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# Expression, secretion and functional characterization of three laccases in *E. coli*

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ABSTRACT

Endocrine Disrupting Chemicals (EDCs) are a group of molecules that can influence hormonal balance, causing disturbance of the reproductive system and other health problems. Despite the efforts to eliminate EDC in the environment, all current approaches are inefficient and expensive. In previous research, studies revealed that laccase-producing microorganisms may be a potential candidate for EDC degradation, as laccases have been found to be able to degrade many kinds of EDCs effectively and steadily. Here, we created two recombinant laccases, each fused with secretion peptide, Novel Signal Peptide 4 (NSP4), and expressed them in *Escherichia coli* (*E. coli,* BL21), together with one laccase without secretion peptide. We first optimized the culture condition of expressing these laccases. Then, we test the activity of the recombinant laccases of decolorizing of a synthetic dye, indigo carmine. Finally, we confirmed the secreted can degrade one of the EDCs, β-estradiol, showing the potential of using the laccase secretion system to degrade toxic compounds.

## 1. Introduction

Endocrine Disrupting Chemicals (EDCs) are a group of organic compounds that influence the normal function of our endocrine system, thereby harming our cardiovascular, neurological, reproductive, and other organ systems in humans. Although the effects of EDCs are complex and diverse (as EDCs consisted of a large group of chemicals), they are associated with many diseases such as diabetes, breast cancer and ovarian cancer [1–3]. In addition, previous studies also show that the EDCs can interfere with the development of children, increasing their risk of getting neurodegenerative diseases, learning disabilities and reproductive disorders years after they grew up.

EDCs are usually produced, as a byproduct, in the process of mass production of industrial and consumer products, such as dye, pesticides and plastics. Because many EDCs have chemical structures similar to natural hormones, they can activate or antagonize hormone receptors and thereby disrupt the endocrine system. More importantly, large amounts of EDCs have been found entering the ecosystem through domestic and industrial sewage, and in turn, enter the human body after biomagnification [4]. Therefore, removing EDCs has become a critical health and environmental problem.

Laccases are copper-containing enzymes that could oxidize phenolic and non-phenolic aromatic compounds [5,6]. Laccases are found in wide spectrum of organisms including plants, fungi, bacteria, and insects. Because of their high efficiency and stability, laccases are widely used in many industries, such as textile (decolorize textile), paper (bleach papers), and chemicals production [6,7]. Moreover, laccases have been used to help removing harmful substances from the wastewater [6,7], degrading many industrial pollutants, such as biocides, halogenated pesticides, analgesic and anti-inflammatory drugs, and antibiotics, etc. [8–13]. Previous research also shown that laccases can degrade some of the common EDCs, such as BPA, nonylphenol, Triclosan. In fact, some of the laccases are highly effective in catalyzing the degradation of specific types of EDCs. For example, commercial laccase Novozym 51,003 (from *Aspergillus Oryzae*) can remove BPA(110,0 ± 10 μg/L) after 8 h incubation (pH = 7, 28 °C), while laccase from *Coriolopsis Polyzona* can completely eliminate nonylphenol (5 mg/L) after incubating for 4 h (pH = 5, 50 °C) [8,14].

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Here, we studied the characteristics of three laccases from different organisms, namely Lac1326 from marine sediment samples [15], fungal tvel5 laccase from *Trametes versicolor* [16], and bacterial BPUL laccase from *Bacillus pumilus* [17]. The Novel Signal Peptide 4 (NSP4) was fused to the upstream sequence of the Lac1326 and tvel5 laccase to allow secretion of the laccase from the recombinant *E. coli* BL21 strains [18]. The degradation efficiency of these recombinant enzymes was tested through decolorizing indigo carmine and degrading β-estradiol (one type of EDCs).

## 2. Material and methods

### 2.1. Strains and plasmids

*E. coli* One Shot™ TOP10 (DH10B) from Invitrogen and *E. coli* DH5α used for cloning, were kindly provided by Prof. Leo Tsz On Lee. *E. coli* BL21 (DE3), used for plasmid expression, was kindly provided by Prof. Vivian Ya-Fan Wang (Faculty of Health Sciences, University of Macau). Detailed descriptions of recombinant plasmids used in this study are described in Table 1. Luria-Bertani (LB) broth powder (BD Difco™; USA) was prepared as described in Manual, and then used to culture *E. coli*.

### 2.2. Chemicals

2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) (ABTS), indigo carmine and β-estradiol were purchased from Sigma (USA). Anti-His antibody, *Anti*-RNA polymerase beta and Anti-Rabbit lgG (abcam) were kindly provided by Prof. Ruiyu Xie (Faculty of Health Science, University of Macau).

### 2.3. Cloning of recombinant laccases in E. coli

Lac1326 and tvel5-Laccase were synthesized in accordance with iGEM registry (iGEM Part: BBa_k863030) and Genbank (Lac1326, Accession No.: KP752045). A 6xHis-tag was inserted into the downstream sequences. The tvel5-Laccase gene was codon optimized to meet the iGEM RFC10 requirement.

Lac1326 and NSP4 gene fragments were assembled using NEBuilder Master Mix as described in the manual. The assembly product was then amplified with Q5 Mix PCR for adding two NheI restriction sites. The PCR product was digested with NheI restriction enzyme at 37 °C for 4 h, and was inserted in pET-11a expression vector by using T4 Ligase at room temperature for 15 min; tvel5-Laccase was amplified with Q5 Mix PCR for adding the NheI and BamHI restriction sites. PCR products and pET-11a plasmid were double digested with restriction enzymes (NheI and BamHI), and ligated by T4 ligase at room temperature for 15 min.

Ligation products were transformed into DH5α competent cells and cultured on LB agar plate containing ampicillin (100 μg/mL) at 37 °C for 18 h. Single colonies were picked and cultured in LB medium with ampicillin under the same condition and being shaken at 250 rpm, plasmids were then extracted using QIAprep Spin Miniprep Kit (QIAGEN). NSP4 and the extracted plasmids were amplified for overhangs with PCR, and assembled using NEBuilder. The resulting plasmids were subsequently transformed into DH5α competent cells. These cells then were cultured and the plasmids were extracted as mentioned above. Sequencing analysis was then performed by Sangon (Shanghai, China) for confirmation.

**Table 1**
Plasmids used in this study.

| Plasmid | Description | Antibiotic resistance | Reference |
|---|---|---|---|
| pET-11a | High copy number vector for IPTG-inducible expression, lacI, $P_{T7}$, ori: pBR322 | $Amp^R$ | NovoPro |
| NSP4-Lac1326-6xHis-tag-pET-11a | Lac1326 (laccase from marine sediment sample) with NSP4 secreting peptide in pET-11a expression vector, under T7 promoter regulation | $Amp^R$ | iGEM Part: BBa_K3021004 |
| NSP4-tevl5-laccase-6xHis-tag -pET-11a | Condon optimized laccase from *Trametes versicolor* with NSP4 secreting peptide in pET-11a expression vector, under T7 promoter regulation | $Amp^R$ | iGEM Part: BBa_K3021002 |
| T7 promoter BUPL-laccase-6xHis-tag-pSB1C3 | Laccase from *Bacillus pumilus* in pSB1C3 expression vector, under T7 promoter regulation | $Cam^R$ | iGEM Part: BBa_K863000 |

### 2.4. Expression of the recombinant laccases in E. coli

The recombinant laccase plasmids were extracted and transformed into *E. coli* BL21 (DE3) competent cells. The cells were cultured with 100 mL LB medium containing ampicillin under different conditions. The bacteria culture was centrifuged for 10 min at 6000×*g* when $OD_{600}$ reached 0.4 or 1.2. The supernatant was transferred to another tube for later use. The bacterial pellet was then resuspended in 2500 μL of 50 mM HEPES. All protein was extracted through microtip sonication at 40% AMPL, 45 s processing, 1 s on-off for three times. The processed samples were centrifuged at 12000×*g* for 10 min, where the supernatants (protein extracted) were transferred to another centrifuge tube and stocked at −20 °C.

### 2.5. Nickel-pulldown assay

The secreted recombinant laccases in the supernatant of bacterial culture (with LB medium) were extracted through Nickel-pulldown assay. 20 mL supernatant was thawed on ice and 500 μL was pipetted to resuspend the nickel resin, which subsequently was pipetted back into 20 mL supernatant. After incubating and rotating at 4 °C for 45 min, the mixture was centrifuged at 3900 rpm for 10 min. The supernatant was removed carefully, with an average of 1 mL fraction was left to resuspend the resin. The resin was then transferred to a 1.5 mL centrifuge tube, and was spun down by centrifugation at 4000×*g* for 2 min, supernatant was decanted. The resin was washed with 750 μL binding buffer (20 mM Tris 7.5, 150 mM NaCl, 1% Triton-X100). Buffer was removed as much as possible then, 30 μL of 2x SDS dye was added into the resin and heated at 98 °C for 10 min.

### 2.6. Protein concentration determination

Western blot was performed as described in the General Western Blot Protocol (abcam). The extracted proteins were added into 4x loading, then boiled for 10 min 12% SDS-gel was prepared and used according to the manual of SureCast Handcast System (Invitrogen). Anti-His rabbit developed (1:2000 diluted) was used as primary antibody, while anti-rabbit conjugated HRP (1:5000 diluted) was used as the secondary antibody.

### 2.7. Indigo carmine decolorization

20 μL BR buffer (pH = 5), 4 μL indigo carmine (500 mg/L), and 33.4 μL protein samples (cell lysed total protein, diluted to 3 μg/μL) were added to each well of the 96-well plate. 2 μL ABTS (100 mM) was added as a control. HEPES (50 mM) was then added to each well to 200 μL. Each experiment group was triplicated. The 96-well plate was sealed and incubated at 37 °C, and the optical density (610 nm) was measured every 24 h.

We calculated the enzyme activity based on the absorbance changes in the indigo carmine decolorization. Using formula:

$$U / L = (\Delta E \times Vt)/(\varepsilon \times d \times Vs)$$

where molar absorbance coefficient ($\varepsilon = 8080\ M^{-1}cm^{-1}$,610 $nm$[19]) of indigo carmine and the pathlength ($d = 0.56\ cm$), equivalent to 200 μL liquid in 96-well plate, were used.

### 2.8. Degradation of β-estradiol

A mixture of 500 μL sodium citrate buffer (pH = 5), 500 μL protein sample or nickel-pulldown product (3 μg/μL), 1.6 μL ABTS (200 μM), and 0.8 μL β-estradiol (100 μM) was prepared and shook at 25 °C for 36 h, then 100 μL of the mixture was mixed with 400 μL acetonitrile and was centrifuged at 10,000 rpm for 5 min to remove precipitated protein. The supernatant was extracted and transferred to another tube for drying via nitrogen blow. The dried sample was then re-dissolved in an appropriate volume of acetonitrile depending on the concentration of the targeted molecule in the sample by a vivid vortex. Insoluble salt was removed by centrifugation at 10,000 rpm for 5 min. The supernatant was then ready for LC-MS analysis.

### 2.9. LC-MS qualification of β-estradiol

To extract β-estradiol for quantification analysis, 200 μl laccase reaction product (cell lysed total protein, 3 μg/μL) were mixed with 800 μl ice-cold methanol by vigorous votexing. The mixture was then undergone centrifugation (12,000 g for 10 min at 4 °C) to pellet denatured proteins. The aqueous fractions were retrieved and dried in the microtubes by nitrogen gas using a Pressured Gas Blowing Concentrator (Eyela, MGS-2200E). The dried sample was resuspended with 200 μl of ice-cold methanol, which was ready for ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) analysis.

For quantification, 2 μl of sample was subjected to UPLC–MS analysis using an ACQUITY UPLC CSH C18 (1.7 μm, 2.1 × 100 mm) column on the Waters ACQUITY UPLC H-Class System. A gradient solvent analysis was carried out with a mobile phase of acetonitrile: water with 0.1% v/v formic acid at a flow rate of 0.5 mL $min^{-1}$ with 6-min running time under the following conditions: the injection cycle was initiated with 50% acetonitrile for 1 min, then increased to 90% for 3 min, maintaining at 95% for 1 min, returning to 50% within 0.1 min and balanced for another 0.9 min. β-estradiol tandem mass spectrometry (MS/MS) detection was performed using pairs of positive ions ($m/z$: 273.04/116.97) through a Waters Xevo TQD equipped with an electrospray ionization source. The capillary voltage was 3.8 kV. Nitrogen gas was flowed as the cone and desolvation gas at rates of 50 and 1000 L $h^{-1}$, respectively. The source and desolvation temperatures were 200 and 500 °C, respectively. Cone and collision voltages were optimized to 10 and 53 V for β-estradiol with a dwell time of 25 ms. Data were processed with the Waters MassLynx V.4.1 software to calculate peak areas for quantification.

## 3. Results

### 3.1. Validate laccase expression in recombinant Escherichia coli strains

The expression of the recombinant laccases from cell lysed, were analyzed by Western blot analysis with Anti-His antibody (Fig. 1). RNA polymerase beta was served as a loading control. The protein expression levels (Fig. 1B) were standardized with RNA polymerase beta and then further normalized with pET-11a being 1-fold.

The results suggested all of the laccase genes, NSP4-Lac1326, NSP4-tvel5 laccase and BPUL Laccase, were successfully expressed in *E. coli* BL21 (DE3) after IPTG induction (Fig. 1A). Analysis with Western blot demonstrated expression of the his-tagged NPS4-Lac1326, where two bands close to 52 kDa was found. The his-tagged NSP4-tvel5 laccase and BPUL Laccase has molecular weight 58 kDa and 59 kDa, respectively, consistent with the expected size. The quantitively analysis suggested

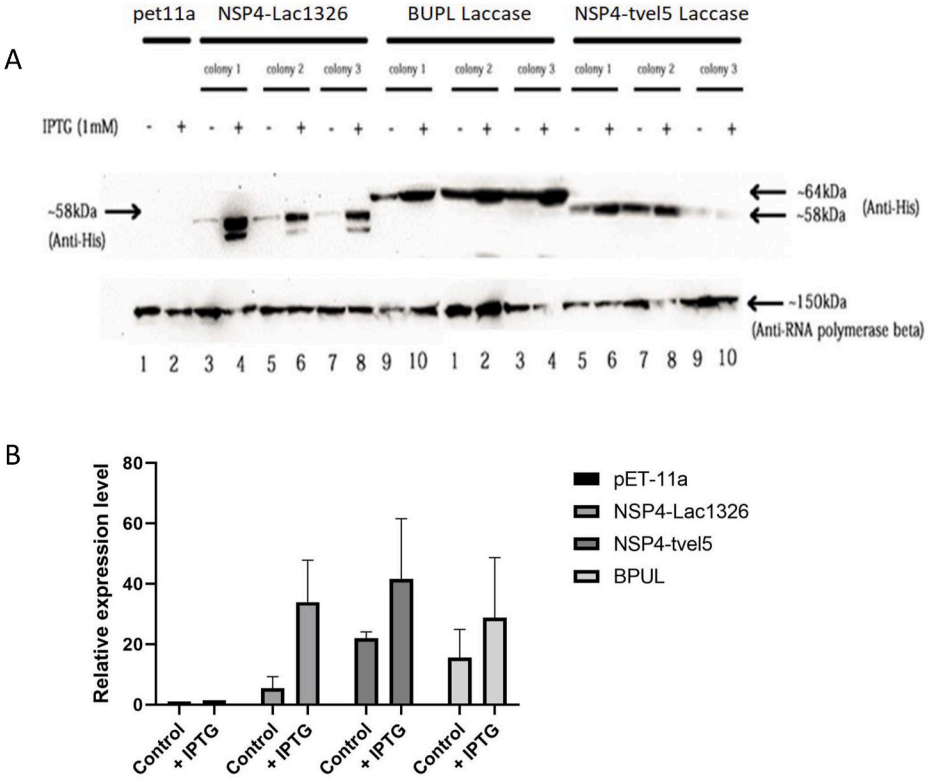


**Fig. 1. Western Blot analysis on different laccases. A)** Western blot analysis on the expression of NSP4-Lac1326, BPUL Laccase, and NSP4-tvel5 laccase **B)** Relative expression level of laccases under 1 mM IPTG induction. Recombinant proteins containing C-terminal His-tagged residues were expressed in *E. coli* BL21 (DE3), followed by purification by HEPES solution. *Anti*-RNA polymerase beta was used as a loading control. Error Bar represented SD of the mean.

that all three laccases can be induced by the IPTG as expected (Fig. 1B). Significant increase were found in the NSP4-Lac1326 expressions (unpaired *t*-test; $p < 0.05$) after IPTG induction. We also found significant laccases expressions even without IPTG induction (Fig. 1A; Fig. 1B), suggesting that the T7 promoter is leaky, despite all of them can be induced by IPTG.

### 3.2. Optimizing condition for laccase production and secretion

To maximize the yields of laccases production, we tested the protein expression under different culturing temperature, IPTG concentration, and cell growth phase. pET-11a empty vector under $OD_{600}$ 0.4 at 30 °C without IPTG induction was used as a control. BL21 (DE3) were cultured under four different culturing conditions and the expression of proteins were analyzed by Western blot (Fig. 2). Compared to the control, cell lysed NSP4-Lac1326 with starter culture at $OD_{600}$ 0.4 + 0.4 mM IPTG at 25 °C, BPUL Laccase, NSP4-tvel5 laccase with starter culture at $OD_{600}$ 0.4, 0.4 mM IPTG at 30 °C have the highest expressions, with the fold changes (His-tagged band intensity divided by the *Anti*-RNA Polymerase beta band intensity) of 24.59, 51.33 and 44.16, respectively (Fig. 2A). These culturing conditions were used for subsequent laccase production for following functional tests.

We also tested the secretion of laccases to the cell medium (Fig. 2B). The result confirmed that NSP4-Lac1326 and NSP4-tvel5 laccase can be secreted to the cell medium as expected. Based on the band intensity. The culturing condition that works well for intracellular laccase production ($OD_{600}$ 0.4 + 0.4 mM IPTG at 25 °C for NSP4-Lac1326; and $OD_{600}$ 0.4 + 0.4 mM IPTG at 30 °C for NSP4-tvel5), also consistently performed well for the production of the secreted laccases.

### 3.3. Laccase-catalyzed decolorization of synthetic dyes

The laccase-mediator system for textile dye decolorization has been continually improvised. In this kind of system, the mediator acts as an electron carrier and oxidizes substrates. Similar to previous studies [13,20,21], we determined the laccase activity by the dye decolorization with ABTS as the mediator. To test the decolorization rate, a water-soluble blue acid dye, indigo carmine, was used. Cell lyses (100 ng) containing different laccases were incubated with the dye and ABTS at 37 °C for 96 h. As shown in Fig. 3, all laccases could decolorize indigo carmine, in which BPUL laccase showed the best decolorization efficiency at day 4, removing 81.09% indigo carmine, followed by NSP4-Lac1326 and NSP4-tvel5 laccase with 47.62% and 22.01% removal of the dye respectively (Fig. 3A). Unpaired *t*-test showed significant differences comparing the pET-11a (control) with the BPUL laccase, NSP4-Lac1326 and NSP4-tvel5 laccase at day 4 (p values = $3 \times 10^{-6}$, $2 \times 10^{-6}$, and $2 \times 10^{-6}$ respectively.)

We calculated the enzyme activity of our crude laccase proteins across different time points based on the decolorization of indigo carmine and ABTS mediator (see Methods for details). As shown in Fig. 3B, the activity of BPUL laccase was significantly higher than the other two, reaching a peak of about 0.12 U/g after incubating for 24 h. The activity of NSP4-Lac1326 and NSP4-tvel5 remained roughly stable, with a slight decrease of about 0.01 U/g at the end of the 96-h interval, and the activity of NSP4-Lac1326 was significantly higher than that of NSP4-tvel5, with the highest activity at 0.065 U/g and 0.035 U/g respectively.

### 3.4. β-estradiol degradation by secreted laccase

To validate the idea of using secreted laccases to degrade EDCs, biodegradation experiment was conducted. β-estradiol was selected as the target substrate due to the increasing concern from the public. After incubation of cell lysed or cell-free medium extracted laccases with β-estradiol for 36 h, we analyzed the amount of remaining β-estradiol with Liquid Chromatography-Mass Spectrometry (LC-MS) based on the peak area.

As shown in Fig. 4A and B, the concentration of β-estradiol decreased after treatment with all recombinant laccases, compared with pET-11a (empty vector). This result successfully demonstrated our concept of

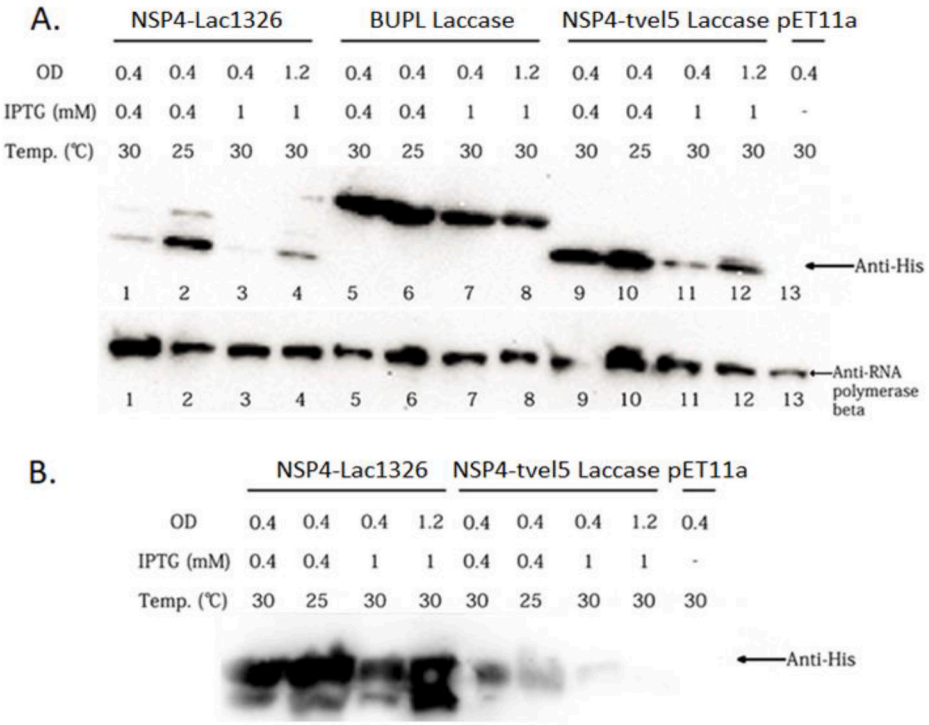


**Fig. 2. Expression and secretion of laccases under different culturing conditions. A)** Western blot analysis of Laccases purified from the lysed cells. **B)** Western blot analysis of Laccases purified from the cell-free medium.

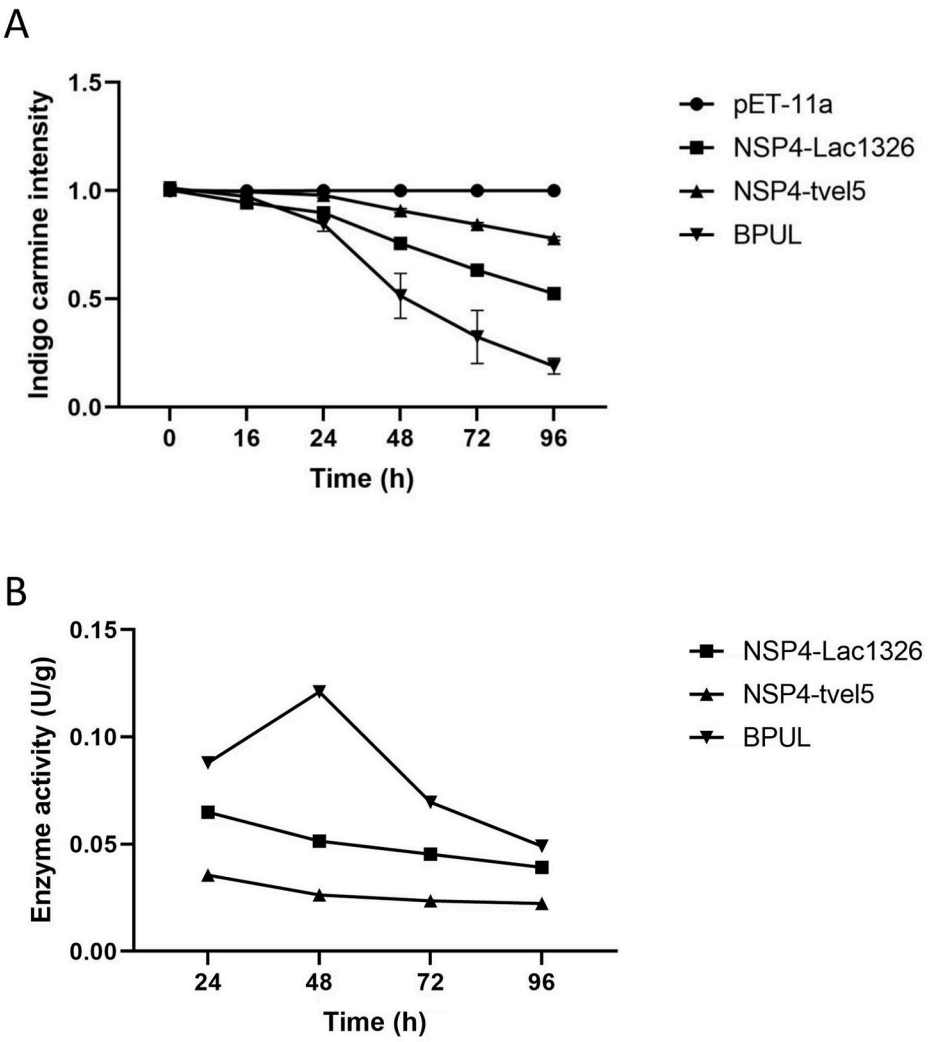


**Fig. 3. Decolorization of indigo carmine by different recombinant laccases at 37 °C. (A)** Decolorization of the indigo carmine dye across 4 days as measured with OD (610 nm). The experiment was triplicated with mean and SD was shown in the error bar. **(B)** Enzyme activity of the three laccases, estimated with indigo carmine decolorization, under 37 °C incubation in a 96-h interval.

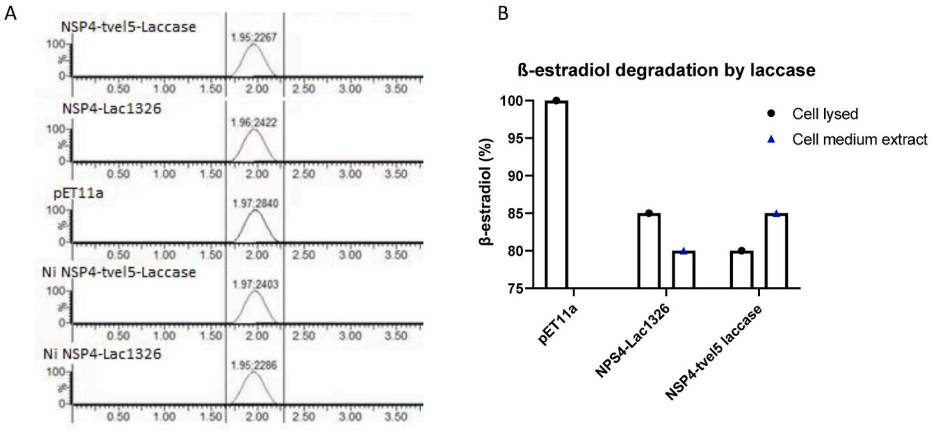


**Fig. 4. LC-MS analysis of β-estradiol degradation by recombinant laccases. A)** Chromatogram of β-estradiol by NSP4-Lac1326 and NSP4-tvel5 Laccase from cell lysates and cell-free medium extraction, with pET-11a as control **B)** LC-MS analysis of β-estradiol degradation by different laccases. Protein expression from cell lysed and cell free medium (Ni: Nickle Pulldown Assay) was from *E. coli* induced under the condition of $OD_{600}$ = 0.4, IPTG = 0.4 mM and 25 °C.

utilizing NSP4, for laccase secretion, in EDC degradation.

## 4. Discussion

EDCs are highly concerned bioaccumulating pollutants, which could contaminate our drinking water and food. However, methods to remove or prevent EDC pollution are limited and not efficient—even the EDC pollution from wastewater plant effluent have yet remained unsolved. We here tested and characterized the possibility of deploying laccases, secreted from microorganisms, in tackling EDCs contamination. We engineered two recombinant *E. coli* to allow the secretion of laccases. This allowed the cell to consistently produce laccases and secrete them to the environment, avoiding the determinant effect of laccases accumulation within the cell, and preventing proteolysis by cytoplasmic or periplasmic proteases. In this study, NSP4 was used as the inducible secretion system, as previous research shown that it can improve secretion in fed-batch fermentation by 3.5 fold in comparison with the conventional hlyA, dsbA or pelB signal peptides [11]. We believe these synthetic biology designs could improve the cost-effectiveness of the laccase-based EDC degradation system, which worth further investigation.

We validated the feasibility of using the laccase secreting system via the decolorization experiment, to demonstrate the degradation efficiency of our recombinant laccases. As shown in Fig. 3, cell lysed NSP4-Lac1326 and NSP4-tvel5 Laccase show lower degradation efficiency in comparison with the BUPL Laccase. However, this result might be due to the fact that a significant portion of the NSP4-Lac1326 and NSP4-tvel5 Laccase protein were secreted to the cell medium, as validated by other experiments (Western blot and LCMS on EDC degradation) in the study.

Interestingly, our data also suggest that the NSP4-Lac1326 laccase can be secreted into the cell medium more easily than the NSP4-tvel5 laccase. As shown in Fig. 2B, Western blot analysis demonstrated higher expression of NSP4-Lac1326 in the cell medium. Consistent with this, we also found that in the EDCs degradation experiment. Although both of the secreted NSP4-Lac1326 and NSP4-tvel5 laccases can degrade β-estradiol, NSP4-Lac1326 cell medium degrade EDC better than the cell lysates, while we found an opposite effect in NSP4-tvel5. Taken together, these results suggest that NSP4-Lac1326 has a stronger laccase secretion profile than NSP4-tvel5 Laccase, although both of them are secreted using NSP4 in *E. coli* BL21 strain. Further research may, therefore, investigate what might causes the potential differences in the secretion level of these laccases. This would be interesting not only to researchers studying laccases or signal peptides, but also to other researchers working on synthetic biology or molecular biology research, where protein secretion is an important topic.

In this study, three recombinant laccase genes were selected, and two of them were fused with the NSP4 to secretion. In Western blot analysis, our results show two bands for NSP4-Lac1326, while NPS4-tvel5 laccase and BUPL laccase only have one band. In a previous publication, a single band of Lac1326 was found in the SDS-PAGE analysis, indicating Lac1326 is a monomeric enzyme [8]. Therefore we think the two bands in NSP4-Lac1326 enzyme may indicate a post-translational modification, such as glycosylation, which appeared in some laccases [22–24]. As shown in a research performed by Schulze and his colleague, Laccase Lcc8 indeed consists of two bands with the sizes 64 kDa and 77 kDa, where further confirmation with their LC-MS analyses demonstrated protein products from both bands were Lcc8 [21]. However, the follow-up deglycosylation experiment of the purified laccase rejects the hypothesis of glycosylation impact on the Lcc8 molecular weight difference. Therefore, the reason for coexisting in the Lcc8 two bands still remain unclear. For NSP4-Lac1326, our current study here did not conduct LC–MS and deglycosylation experiments of purified laccase, therefore we cannot reject the influence of glycosylation, which might worth further investigation. As our data suggested that NSP4-Lac1326 have a higher secretion level than NSP4-tvel5 laccase, it would be interesting to study whether the post-translational modification is the reason for both higher secretion and the two bands in the Western blot analysis.

Although we have demonstrated the feasibility of using secreted laccases for EDC degradation, there are still a lot of things one could investigate. For example, in future studies, researchers may investigate the secretion level of different secretion signal peptides with laccase to test whether NSP4 is a good choice. Also, similar to some of the other studies [25–28], we have not tried to use purified laccase from culture medium to conduct the decolorization test, but only use the protein crude extract of recombinant *E. coli*, so we have not been able to determine the enzyme activity of the pure laccase accurately. Further studies can try to purify the laccase in the culture medium for experiments to get the exact enzyme kinetic parameters. In addition, we only tested the degradation effect of laccases on β-estradiol but not other EDCs. Even though EDCs have a similar structure with estradiols, it is still necessary and interesting for further researchers to conduct more experiments, testing the efficiency of laccases and secreted laccases in different EDCs. The constitution and phytotoxicity of the laccase catalyzed degradation products of β-estradiol can also be further investigated in the future to ensure the safety and the feasibility of this degradation system. Some of the previous studies showed that some laccase degrade one or few specific EDCs better under specific conditions. Therefore, it would be very useful for further research to look for the best combination of laccases with synthetic biology techniques. By testing three laccases (two with secretion peptides) here, we here provide a basis for other synthetic biology research in laccases. Besides that, finding more laccases that can tolerate the harsh environment, such as wastewater environment, is also important to turn laccase research into applications. The Lac1326 laccase we tested here was known also a wide adaptability (for example, with high activity at up to 60 °C). By characterizing this laccase, we hope to provide some insights for further studies testing environment-tolerating laccases.

## 5. Conclusions

The environmental harm caused by EDCs have yet to be properly addressed. In tackling this issue, we engineered *E. coli* BL21 (DE3), to produced laccases with NSP4 secretion peptide upstream, to degrade the EDCs. Here, we first validated expression and secretion, and further optimized the production of these laccases in *E. coli*. Then, we confirmed that the produced laccases can be functional in decolorization of indigo carmine and EDC (β-estradiol) degradation. Taken together, by proving the expression, secretion and functional characterization of laccases in *E. coli*, we show a potentially feasible approach in tackling EDC pollution problems.

## Credit author statement

Yating Mo: Data curation, Writing – original draft preparation, Visualization, Investigation. Hou Ip Lao: Data curation, Investigation. Sau Wa Au: Data curation, Investigation. Ieng Chon Li: Data curation, Investigation. Jeremy Hu: Data curation, Investigation. Hoi Man Yuen: Data curation, Investigation. Wai Man Cheong: Supervision, Methodology, Data curation, Investigation, Funding acquisition. Owen Lok In Lo: Supervision, Methodology, Data curation, Writing – review & editing, Funding acquisition. Leo Chi U Seak: Supervision, Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

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