column (BEH C18, 100 × 2.1 mm, 1.7 μm; Waters) and a refractive index detector (Waters Acquity PDA) was used. The column temperature was 45°C, and the detection wavelength ranged from 200 to 600 nm. The mobile phase consisted of acetonitrile (solvent A) and 0.1% aqueous formic acid (solvent B). Analyses were performed at a flow rate of 0.3 mL/min using the following gradient: 0–17 min, 5% A and 95% B; 17–20 min, 60% A and 40% B; 20–22 min, 100% A; and 22–25 min, 5% A and 95% B.

2.5. Cultivation of the strain ZH34

The strain ZH34 was first cultured in sterile seed medium containing 200 g/L potato extract, 20 g/L glucose, and 3 g/L yeast extract and incubated at 110 r/min and 30°C for 18 h. Then, different inoculation amounts of seed culture varying from 2% to 15% was inoculated into the culture medium containing 5 g/L glucose, 3 g/L yeast extract, 2 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.2 mg/L $FeSO_4 \cdot 7H_2O$, 0.3 mg/L H_3BO_3, 0.04 mg/L $CuSO_4 \cdot 5H_2O$, 0.1 mg/L KI, 0.4 mg/L $MnSO_4 \cdot 7H_2O$, and 0.2 mg/L $Na_2MoO_4 \cdot 2H_2O$ at pH 7.0. The culture medium mentioned above was not optimized and hence needed to be optimized for a higher coniferyl aldehyde production. The cultivation process was carried out at 110 r/min and 30°C for different culture ages varying from 12 to 40 h. Each experiment was run three times, and the data shown are the means of three separate experiments with standard deviation.

2.6. Production of coniferyl aldehyde by the strain ZH34

After the cultivation process, biotransformation was conducted by adding eugenol emulsion into the biotransformation culture. Different co-solvents, namely, Tween 80, Span 80, glyceryl trioleate, dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (SDS) were added into water and mixed with eugenol to make the emulsion. Biotransformation was carried out in the shaker at 110 r/min. The temperature varied from 20 to 45°C. To evaluate the effect of initial eugenol concentration on coniferyl aldehyde production, varying concentrations of eugenol (0.5, 1.0, 1.5, 2.0, and 2.5 g/L) were adopted. Samples were drawn from the biotransformation mixture at 5 h time intervals and analyzed by HPLC for eugenol substrate and intermediary metabolites. A fed-batch strategy was used with eugenol feeding time intervals of 6 and 8 h, and a total of 6 g/L eugenol was added. Samples were withdrawn for analysis before each feeding. The coniferyl aldehyde yield was defined as the amount of coniferyl aldehyde produced from 1 g of eugenol consumed (expressed as

percentage). Each result was the average of values from at least three parallel experiments.

2.7. High-performance liquid chromatography (HPLC) analysis

The biotransformation mixtures were centrifuged at 1000 r/min for 10 min to remove cells and then filtered through a 0.45 μm filter before quantification by HPLC with a gradient elution method. A Hitachi L2000 HPLC system (Hitachi, Tokyo, Japan) equipped with a column (Amethyst C18-H, 250 × 4.6 mm, 5 μm, Sapax Technologies, Delaware, USA) and a refractive index detector (Hitachi L2000) was used. The mobile phase consisted of acetonitrile (solvent A) and 0.1% aqueous formic acid (solvent B). Analyses were performed using the following gradient: 0–4 min, 10% A and 90% B at a flow rate of 1.0 mL/min; 4–20 min, 20% A and 80% B at a flow rate of 0.9 mL/min; 20–30 min, 75% A and 25% B at a flow rate of 1.0 mL/min; and 30–35 min, 10% A and 90% B at a flow rate of 1.0 mL/min. The column temperature was 30°C, and the detection wavelength was set at 280 nm.

3. Results and discussion

3.1. Screening and characterization of the strain ZH-34

Among the filamentous fungi stored in our laboratory, 16 were found to use eugenol as a carbon and energy source, and hence, they were examined for the biotransformation of eugenol to related methoxyphenols (partially shown in Fig. S1a). Substrate biotransformation was followed by HPLC analyses. Fig. S2b showed chromatograms of mixed standards of vanillic acid (retention time at 11.37 ± 0.2 min), coniferyl alcohol (14.32 ± 0.2 min), vanillin (15.07 ± 0.5 min), ferulic acid (16.31 ± 0.3 min), coniferyl aldehyde (22.86 ± 0.2 min), and eugenol (26.41 min ± 0.4). Reaction metabolites were also clearly separated by the gradient elution method we adopted (Fig. S2c). Referring to the consistent retention time, three possible metabolites were observed: coniferyl alcohol, vanillin, and coniferyl aldehyde; each of them can be quantified by comparison with standards. Among the strains that can degrade eugenol, one strain designed as ZH-34 was found to produce more coniferyl aldehyde than the other 15 filamentous fungi. As a result, the strain ZH-34 was chosen for further studies.

This strain was analyzed combining its morphological characteristics (Fig. S2) and ITS-rDNA gene sequence analysis. The central texture of the colony is uniform, dense, and velvet, with a pale pink color. The hyphae have diaphragms and are capable of budding. Conidia are produced from hyphal tips and are single celled and round. On the basis of the phylogenetic analysis of near to full ITS-rDNA sequence obtained from the strain SPR1, the sequence matched with those of *Fusarium* sp. and *Gibberella* sp., with similarities in both higher than 98.0% based on 561 bp. Phylogenetic tree obtained by the neighbor-joining method demonstrated that the position of the strain ZH-34 was most close to the *Gibberella intermedia* strain DG-10-4-3-2 (Fig. 1). On the basis of the results of morphological characteristics and also ITS-rDNA sequence analysis, the strain ZH-34 was identified as *G. fujikuroi* ZH-34 and was stored at China Center for Type Culture Collection (number CCTCC M2016171).

3.2. Metabolic pathway of eugenol in G. fujikuroi ZH-34

Reaction metabolites from *G. fujikuroi* ZH-34 were analyzed by HPLC-MS by comparing their retention times and mass spectra with those of the authentic standards. The metabolites b, c, d, and e with retention times at 10.31, 12.58, 12.00, and 9.51 min, respectively, were accumulated in the biotransformation mixture with eugenol as shown in Fig. 2a. These metabolites were identified as coniferyl alcohol, coniferyl aldehyde, ferulic acid, and vanillin, respectively, with $[M-H]^-$ ion peaks appearing at m/z values of 179.0, 177.0, 193.0, and 151.0, respectively.

The metabolic pathway of eugenol was investigated by biotransformation kinetics. A model fermentation and biotransformation process was conducted with the following conditions: unoptimized fermentation medium, 50 mL culture in 500 mL flask, 8% inoculation amount (which means 4 mL seed was added into 50 mL culture medium), 20 h culture age, 1.0 g/L eugenol concentration with Tween 80 as the co-solvent, and 30°C biotransformation temperature. The curves generated for metabolite production after adding eugenol are depicted in Fig. S3. Eugenol was depleted after 6 h, leading to 0.26 g/L coniferyl alcohol and 0.54 g/L coniferyl aldehyde. Then, the concentration of coniferyl aldehyde increased to 0.71 g/L at 12 h together with a rapid decrease in coniferyl alcohol concentration. At this time, no ferulic acid or vanillin was detected in the biotransformation mixture. After 12 h, coniferyl aldehyde concentration decreased and ferulic acid was produced slowly with vanillin. After 36 h, concentrations of both coniferyl alcohol and coniferyl aldehyde decreased rapidly. The concentration of ferulic acid reached the highest value of 0.32 g/L at 42 h. This result indicated that the metabolic pathway was from coniferyl alcohol, coniferyl aldehyde, and ferulic acid to vanillin. Moreover, each intermediate was also fed to cultures of the strain
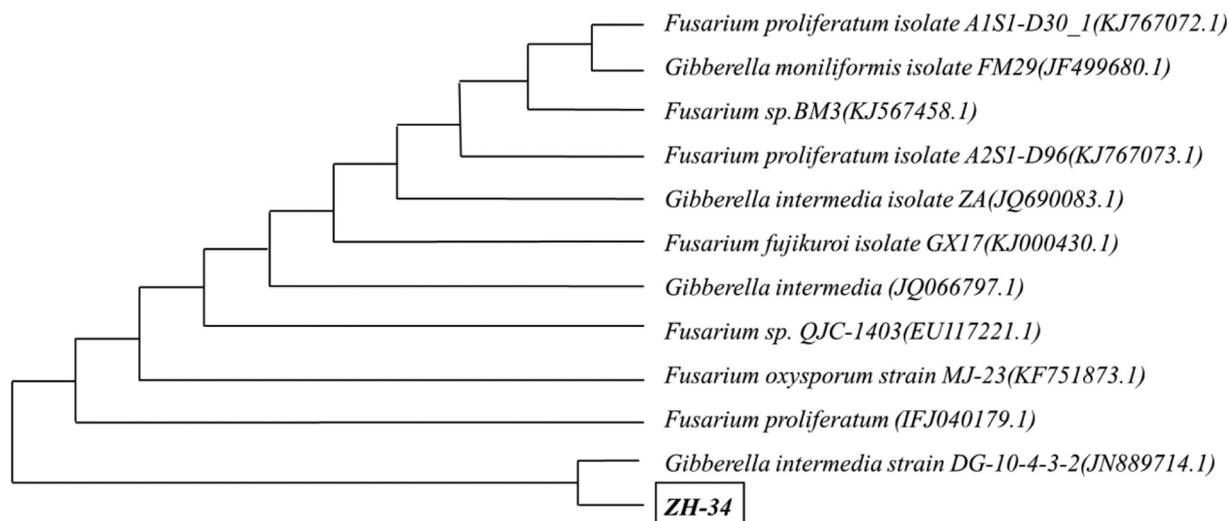

Fig. 1. Phylogenetic tree of *G. fujikuroi* ZH-34 based on ITS-rDNA sequences. GenBank accession numbers are given in parentheses.
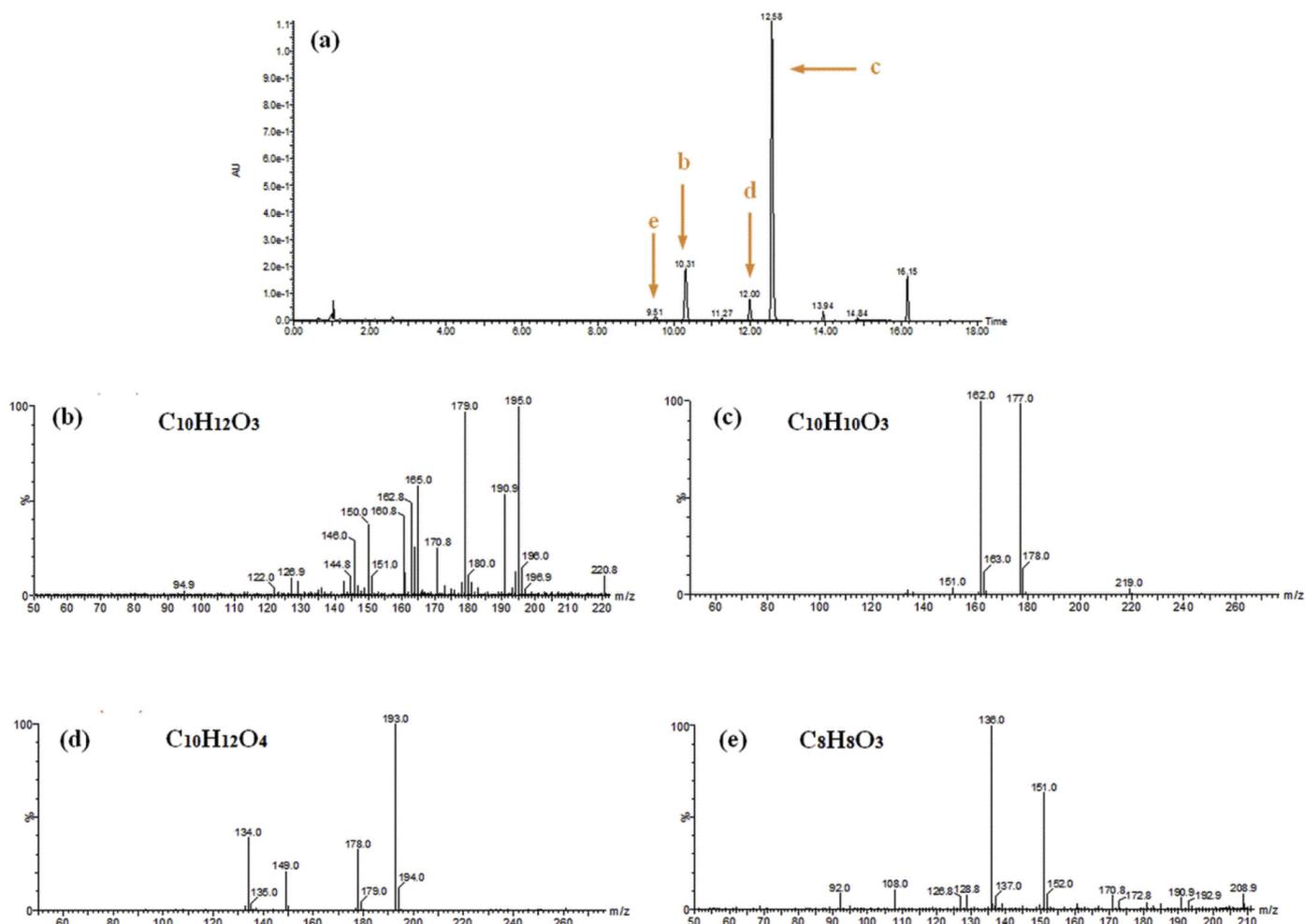
Fig. 2. HPLC-MS spectra of eugenol-produced metabolites (a). (b) to (e) are mass spectra of the substances indicated by b, c, d, and e in (a).

ZH34. We observed that spots of the metabolite disappeared and spots of vanillin appeared in TLC analysis (data not shown), hence showing that these compounds could also support the growth of the strain
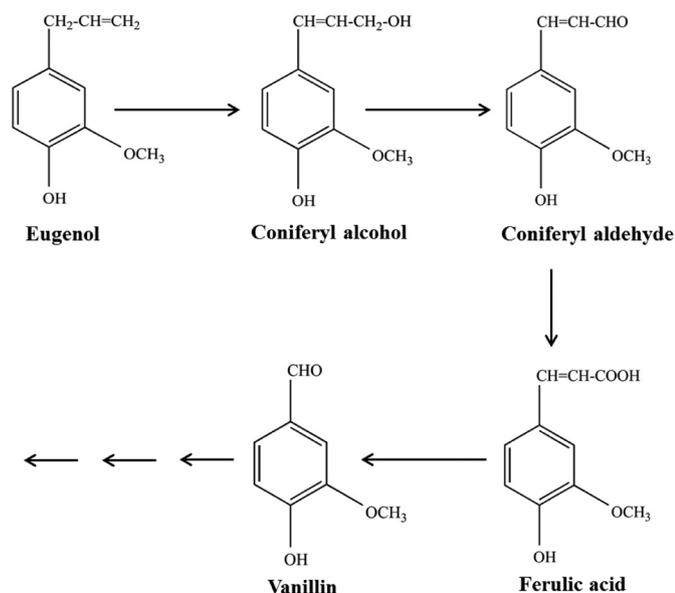

Fig. 3. Hypothetical pathway for the biotransformation of eugenol by *G. fujikuroi* ZH-34.

ZH34 as the sole carbon and energy source. The metabolic pathway of *G. fujikuroi* ZH-34 is proposed in Fig. 3. The eugenol catabolism in this strain was found to be similar to those found in *Pseudomonas* sp. HR199 and *Pseudomonas nitroreducens* Jin1 [26,28]. Notably, according to the results, one can accumulate different target products by tailoring the fermentation and biotransformation conditions.

3.3. Effect of culture medium and cultivation condition on coniferyl aldehyde production

First, different carbon and nitrogen sources were used for strain growth, and the corresponding metabolite concentrations are summarized in Table S1. According to this table, glucose and yeast extract performed better in coniferyl aldehyde production than others at the same concentration. Glucose and yeast extract concentrations were optimized, as shown in Fig. S4a and b, thus indicating that 10 g/L glucose and 3 g/L yeast extract should be used in the fermentation medium. Changes in pH can influence nutrient uptake by the strain and mass transfer of metabolites, hence influencing both strain growth and target product production. Culture media with different pH values were used, and the corresponding metabolite concentrations are shown in Fig. S4c. Coniferyl aldehyde can be produced at a pH range of 6.5 to 10.0. At pH 7.5, 0.83 ± 0.04 g/L coniferyl aldehyde was obtained; further increasing pH values led to a decrease in coniferyl aldehyde concentration. As a result, the pH value of the fermentation medium was kept at pH 7.5.

Cultivation conditions were optimized, and the results are shown in Fig. 4. Oxygen influences strain growth and the corresponding enzyme
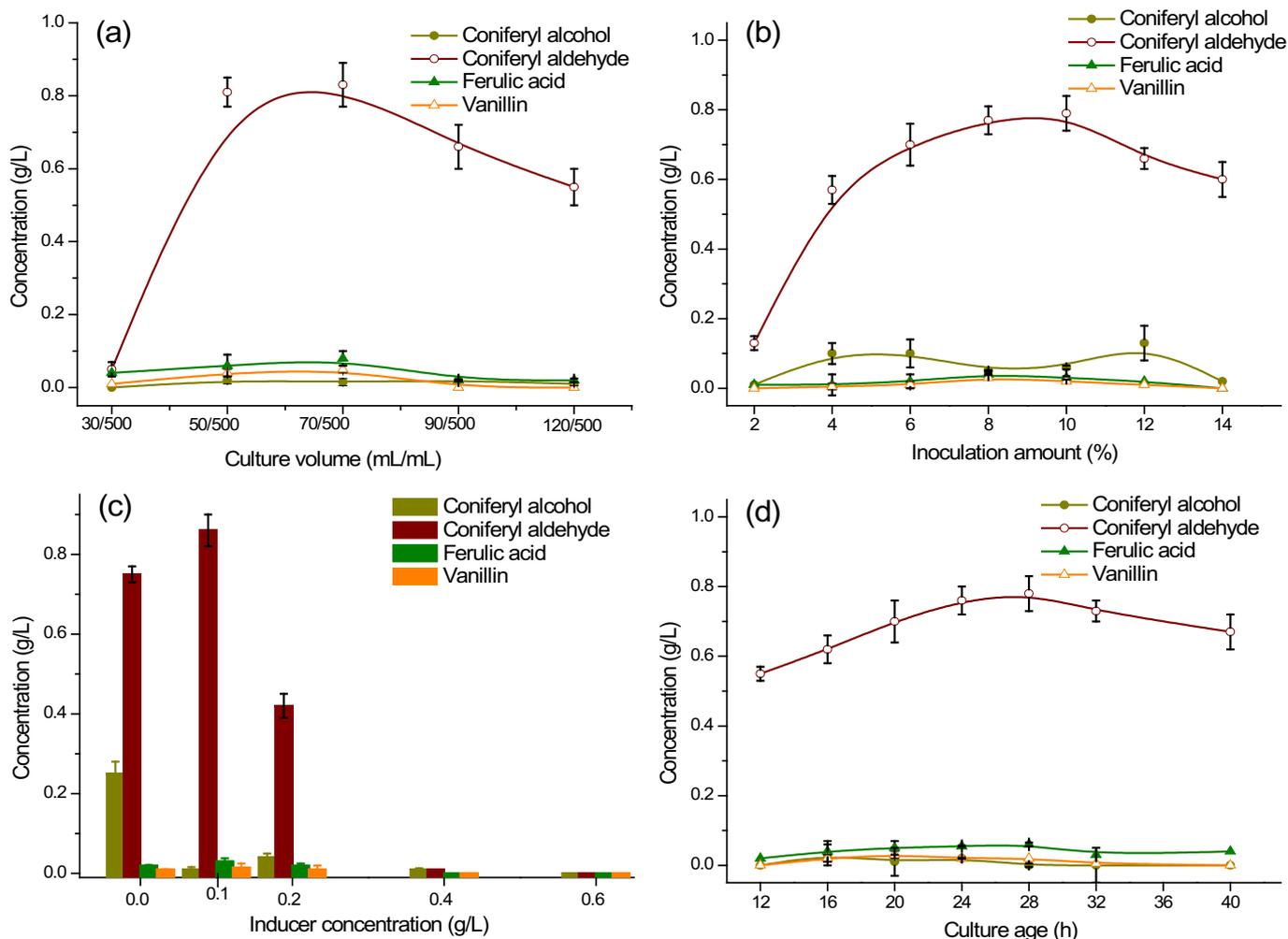
Fig. 4. Effects of culture volume (a), inoculation amount (b), inducer concentration (c), and culture age (d) on coniferyl aldehyde production by *G. fujikuroi* ZH-34.

activity, thus influencing product accumulation [33,34]. In this work, oxygen supply was studied by considering culture volume of the media/volume of the bottle. In Fig. 4a, a little amount of coniferyl aldehyde was accumulated with a 30/500 (mL/mL) culture volume. In this circumstance, too much oxygen in the flask can degrade eugenol quickly, and the intermediate products such as coniferyl alcohol and coniferyl aldehyde were also degraded quickly; as a result, the target product cannot be accumulated. Increasing the culture volume led to an increase in the coniferyl aldehyde concentration, and when the culture volumes were 50/500 and 70/500 (mL/mL), the yield was 0.81 ± 0.04 and 0.83 ± 0.06 g/L coniferyl aldehyde, respectively. Further increasing the culture volume produced less coniferyl aldehyde, thus indicating that eugenol degradation with *G. fujikuroi* ZH-34 was an aerobic process, and an appropriate amount of oxygen was needed to biotransform eugenol to coniferyl aldehyde. Fig. 4b presents the effect of inoculation amount on coniferyl aldehyde production. Two percent inoculation amount led to a little amount of metabolites in the biotransformation mixture, which was because the cell concentration was low and the cells were sensitive to the environment. Increasing inoculation amount witnessed an increase in the coniferyl aldehyde concentration; when the inoculation amount was 8% and 10%, the corresponding coniferyl aldehyde concentrations were 0.77 ± 0.04 and 0.79 ± 0.05 g/L, respectively, and no eugenol was detected in the biotransformation mixture. An inoculation amount higher than 10% resulted in a decrease in the coniferyl aldehyde concentration, and there was eugenol remaining in the

biotransformation mixture. This was because cells can grow quickly at a high inoculation amount in a short time, which may change the culture composition and cause an accumulation of toxic substances, thus leading to a hindrance to the performance of vanillyl alcohol oxidase, coniferyl alcohol dehydrogenase, or eugenol hydroxylase participating in the biotransformation of eugenol into coniferyl aldehyde [35,36]. Moreover, the mass transfer limitation of dissolved oxygen may affect the metabolism and the concentration of the target product. As a result, an inoculation amount of 10% was adopted.

Inducer concentration and culture age were also optimized, as indicated in Fig. 4c and d. Eugenol was used as the inducer in order to increase the amount of enzymes participating in the biotransformation. However, eugenol was toxic and can hinder cell growth; hence, its concentration should not be too high. Eugenol concentration of 0.1 g/L performed best for the final coniferyl aldehyde production. Culture age is related to seed vitality. According to this figure, when the culture age was 12 h, cells were still growing and could not produce a large amount of metabolites. Increasing the culture age helped to improve metabolite concentration, and when it was 28 h, 0.78 ± 0.05 g/L coniferyl aldehyde could be produced. Further increase in the culture age caused a decrease in metabolite concentrations.

As a result, the optimized culture medium and cultivation condition were 10 g/L glucose, 3 g/L yeast extract, pH 7.5, 70/500 (mL/mL) culture volume, 10% inoculation amount, 0.1 g/L eugenol as the inducer, and 28 h culture age.
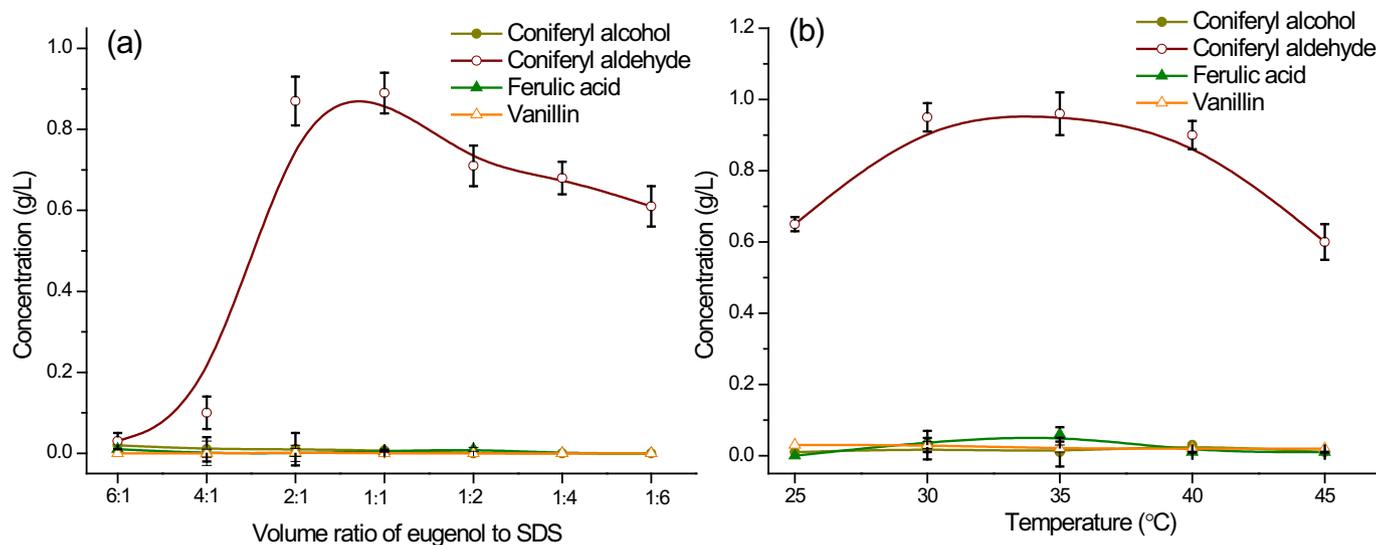
Fig. 5. Effects of volume ratio of eugenol to SDS (a) and temperature (b) on coniferyl aldehyde production by *G. fujikuroi* ZH-34.

3.4. Effect of biotransformation condition on coniferyl aldehyde production

Co-solvents were mixed with eugenol to form a substrate emulsion to decrease the mass transfer resistance of eugenol and form the contact between eugenol and cells more feasible [37,38,39]. Five different co-solvents were used, and the corresponding coniferyl aldehyde yield is shown in Fig. S5. According to this figure, SDS performed the best. It is an ionic surfactant and can improve cell membrane permeability, which facilitates the entry of eugenol into cells. The usage of SDS was optimized as shown in Fig. 5a: when the volume ratio of eugenol to SDS was 6:1 and 4:1, eugenol was depleted at the end of biotransformation, but the concentrations of coniferyl aldehyde, coniferyl alcohol, and ferulic acid were very low, thus indicating eugenol was metabolized into other substances; increasing the SDS portion can increase the production of coniferyl aldehyde, and when the ratio was 1:1, the highest coniferyl aldehyde concentration of 0.89 ± 0.05 g/L was produced; further increasing the SDS portion led to a decrease in coniferyl aldehyde concentration, which was probably due to a severe damage to the cell membrane. According to these results, a volume ratio of eugenol to SDS of 1:1 was chosen.

Temperature also plays an important role in biotransformation. Generally, increasing the temperature favors the enzymatic catalytic reaction rate; however, when the temperature is too high, the enzyme can be deactivated. The effect of temperature on coniferyl aldehyde production is shown in Fig. 5b, thus suggesting the temperature should be controlled within 30–35°C.

Eugenol concentration and bioconversion time also affected the production of coniferyl aldehyde, as shown in Table 1. From an economic point of view, it is encouraged to obtain more amount of target product with less substrate. When an initial eugenol concentration of 0.5 g/L was added, 0.15 ± 0.01 g/L coniferyl aldehyde was produced, and eugenol was depleted within 10 h; after 10 h, coniferyl aldehyde was depleted with little vanillin remaining. In the range of 1.0–2.5 g/L eugenol concentration, the concentration of coniferyl aldehyde increased with bioconversion time; at 50 h, the corresponding coniferyl aldehyde yield was 94.0%, 72.0%, 2.03%, and 16.8%. Eugenol concentration 1.0 g/L produced the highest coniferyl aldehyde yield; hence, the substrate concentration was fixed at 1.0 g/L. Under this concentration, 0.92 ± 0.06 g/L coniferyl aldehyde was obtained at 35 h. As a result, eugenol concentration and bioconversion time was set at 1.0 g/L and 35 h.

Under the above-mentioned optimized conditions, a trial of improving coniferyl aldehyde production was conducted by intermittent addition of eugenol. The product concentration curves at a 6 h time interval of eugenol addition are shown in Fig. 6a. Coniferyl alcohol started to accumulate after 6 h, and the concentrations of coniferyl alcohol and coniferyl aldehyde increased after eugenol addition. According to the metabolic pathway for the biotransformation of eugenol by *G. fujikuroi* ZH-34, coniferyl aldehyde is obtained from coniferyl alcohol; the 6 h time interval was not enough for all coniferyl alcohols to be converted into coniferyl aldehydes, and thus, coniferyl alcohol was accumulated. Under this

Table 1
Effects of eugenol concentration and bioconversion time on the production of coniferyl aldehyde (g/L).

| Bioconversion time (h)	Eugenol concentration (g/L)				
	0.5	1.0	1.5	2.0	2.5
5	0.15 ± 0.01	0.51 ± 0.03	0.55 ± 0.03	0.17 ± 0.01	0.16 ± 0.01
10	0	0.68 ± 0.02	0.71 ± 0.03	0.22 ± 0.02	0.20 ± 0.02
15	0	0.75 ± 0.04	0.87 ± 0.04	0.29 ± 0.01	0.25 ± 0.03
20	0	0.79 ± 0.05	0.93 ± 0.03	0.30 ± 0.02	0.28 ± 0.02
25	0	0.85 ± 0.05	0.96 ± 0.06	0.34 ± 0.03	0.32 ± 0.03
30	0	0.88 ± 0.07	1.00 ± 0.08	0.35 ± 0.03	0.34 ± 0.03
35	0	0.92 ± 0.06	1.04 ± 0.07	0.38 ± 0.02	0.35 ± 0.02
40	0	0.93 ± 0.07	1.05 ± 0.08	0.40 ± 0.03	0.38 ± 0.03
45	0	0.94 ± 0.09	1.06 ± 0.10	0.43 ± 0.03	0.40 ± 0.04
50	0	0.94 ± 0.06	1.08 ± 0.09	0.46 ± 0.04	0.42 ± 0.04
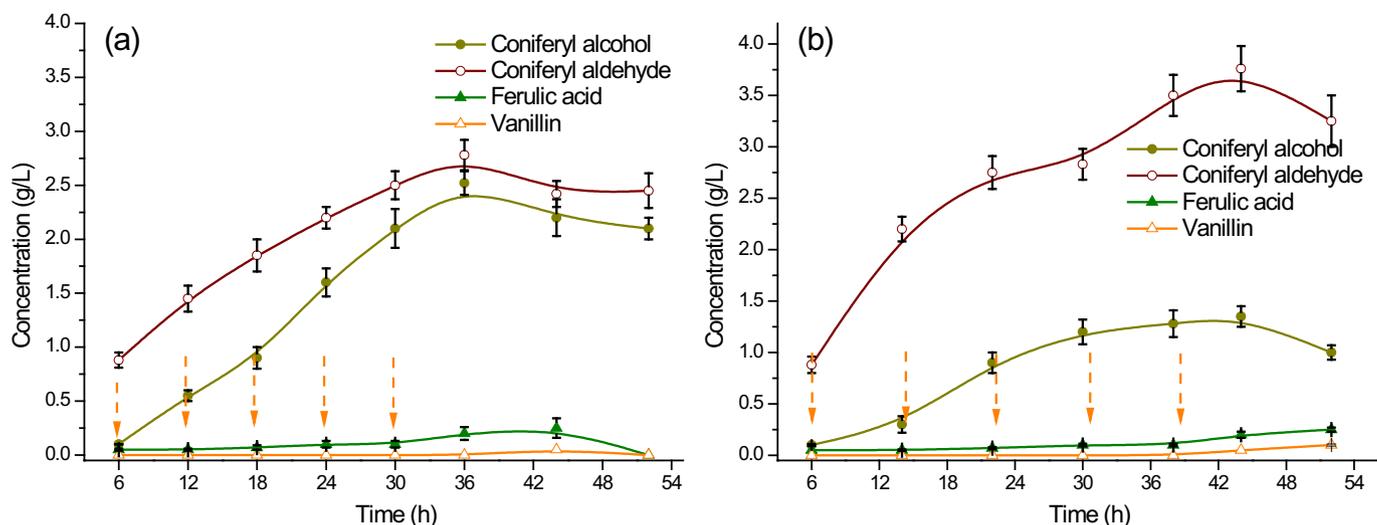
Fig. 6. Coniferyl aldehyde production under eugenol feeding intervals of 6 h (a) and 8 h (b). Dotted arrows indicate that eugenol was added at this time.

fed-batch strategy, 2.78 ± 0.11 g/L coniferyl aldehyde and 2.52 ± 0.14 g/L coniferyl alcohol were obtained. On the basis of this result, we thought that extending the eugenol feeding time interval can promote the conversion of coniferyl alcohol to coniferyl aldehyde. Fig. 6b depicts coniferyl aldehyde production under a eugenol feeding interval of 8 h. From this figure, we can see that the accumulation of coniferyl alcohol was slow, and the concentration of coniferyl aldehyde was significantly higher than that of 6 h time interval. In this trial, 1.35 ± 0.1 g/L coniferyl alcohol and 3.76 ± 0.22 g/L coniferyl aldehyde were obtained with the corresponding coniferyl aldehyde yield of 57.3%.

In this work, we screened a strain of *G. fujikuroi* ZH-34, which was capable of transforming eugenol into coniferyl aldehyde. At a 6 h time interval of eugenol fed-batch strategy, 3.76 ± 0.22 g/L coniferyl aldehyde with the corresponding yield of 57.3% were obtained. Moreover, this work indicates that different products such as coniferyl alcohol, coniferyl aldehyde, and ferulic acid can be accumulated by tailoring the fed-batch strategy.

Conflicts of interests

The authors declare no conflicts of interest in the manuscript.

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Supplementary material

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