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Detection of catechol using an electrochemical biosensor based on engineered *Escherichia coli* cells that surface-display laccase

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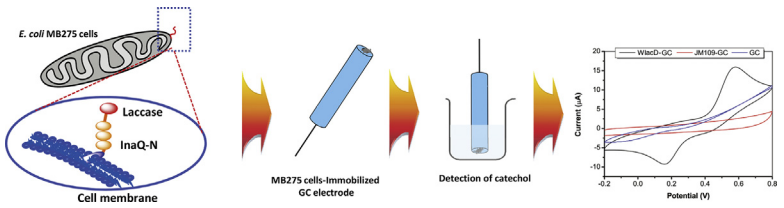
HIGHLIGHTS

- A novel biosensor based on live bacterial cells with surface-targeted laccases.
- Easy electrode preparation by simple adsorption.
- Comparable LOD with other chemically-modified laccase based biosensors.
- High stability, reproducibility, and accuracy for catechol detection.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we report an electrochemical microbial biosensor that was made by immobilizing a bacterial laccase on the surface of *Escherichia coli* cells followed by adsorption of modified live cells onto a glassy-carbon electrode. Expression and surface localization of laccase on target cells were confirmed by Western blotting, flow cytometry assays and immunofluorescence microscopy observation. Increased tandem-aligned anchors with three repeats of the N-terminal domain of an ice nucleation protein were used to construct a highly active *E. coli* whole cell laccase-based catalytic system. When the proposed biosensor was used to detect catechol, the electrochemical response under optimized pH conditions was linear within a concentration range of 0.5 μM–300.0 μM catechol. Metal ions (Mn^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+}, Al^{3+} and Zn^{2+}) at concentrations from 1 to 10 mg L^{-1}, bovine serum albumin and glucose at concentrations from 0.1 to 10 g L^{-1}, and ascorbic acid at concentrations from 0.01 to 0.1 g L^{-1} did not cause a noticeable interference effect. The detection limit of 0.1 μM catechol was comparable to those of other biosensors based on purified chemically modified laccases. When used to detect catechol in real red wine and tea samples, the biosensor offered a considerable level of accuracy comparable to the HPLC method as well as high recovery rates (98.2%–103.8%) towards all of the tested samples. Moreover, the developed system also exhibited high stability and reproducibility.

1. Introduction

Over the past few decades, a variety of biological materials, such as enzymes, antibodies, microbial cells, eukaryotic cells and organelles, have been extensively used for the construction of various biosensors [1,2]. Of these available biologically active materials, microbial enzymes, especially oxidoreductases, are the most commonly used materials because of their high reactivity, high selectivity, and relatively low cost [3,4]. Recently, biosensors based on microbial laccases have received increasing attention because of

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the advantages of laccase, such as its direct reduction reactivity of oxygen in water without accumulation of intermediate H_2O_2 (unlike the peroxidases) and non-requirement for an additional mediator, enabling the non-bypass electrochemical detection of electron transfer from substrates to corresponding oxides [3,5].

A technical problem of laccase-based biosensors is the efficient and facile immobilization of free enzyme onto the electrode surface without significant activity loss. Conventional strategies for laccase–electrode immobilization often use various inorganic or organic matrixes that chemically modify the electrode surface, thereby binding laccase through covalent or non-covalent linkages [6,7]. Various chemically modified laccase electrodes have been successfully applied for phenolic compound monitoring [8], biofuel cell construction [9], pesticide residue quantification [10], oxygen or azide detection [11] as well as in other fields. Despite the generally improved stability and applicable detection levels these chemically modified electrodes, their cost-consuming preparation processes should be considered alongside their limited regenerability and possible enzyme activity loss that results from multistep *in vitro* manipulation. Immobilized enzymes using live microbial cell platforms have also gained great attention because of the ease and effectiveness of these available methods, which allow for simple immobilization of cells onto electrodes by adsorption or other similar approaches [12,13].

Displaying intracellularly expressed enzymes on the surface of live microbial cells allows for a direct enzymatic reaction on the cell surface-substrate interface, thereby eliminating mass transfer limitations and increasing reaction rates [14,15]. Most profitably, the stable cell platform facilitates enzyme activity retention and stabilizes surface-displayed enzymes under harsh conditions. Thus, such cellular enzyme components are easily regenerated in unlimited quantities [13,16]. The potential of live bacterial cell-mediated biosensors using microbial cell surface display technology has been recently investigated [17–19]. These biosensors basically have the advantages of stability, specificity, reproducibility and cost-effectiveness compared with those based on purified enzymes. However, immobilization of these surface-displayed cells on the electrode surface has often been achieved through certain chemical modification processes. Thus, complex and time-consuming procedures should be considered.

In Gram-negative bacteria, ice nucleation protein (INP) from *Pseudomonas syringae* primarily functions as an anchor protein to display various other proteins [20]. Cell surface-immobilized functional proteins have been successfully achieved by using full-length INPs [21,22] and truncated variants [16,22] in *Escherichia coli* and other bacterial systems. Previously, we demonstrated the feasibility of displaying a bacterial laccase on *Bacillus thuringiensis* cells without altering its catalytic activity [23]. Moreover, we have demonstrated that increased tandem repeats of a truncated INP domain (the N-terminal domain of INP) in *E. coli* systems significantly increased the display efficiency of some proteins [24]. However, the INP-anchored surface display approach has not yet been used to investigate the functionality of bacterial laccases as biosensors thus far.

Several previous studies have demonstrated the feasibility of immobilizing living bacterial cells onto an electrode surface by direct adsorption [12,25,26]. These biosensors retain the activity of adsorbed cells for several weeks or even months [13]. Consequently, adsorption is an attractive approach to bacterial biosensor development. In the current study, as illustrated in Scheme 1, a mutated bacterial laccase, WlacD [27], was engineered onto the *E. coli* cell surface using previously optimized bacterial cell surface display strategies [22]. Recombinant *E. coli* cells with surface-immobilized laccase were directly dropped onto and adsorbed by a glassy-carbon (GC) bare electrode to construct a biosensor to detect catechol. The effects of cell loading, pH and the scan rate on the whole-cell enzymatic activity and oxidation peak current of the biosensor were investigated, as were the response of biosensor cyclic voltammetry (CV) currents and limits of detection (LOD). The proposed system was applied to determine the presence of catechol in real red wine and tea samples. The catechol content and detection recovery rates were subsequently analyzed.

2. Experimental

2.1. Chemicals and reagents

Catechol, guaiacol, ferulic acid, 1,4-hydroquinone, phenol, ascorbic acid, and isopropyl-β-D-thiogalactoside (IPTG) were purchased from Sigma Chem. Co. (St. Louis, MO, USA). 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was purchased from AMRESCO LLC (Solon, OH, USA) and was used as a substrate to measure laccase enzymatic activity. All of the chemicals were of analytical grade.

2.2. Bacterial strains, plasmids, and culture conditions

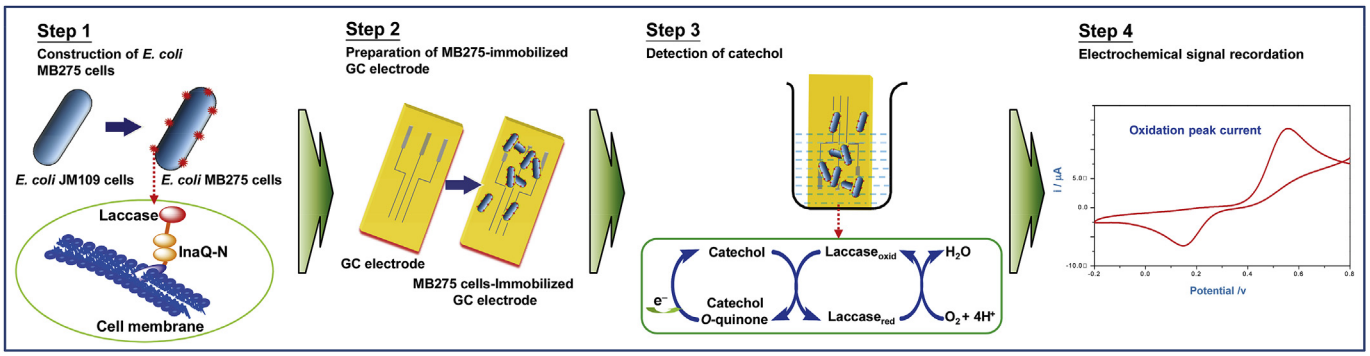
E. coli JM109 was used as the recipient strain to construct the recombinant plasmids. This strain was likewise used as the host strain for surface display, localization experiments, and electrochemical analyses. The previously constructed recombinant plasmids pMB110 [24] and pMB172 [27], which harbor three tandem aligned repeats of the N-terminal domain of an *inaQ* gene (*inaQ-N*) and the mutated laccase gene *wlacD*, respectively, were used as the parent plasmids to construct plasmid pMB275, which contained the fusion gene (*inaQ-N*)$_3$-*wlacD*. All of the strains were grown in Luria–Bertani (LB) medium [28]. Recombinant *E. coli* cells were cultured at 37 °C in LB containing 100 μg mL^{-1} ampicillin. For expression of the target proteins in *E. coli*, when the optical density at 600 nm (OD_{600}) of the cell suspension reached 0.6, IPTG was added to a final concentration of 0.1 mM.

2.3. Plasmid construction and transformation

Bacterial plasmid DNA was extracted using a standard procedure [28]. The *wlacD* gene was amplified from plasmid pMB172 using polymerase chain reaction (PCR) with the primers F_{lac}: 5'–CCGAGATCTATGCAACGTCGTGATTTC–3' (*Bgl* II site underlined) and R_{lac}: 5'–AAAGAATTCTTATACCGTAAACCCTAAC–3' (*Eco*R I site underlined). Before *Bgl* II and *Eco*R I digestion, the PCR-amplified fragment was sequenced to verify its identity with the parent sequence. The digested fragment was then ligated to the *Bgl* II/*Eco*R I site of pMB110, thereby yielding plasmid pMB275 (Fig. S1). *E. coli* transformation was performed as in a previously described protocol [28]. Recombinant *E. coli* harboring pMB275 was named MB275.

2.4. Surface localization of fusion proteins

Approximately 500 μg of the purified WlacD protein expressed in *E. coli* cells [27] was injected into a New Zealand rabbit to prepare polyclonal WlacD antiserum following previously described procedures [23]. *E. coli* MB275 cells expressing the WlacD-fusion protein were fractionated to prepare whole cell, cell cytoplasmic and cell outer membrane fraction samples following procedures described previously [24]. Western blot analyses of the fractioned samples and intact MB275 cells, as well as immunofluorescence microscopy and flow cytometry of MB275 cells, were essentially performed as described previously [23] except that anti-WlacD polyclonal antiserum (approximately 1:100 diluted, v/v) was used as the primary antibody and Cy5-conjugated goat anti-mouse IgG



Scheme 1. Schematic diagram of the construction of engineered *E. coli* MB275 cells adsorbed onto a GC electrode and their use for the electrochemical detection of catechol.

(1:100, v/v) (Invitrogen, Carlsbad, CA) was used as the second antibody for these assays.

2.5. Assay of whole-cell laccase activity

Whole-cell laccase enzymatic activity was measured using ABTS as the substrate at 25 °C as previously described [27]. The reaction mixture contained 0.5 mM ABTS, 0.1 M sodium acetate buffer (pH 3.0), and a suitable amount of recombinant *E. coli* cells. The oxidation rate of ABTS was calculated based on the net absorbance increase of each reaction mixture at 420 nm ($\varepsilon = 36{,}000\ M^{-1}\ cm^{-1}$, referred to ABTS concentration) as determined by a UV–Vis spectrophotometer (DU-800 Nucleic Acids/Protein Analyzer, Beckman Coulter). Whole-cell WlacD enzymatic activity was expressed in units, wherein one unit of enzyme activity was defined as the amount that could oxidize 1 μmol of ABTS per min.

2.6. Preparation of MB275-immobilized GC electrodes

The preparation and use of the biosensor with cell surface-immobilized laccase are illustrated in Scheme 1. To prepare *E. coli* MB275 cells for immobilization, 1 L of recombinant *E. coli* MB275 cells was initially cultured for an additional 6 h (approximately 1.5×10^8 cells mL^{-1}) under IPTG induction. The cells were harvested and washed three times with phosphate-buffered saline (PBS; pH 7.0). The cells were then resuspended in an appropriate volume of PBS to obtain a concentrated cell suspension (approximately 4×10^{10} cells mL^{-1}). A GC electrode (2.0 mm in diameter) was initially polished with an aqueous suspension of alumina paste (0.05 mm in diameter) before biosensor preparation. The electrode was ultrasonically cleaned for 10 min in a 1:1 ethanol-water solution and was then rinsed with distilled water and allowed to dry at room temperature (RM). To prepare the *E. coli* MB275 cell-immobilized GC electrode (namely, WlacD-GC), 10 μL of the previously prepared MB275 cell suspension (approximately 4×10^8 cells) was dropped onto the GC electrode surface and dried at RM for 0.5 h. A reference GC electrode using *E. coli* JM109 cells, namely, JM109-GC, was prepared in parallel using the above described procedures. The as-prepared WlacD-GC and JM109-GC electrodes were then stored at 4 °C until use.

2.7. Electrochemical measurement

CV measurements were performed using a Chi660C electrochemical workstation (Shanghai Chenhua Co., China) with three electrode cells (each with a 10-mL loading volume and a 3-mL working volume). All of the measurements were conducted using a conventional three-electrode system: an MB275 cell-immobilized GC electrode as the working electrode, platinum wire as the counter electrode, and calomel electrode as the reference electrode. The CV responses were recorded from a 3-mL reaction mixture containing catechol at different concentrations in 0.1 M Na_2HPO_4-citric acid buffer (pH 3.0). The electronic potential was scanned over the range of −0.2 V to +0.8 V. All of the voltammograms were recorded at a scanning speed rate of 5 mV s^{-1}.

2.8. Real sample detection

Five commercially bottled red wines and five canned "ready-to-drink" teas were selected as real catechol-containing biological samples for the detection of their catechol content using the proposed biosensor. Standard high-performance liquid chromatography (HPLC) determinations using a reversed-phase Waters ACQUITY BEH C-18 column (2.1 mm × 50 mm × 1.7 μm)(Waters Corporation, MA, USA) on an Agilent 1200/6460 LC/QQQ system were performed in parallel with control methods following a previously described method [29]. Prior to the introduction of each sample into the HPLC column, the sample solution was filtered through a 0.45-μm membrane filter and was degassed, and all of the samples were diluted with 0.1 M Na_2HPO_4-citric acid buffer (pH 3.0) prior to the biosensor measurements. Additional pure catechol at final concentrations from 10 to 100 μM was randomly added to the red wine and tea samples to determine and calculate the catechol recovery rate.

3. Results and discussion

3.1. Construction of E. coli MB275 cell-adsorbed glassy-carbon electrode

To project WlacD onto the surface of *E. coli* JM109, the recombinant *E. coli* strain MB275 was constructed to harbor plasmid pMB275, which encodes the fusion protein $(InaQ\text{-}N)_3$-WlacD (Fig. S1). Expression of $(InaQ\text{-}N)_3$-WlacD in *E. coli* MB275 cells was confirmed by Western blotting (Fig. 1A, lane WC), which showed that the fusion protein was accurately synthesized with a predicted molecular mass of approximately 112 kDa.

The surface localization of $(InaQ\text{-}N)_3$-WlacD in MB275 cells was analyzed by Western blotting, immunofluorescence microscopy, and flow cytometry. The Western blot profile indicated that a certain amount of $(InaQ\text{-}N)_3$-WlacD fusion protein was detected in the outer membrane fraction (Fig. 1A, lane OM) of transformed cells. Immunofluorescence microscopy visualized the cell-surface fluorescence of MB275 cells (Fig. 1B), which resulted from the binding of Cy5 to the surface-immobilized $(InaQ\text{-}N)_3$-WlacD. A follow-up flow cytometry assay further verified the surface

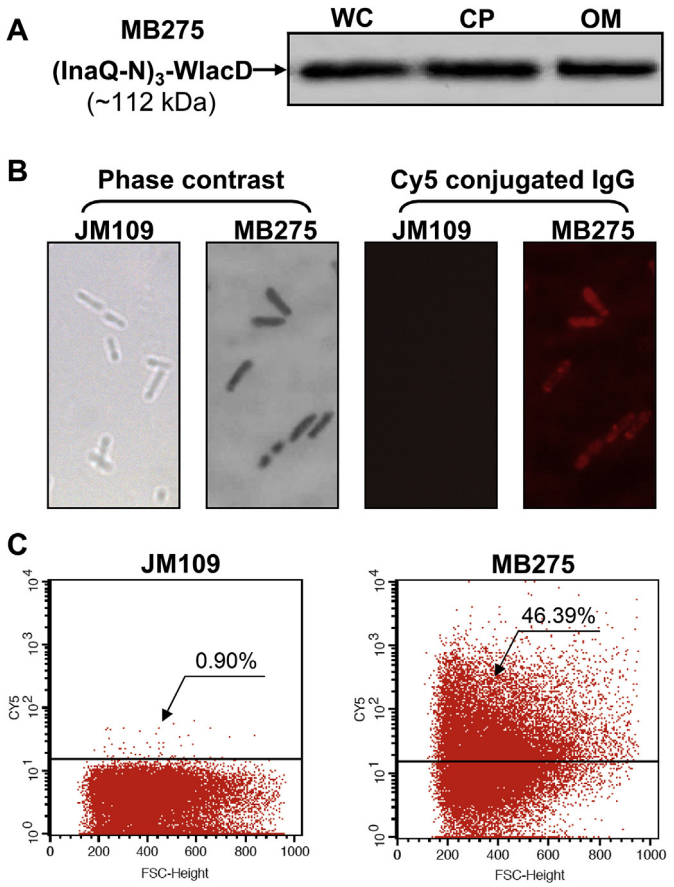


Fig. 1. Surface localization analysis of immobilized $(InaQ\text{-}N)_3$-WlacD in recombinant *E. coli* MB275 cells. **A**, Western blot analysis of MB275 cell fractions. WC, whole cell fraction; CP, cell cytoplasmic fraction; OM, cell outer-membrane fraction. **B**, Immunofluorescence microscopy of intact *E. coli* MB275 and control cells (*E. coli* JM109). **C**, Flow cytometry of intact *E. coli* MB275 and control cells (*E. coli* JM109). The upper histogram of each figure depicts the total number of Cy5-labeled fluorescent cells; the values indicate their percentage in the total cell counts.

localization of the protein. Compared with the limited Cy5 fluorescence intensity of the *E. coli* JM109 control strain, MB275 cells exhibited a prolonged Cy5 fluorescence intensity (Fig. 1C). These results demonstrated that the laccase protein (WlacD) was successfully immobilized on the outer membrane of MB275 cells.

3.2. Effect of pH on whole-cell laccase activity

The whole-cell enzymatic activity of laccase in *E. coli* MB275 was determined using ABTS as the substrate over a pH range of 2.0–5.5. The strain exhibited stable whole-cell enzymatic activity from pH 2.0 to 3.0; however, the enzyme activity rapidly decreased beyond pH 3.0 (Fig. S2). Compared with immobilized *B. thuringiensis* vegetative cells (by approximately a highest activity of 15.3 U mL^{-1} at pH 2.5) [23] or *P. putida* cells (by approximately a highest activity of 7.5 U mL^{-1} at pH 2.5) [16], the WlacD protein immobilized on the *E. coli* MB275 surface enabled higher enzyme activity, with a maximum activity of 33.4 U mL^{-1} at pH 2.5. The observed higher level of whole-cell activity in *E. coli* MB275 was expected, given that the strong IPTG-inducible promoter (P_{trc}) in MB275 cells is apparently conducive for the increased expression of WlacD proteins, whereas only constitutive and low-level promoters exist in both *B. thuringiensis* (P_{3Aa}) and *P. putida* (P_{oprL}) cells. These results suggest that *E. coli* MB275 could be a preferable platform for developing whole-cell laccase-based biosensors. Therefore, *E. coli* MB275 was selected as the donor strain for preparing the laccase-based electrode WlacD-GC.

3.3. Response of the WlacD-GC electrode to catechol

Five representative phenolic compounds, catechol, guaiacol, ferulic acid, 1,4-hydroquinone, and phenol, were selected as the substances to investigate the responses of the WlacD-GC electrode. The electrode did not sense guaiacol, ferulic acid, 1,4-hydroquinone, or phenol at a potential of 0.54 V (data not shown). However, as shown in Fig. 2, the WlacD-GC electrode exhibited a typical oxidation current profile toward catechol at a potential of 0.54 V with 20 μM catechol as the substrate, whereas the JM109-GC and sole GC control electrodes did not sense catechol in terms of the oxidation current. We speculate that this significant discrepancy of sensing might be a direct consequence of the different molecular structures of the substances. In fact, phenol is highly similar to catechol in molecular structure, and both of them are normal reaction substrates of laccases [30], whereas guaiacol, ferulic acid, and 1,4-hydroquinone are structurally different from catechol.

3.4. Effects of MB275 cell loading, pH and scan rate on the biosensor response

The correlation between the CV current and total cell loading was analyzed using various working electrodes with different total loading values of *E. coli* MB275 cells, with 20 μM catechol as the substrate. Fig. 3A shows that a steadily increasing pattern of the oxidation peak currents was observed with increased cell loading over the range of 5×10^7 cells to approximately 4×10^8 cells, and then was maintained without significant variation despite the increase in cell loading up to 3×10^9 cells. We therefore selected a loading of 4×10^8 cells for direct adsorption onto the GC electrode for each experiment.

Fig. 3B shows that the pH range of 2.0–3.0 caused the highest oxidation peak current with 20 μM catechol as the substrate, but the peak current significantly declined beyond pH 3.5. Fig. S3 shows that catechol maintained a considerably stable state when incubated for 60 min under pH 3.0 and pH 6.5, with a slight content loss of less than 2% upon HPLC determination. Based on these results and the fact that the optimum pH values for the whole-cell

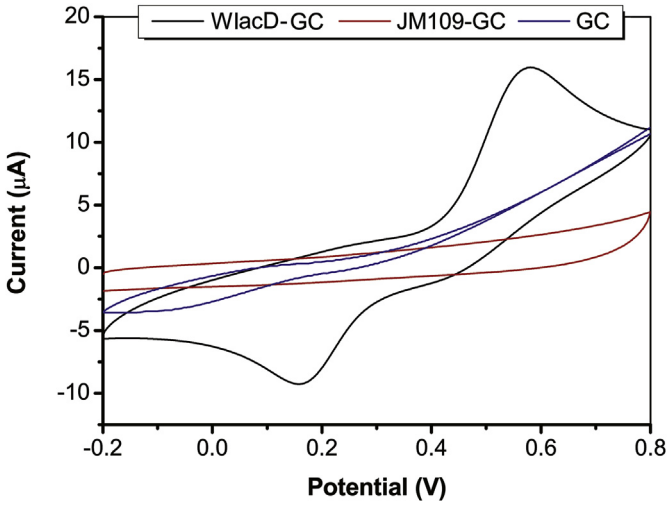


Fig. 2. Cyclic voltammograms of the WlacD-GC electrode for the detection of catechol in 0.1 M sodium acetate buffer (pH 3.0) at a scan rate 100 mV S^{-1}. Catechol was at a concentration of 20 μM, and the GC and JM109-GC electrodes were used as controls.

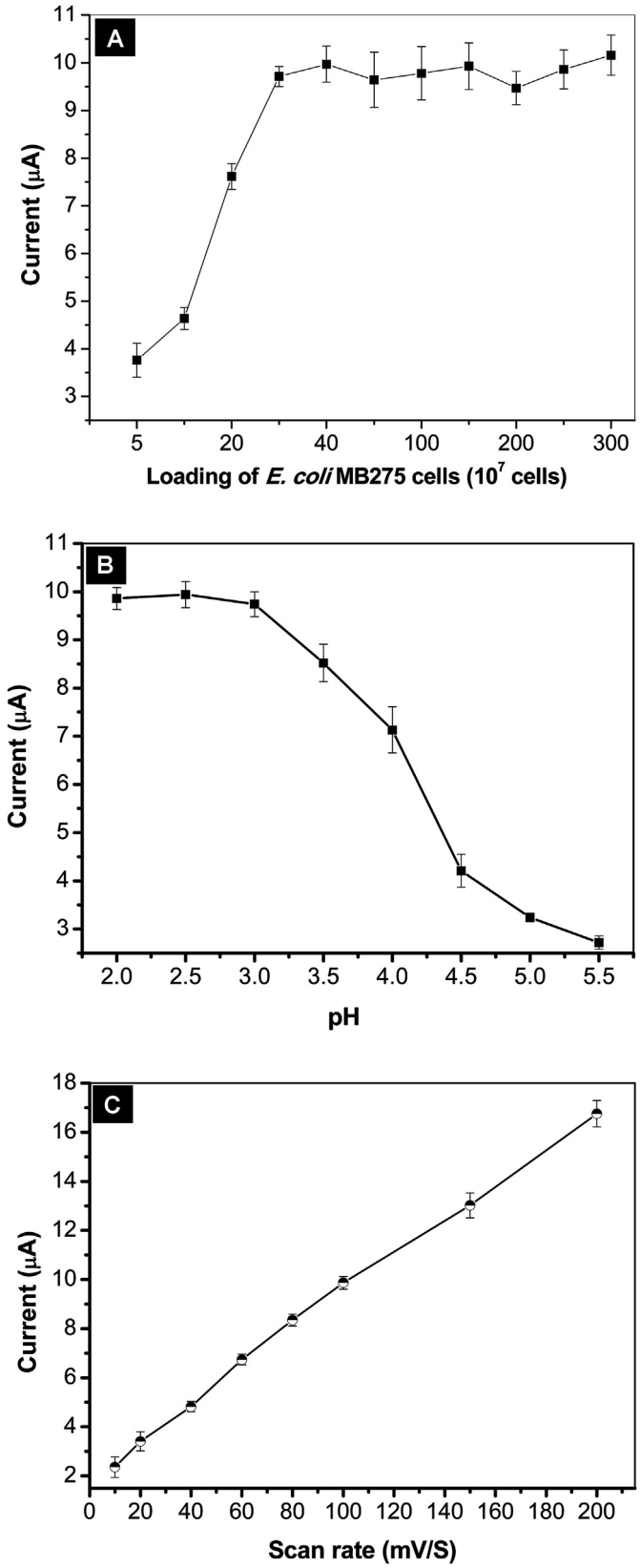


Fig. 3. Effect of *E. coli* MB275 cell loading, pH and scan rate on the electrochemical response of the WlacD-GC electrode. A, MB275 cell loading; B, pH; C, scan rate. All of the CV measurements were performed at room temperature. The oxidation currents were collected at a potential of 0.54 V.

enzymatic activity of MB275 were from pH 2.0 to 3.0 (Fig. S2), we used pH 3.0 as the working pH condition to detect the CV current of catechol.

The effect of the potential scan rate between 10 and 200 mV S^{-1} on the WlacD-GC electrode response to the 20 μM catechol solution was investigated. As shown in Fig. 3C, the oxidation peak current of WlacD-GC for catechol measurement increased with the increase in the scan rate, and a scan rate of 60–100 mV S^{-1} apparently resulted in the most stable peak-current region. Based on these results, the working scan rate in this system was set to 100 mV S^{-1}.

3.5. Effect of O_2, metal ions, protein, glucose and ascorbic acid on the biosensor response

As shown in Fig. S4, O_2 was apparently involved in the redox transformation of catechol, as the CV current was restored to 100% while shifting N_2 to O_2 for both catechol and ABTS. However, the occurrence of a CV current of over 30% in the absence of O_2 also reflected the catalytic oxidation of the substrates by the cells in this system.

The metal ions Mn^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+}, Al^{3+}, and Zn^{2+} along with bovine serum albumin (BSA), glucose and ascorbic acid at certain concentrations were investigated for their effects on the biosensor response for detecting catechol. Fig. 4 shows that, at concentrations of 1 mg L^{-1} and 10 mg L^{-1}, none of the metal ions had an apparent effect on the response currents; however, at a concentration of 100 mg L^{-1}, Cu^{2+} significantly increased the response current, strongly contrasting with the declination effects of other metals, but consistent with a previous investigation that showed that Cu^{2+} enhanced the activity of WlacD [27]. Because it is unlikely that a given sample used for the biosensor would contain metal ions higher than 10 mg L^{-1}, the interference of these metal ions is therefore insignificant.

In various real samples, glucose and ascorbic acid are often found; therefore, the interference effects of such substances need to be verified in terms of the response current for a biosensor. Additionally, given that the WlacD-GC electrode contains abundant proteins and could suffer from interference from certain proteins,

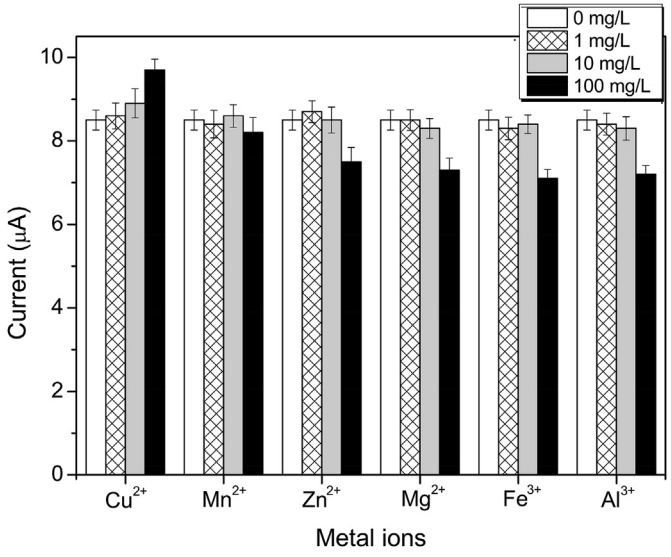


Fig. 4. Effect of metal ions on the oxidation peak current of the WlacD-GC electrode for the detection of catechol at a concentration of 20 μM. The oxidation current values were collected at a potential of 0.54 V.

especially for detecting some biological samples with high levels of various proteins (i.e., urea samples), BSA was selected as an interference substance to test the interference effects. Fig. 5 shows that no noticeable effect was found for either BSA or glucose even at an extreme high concentration of 10 g L^{-1}. Ascorbic acid also did not exert a significant effect at a concentration of 0.01 g L^{-1} or 0.1 g L^{-1}, as the error bars overlapped compared with the control; however, the increased ascorbic acid concentration of 1 g L^{-1} caused an increased response current, a value that was close to the working value (+540 mV) of the current system, showing the potential interference effect of this substance. Fortunately, the content of ascorbic acid in daily foods is normally below mg L^{-1} level [31], so this extremely high concentration of ascorbic acid (1 g L^{-1}) is highly unlikely to occur in the real application process.

3.6. Calibration curves and limit of detection for catechol

The calibration curves generated from the CV current values in response to catechol were determined under the optimum conditions (Cell loading of 4×10^8 cells; pH of 3.0; scan rate of 100 mV S^{-1}). The biosensor response was linear within the concentration range of 0.5 μM–300.0 μM for catechol (Fig. 6 and Table S1). Therefore, the electrochemical detection of catechol by WlacD-GC was reliable within certain concentration ranges, and the linear range of WlacD-GC was even wider compared with some chemically modified biosensors (Table S2). The LOD for the tested catechol was a concentration of 0.1 μM (Table S1), a comparable lower LOD for catechol in contrast to sensors based on chemically modified purified laccase, namely, the derivatized polyethersulfone membrane-laccase-based biosensor, which has a lower LOD of 1.0 μM for catechin [32], and the succinimide/carbodiimide-laccase-based biosensor, which has a LOD of 0.08 mg L^{-1} (~0.73 μM) for catechol [33]. Thus, the proposed system is a sensitive and technically competent sensor.

3.7. Stability of the WlacD-GC electrode in repeated uses

The high stability of a biosensor based on live cells and biologically active enzymes despite its repeated use could enhance its economic feasibility. After the proposed electrode was maintained

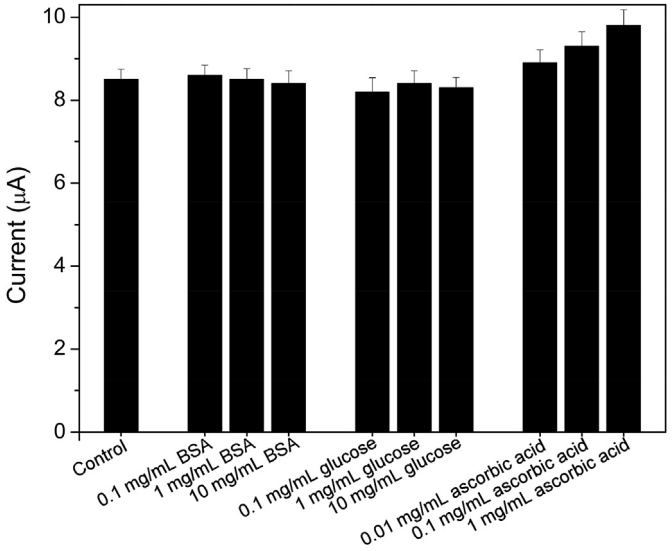


Fig. 5. Effect of BSA, glucose and ascorbic acid on the oxidation peak current of the WlacD-GC electrode for the detection of catechol at a concentration of 20 μM. The oxidation current values were collected at a potential of 0.54 V.

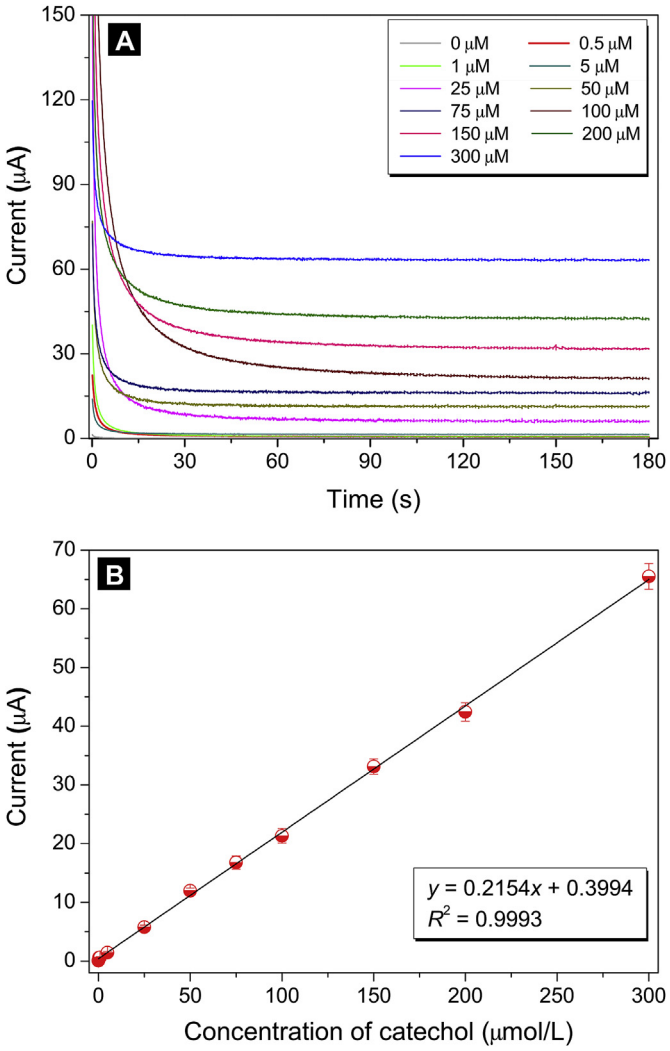


Fig. 6. Current-time curves for different concentrations of catechol (A) and calibration curves of the current values generated by the biosensor in response to catechol (B). All of the current-time curve measurements were performed at pH 3.0 and room temperature. The oxidation currents were collected at a potential of 0.54 V.

for one month under room temperature conditions, the MB275 cells eluted from the electrode retained 89.3% of the initial enzyme activity. Moreover, when the electrode was consecutively used 30 times for catechol index detection in a solution containing 50 μM catechol (with 1-d intervals between measurements), the average determined value of catechol and its relative standard deviation were 49.27 μM and 5.8%, respectively. These results indicated the high stability and reproducibility of the proposed biosensor for catechol detection.

3.8. Determination of catechol indexes in red wine and tea samples

The potential application of the currently developed biosensor for the analysis of catechol was assessed in real red wine and tea samples. The standard HPLC method was performed for a comparison of the accuracy of the developed biosensors in these samples. A recovery study was performed in parallel using the same samples by adding various standard catechol solutions at certain concentrations. As shown in Table 1, the catechol content in each of five different red wines and teas was successfully measured by the biosensor, providing a total content value that was in good

Table 1
Determination of the catechol contents and recovery rates in red wine and tea samples.

Samples		Catechol added (μM)	HPLC determined (μM) (mean ± SD, $n = 5$)	Sensor determined (μM)[a] (mean ± SD, $n = 5$)	Recovery (%)
Catechol in red wine	Sample 1	NA[b]	41.6 ± 0.3	42.1 ± 0.3	102.5
		30	71.4 ± 0.5	73.2 ± 0.6	102.5
		70	111.9 ± 0.6	116.2 ± 1.0	103.8
	Sample 2	NA	22.3 ± 0.1	22.9 ± 0.2	102.7
		50	73.1 ± 0.3	74.6 ± 0.2	102.1
		90	109.2 ± 0.3	107.3 ± 0.6	98.3
	Sample 3	NA	13.6 ± 0.3	13.4 ± 0.3	98.5
		10	23.2 ± 0.4	23.1 ± 0.2	99.6
		30	42.9 ± 0.3	43.4 ± 0.2	101.2
	Sample 4	NA	12.6 ± 0.1	12.8 ± 0.2	101.6
		40	53.1 ± 0.4	52.8 ± 0.3	99.4
		80	93.4 ± 0.4	92.3 ± 0.3	98.8
	Sample 5	NA	31.5 ± 0.2	30.6 ± 0.3	97.1
		50	81.9 ± 0.3	81.3 ± 0.3	99.3
		100	131.9 ± 0.6	132.7 ± 0.5	100.6
Catechol in tea	Sample 1	NA	9.4 ± 0.1	9.6 ± 0.1	102.1
		20	29.6 ± 0.1	29.9 ± 0.2	101.0
		50	59.2 ± 0.3	58.3 ± 0.2	98.5
	Sample 2	NA	22.3 ± 0.3	21.9 ± 0.3	98.2
		10	32.4 ± 0.3	31.9 ± 0.3	98.5
		50	72.9 ± 0.2	72.3 ± 0.2	99.2
	Sample 3	NA	7.3 ± 0.2	7.2 ± 0.2	98.6
		20	27.7 ± 0.2	27.3 ± 0.3	98.6
		40	47.6 ± 0.2	47.5 ± 0.3	99.8
	Sample 4	NA	4.6 ± 0.1	4.5 ± 0.1	97.8
		15	19.4 ± 0.2	19.6 ± 0.1	98.3
		30	34.9 ± 0.1	35.2 ± 0.1	100.9
	Sample 5	NA	32.1 ± 0.2	31.9 ± 0.1	99.4
		60	92.7 ± 0.2	93.1 ± 0.2	100.4
		100	131.4 ± 0.4	132.9 ± 0.6	101.1

[a] The oxidation current values were collected at a potential of 0.54 V.
[b] NA: Not added.

accordance with those determined by HPLC. The significant average recovery rates of catechol in red wine and tea were 97.1–103.8% and 97.8–102.1%, respectively, indicating the high accuracy of the developed biosensor for catechol detection.

To date, fungal laccases have been intensively applied for the development of various biosensors because of their commercial availability and higher catalytic activity compared with those from bacteria. However, bacterial laccases have additional appeal because of their higher production rate and non-glycosylated nature, which can protect efficient electron transfer from non-conducting glycoproteins. The glycoprotein shell and protein framework surrounding the active enzyme core of certain fungal laccases could limit direct electron transfer (DET) from the active site to the electrode [34]. Various electronic vehicle materials have been used to address this problem, including ferrocene monocarboxylic acid [34], ferrocene dimethanol [35] and ABTS [36]. These methods involve a multi-step manipulation and, in some cases, result in an overpotential state of the electrode despite the enabled conducting state between the enzymes and electrode [37]. Based on these results, we investigated the functionality of DET by a bacterial laccase in the current study. The enzyme in the proposed system was genetically modified by site-directed mutagenesis to delete an α-helix domain in its structure. Consequently, the enzymatic activity and thermostability of the biosensor were remarkably increased [23]. When immobilized on the cell surface of a *P. putida* strain, the whole-cell catalyst exhibited high reactivity for degrading certain azo and anthraquinone dyes without requiring Cu^{2+} or a mediator [16]. This phenomenon enabled us to envisage the current use of direct electrochemistry and biosensing by laccase via a cell-surface display strategy, thereby providing a model system of an accurate, directly catalytic biosensor with a facile preparation and high regenerability.

Aside from influencing the enzyme activity, immobilization of microbial cells onto the electrode significantly affects the functionality and reproducibility of a biosensor [12]. Although stability can be enhanced by immobilization with certain inclusions into gel or polymer matrixes or via covalent linkages (mostly for purified enzymes), the possible stress conditions of such methods may decrease cell viability or enzymatic reactivity [13]. By contrast, bacterial cells in the current study were simply adsorbed onto the GC electrode; these cells conferred the functional sensor with a comparable LOD, accuracy, and reproducibility. This observation suggests that adsorption remains a feasible approach for immobilizing living cells with highly active and surface-displayed bacterial laccase. However, as a live cell based system, the limited persistence of activity and detractive efficiency under harsh conditions, such as alkaline pH, higher or lower temperature, among others, should be considered. The development of a capacity-promoted and persistence-improved biosensor is now one of our primary goals.

4. Conclusions

An electrochemical microbial biosensor for catechol detection was successfully constructed by direct adsorption of engineered *E. coli* cells expressing surface-immobilized laccase onto a GC electrode. The surface localization of laccases on the target cells was confirmed by Western blot analysis, immunofluorescence microscopy, and flow cytometry. The proposed biosensor showed single potential values for catechol. Neither metal ions, BSA, glucose, nor ascorbic acid caused noticeable interference effects at their commonly occurring concentrations. Under optimized conditions, the electrochemical response of the biosensor was linear within certain concentration ranges, varying from 0.5 μM to 300.0 μM for

catechol. When used for the detection of several real samples, the high accuracy and high recovery of the biosensor were verified. The biosensor offered a comparable LOD as well as high stability, reproducibility, and accuracy compared with other biosensors based on chemically modified purified laccases. The multiple distinctive features of the proposed biosensor include high enzymatic activity, elimination of mass transfer limits, and simple electrode preparation without using a matrix or a cross-linking reagent. Given these features, the developed biosensor showed potential for simple, accurate, and cost-effective analysis of catechol.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aca.2018.01.008.

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