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The role of lignin in 17β-estradiol biodegradation: insights from cellular characteristics and lipidomics

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Abstract

17β-estradiol (E2) is an endocrine disruptor, and even trace concentrations (ng/L) of environmental estrogen can interfere with the endocrine system of organisms. Lignin holds promise in enhancing the microbial degradation E2. However, the mechanisms by which lignin facilitates this process remain unclear, which is crucial for understanding complex environmental biodegradation in nature. In this study, we conducted a comprehensive analysis using cellular and lipidomics approaches to investigate the relationship between E2-degrading strain, *Rhodococcus* sp. RCBS9, and lignin. Our findings demonstrate that lignin significantly enhances E2 degradation efficiency, reaching 94.28% within 5 days with the addition of 0.25 mM lignin. This enhancement is associated with increased microbial growth and activity, reduced of membrane damages, and alleviation of oxidative stress. Fourier Transform Infrared Spectroscopy (FTIR) results indicate that lignin addition alters lipid peaks. Consequently, by analyzing lipid metabolism changes, we further elucidate how lignin addition promotes E2 degradation.

Keywords 17β-estradiol, Lignin, Biodegradation, Cells characteristics, Lipidomics, Mechanisms of lipid action

Introduction

17β-Estrogen is a widely prevalent endocrine disruptor, raising concern due to its persistence, toxicity, and bioaccumulative nature. It enters the environment primarily through the excretion of animal feces and urine [1, 32, 57]. In recent years, the escalation of industrialized farming and human activities has intensified estrogen pollution [25, 43]. Among various strategies, bioremediation is regarded as a green, cost-effective, and sustainable approach [47]. This approach can be implemented in several ways: (1) introducing exogenous degrading microorganisms, (2) adding other substances to stimulate indigenous microbes, and (3) employing genetically engineered microbes for treatment. However, the efficacy of bioremediation is intrinsically limited by the activity and vigor of microbes affected by estrogen [45, 48]. Thus, exploring methods to overcome these limiting factors remains a critical issue in the bioremediation of estrogen.

Research indicates that the addition of supplementary substances effectively enhances the biodegradation of organic compounds. Resuscitation-promoting factors and Rpf-responsive bacterial communities have been found to amplify the biodegradation of polychlorinated biphenyls [31]. Moreover, studies have shown that the inclusion of humic acid and Tween80 can promote the degradation of organic phenanthrene [54]. Additionally, various low molecular weight organic acids have been

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found to enhance the degradation of organic phenanthrene [55]. Furthermore, the addition of simple carbon sources has been shown to be beneficial for the degradation of 2,4,6-trichlorophenol [37]. Another study suggests that the inclusion of the co-substrate sodium acetate can promote the biodegradation of amoxicillin by Chlorella [52]. Research also shows that the introduction of low doses of low-dose silver can enhance the biodegradation of tetracycline hydrochloride [18].

In our previous research, we isolated a highly efficient estradiol-degrading bacterium, RCBS9, and found that the addition of lignin could enhance the degradation efficiency of estradiol [11]. Lignin, as the second most abundant organic material in natural ecosystems, is rich in sources and can meet the requirements of renewable energy [29]. However, as a waste product in ecosystems, the presence of lignin causes serious environmental pollution. In industrial production, lignin is a major by-product in the pulp and paper industries [14]. The annual global output of industrial lignin is huge, but most lignin cannot be effectively treated and utilized. In agricultural production, the presence of lignin in straw cell walls can improve its toughness, making straw difficult to degrade [10]. In recent years, with the intensive development of the animal husbandry industry and the expansion of production scale, the production of livestock and poultry waste has increased significantly. Livestock waste, such as manure and enclosure bedding materials, contains large amount of lignin, which is difficult to degrade. The widespread application of straw in the animal husbandry industry, especially in the breeding of ruminants, has led to an abundance of waste lignin. Improper handling of this waste causes serious environmental pollution. Therefore, enhancing the decomposition of lignin holds great prospects for environmental protection and the development new beneficial products [9]. In this study, we found that the addition of lignin promoted the degradation of estradiol, allowing for the reasonable utilization of both pollutants. Hence, understanding how lignin promotes the degradation of estradiol by Rhodococcus sp.RCBS9 is of significant importance.

Lipidomics enables a comprehensive and systematic analysis of the lipid composition and expression patterns in biological systems [33]. Lipidomic analysis can efficiently study the changes and functions of lipid families and lipid compounds in various biological processes, thereby elucidating the mechanisms and principles related to biological activities. This study aims to investigate how the addition of lignin promotes the degradation of estradiol, mainly through analyses of cell growth and surface characteristics, cell surface physicochemical properties, and lipidomics, to explain the reasons for enhanced degradation.

The mechanisms by which additional stimuli enhance microbial degradation vary, necessitating the use of diverse methods to uncover the underlying degradation mechanisms. At present, there are fewer studies using lipid metabolism to explain the mechanism of E2 degradation, and lipid metabolomics can elucidate the changes in various functional lipids in strains stimulated under complex environmental conditions. This study investigates the effects of different lignin concentrations on the degradation of estrogen by strain RCBS9, and the effects of lignin addition on E2 degradation under different salinity and pH conditions. Additionally, changes in enzymes related to oxidative stress and energy alterations were also detected.

Materials and methods

Chemicals and culture media

Estradiol and lignin were procured from Solarbio Science Co., Ltd., (Beijing, China) while methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from Fisher Scientific Co. Ltd. (Shanghai, China). All other reagents used were of analytical grade. Cultivation and degradation experiments for the degrading bacteria were conducted using estradiol stock solution (4 g/L), lignin stock solution (10mM), Luria-Bertani (LB) medium, and Mineral Salt Medium (MSM, composed of (NH4)2SO4 1.0 g, K2HPO4 1.0 g, KH2PO4 0.5 g, MgSO4 0.2 g, H2O 1000 mL, pH 7.0). Various working concentrations of E2 and lignin were prepared by diluting the stock solutions with MSM (100 ml).

Design of E2 degradation

The highly efficient estrogen-degrading strain RCBS9 was isolated from the soil of a dairy farm in Changchun [11]. The strain was cultured in LB medium until reaching the logarithmic growth phase, then centrifuged (7500 rpm, 15 min) and washed twice with MSM medium by centrifugation. Subsequently, the cells were resuspended in MSM to achieve an OD600 = 1.5. It was then inoculated at 1% (v/v) into into MSM (100 ml) medium containing 30 mg/L E2. Samples were collected for estrogen degradation detection days 1, 3, and 5. In brief, an equal volume of methanol was added to the degradation system, the mixture was sonicated to dissolve the compounds, and then centrifuged at 10,000 rpm for 5 min. The supernatant was filtered through a 0.22 μm filter, and the presence of estrogen was detected using HPLC/UV. The HPLC/UV column setup included a ZORBAX SB-C18 column (250 mm × 4.6 mm, 5 μm) for the separation and elution of E2 and its metabolites. The injection volume was 25 μL, the flow rate was 1 mL/min, the mobile phase consisted of a mixture of acetonitrile and water (50/50 v/v), the column temperature was maintained at 30 ℃,

and the UV wavelength was set at 280 nm. The E2 content in the culture was calculated by comparing the peak area of each chemical substance with the standard curve, with all R2 values > 0.99. The full trial was replicated three times unless otherwise mentioned.

Cell growth and surface characteristics analysis

The growth of Rhodococcus sp. RCBS9 cells in the control group and lignin-treated groups was monitored at 1 day, 3 days, and 5 days using a microplate reader (Spectra Max M3, China) to measure at an absorbance of 600 nm. On the fifth day, Rhodococcus sp. RCBS9 cells harvested, washed with MSM, and lyophilized using a vacuum freeze dryer (LGJ-12 A, China). The functional groups present on the cell surface were characterized using Fourier Transform Infrared Spectroscopy (FTIR, Nicolet iS5, USA), and the morphology cell surface was examined using a Scanning Electron Microscope (SEM, JSM-IT300, Japan).

Cytophysical and chemical properties

Determination of antioxidant activity

Control and experimental groups of Rhodococcus sp. RCBS9 cells were collected on days 1, 3, and 5, then washed with MSM and subjected to cell disruption under 200 W for 6 min (3 s on: 10 s off pulse) using ultrasonication. Cell walls and fragments obtained by centrifugation were used for enzyme activity measurement. Catalase (CAT) activity was determined as the amount of enzyme that decomposes 1 μmol of H2O2 per minute at 25 °C, and Superoxide Dismutase (SOD) activity was measured as the amount of enzyme that inhibits the reduction rate of cytochrome c by 50% in a Xanthine/Xanthine oxidase system at 25 °C. Specific SOD and CAT activities were expressed as umg/prot. Malondialdehyde (MDA) levels were measured alongside SOD activity assays. The level of SOD activity indirectly reflects the organism's capacity to scavenge oxygen free radicals, while MDA levels indirectly reflects the severity of cellular damage caused by free radical attacks. (SOD, CAT, and MDA were measured using assay kits from Nanjing Jiancheng).

Energy detection

Sample processing followed the procedures outlined in "Energy detection" Section. ATP, as the most important energy molecule, plays a significant role in various physiological and pathological processes of cells. Alterations in ATP levels can profoundly influence cellular functions (ATP were measured using assay kits from Beyotime).

Cell membrane permeability

Changes in cell membrane permeability were assessed using the crystal violet method [5]. Bacterial pellets, prepared in inorganic salt medium at different time points (4 °C, 8000 rpm, 10 min), were washed twice with 50 mM phosphate buffer solution. Untreated cells were used as controls. The pellets were then resuspended in phosphate buffer solution containing 10 μg/mL crystal violet and incubated at 37 °C for 10 min. Subsequently, the suspensions were centrifuged (8000 rpm, 10 min), and the absorbance of the cell-free supernatant was measured at 590 nm. The experiment was performed with three replicates for each group. The percentage of crystal violet absorption was calculated for all samples using the following formula: OD value of the sample /OD value of crystal violet solution * 100%.

Cell membrane hydrophobicity

Cell membrane hydrophobicity was assessed using Microbial Adhesion to Hydrocarbons (MATH) assay [51]. Following centrifugation (4 °C, 8000 rpm, 10 min), the cells were washed twice with 50mM phosphate buffer to remove interfering solutes. Subsequently, the cells were resuspended in the same buffer to obtain an OD600 = 0.6 suspension, which was then transferred to a test tube and covered with 1.5 ml of xylene. The aqueous and organic phases were thoroughly mixed by vortexing and the test tube was left to equilibrate on a rack for 40 min. Afterward, the aqueous phase was carefully removed, and its absorbance at 600 nm was measured. Cell surface hydrophobicity (CSH) was calculated using the formula: $CSH\ (\%) = 100 \times (Ai - Af)/Ai$; where Ai represents the initial optical density at 600 nm of the aqueous phase, and Af represents the final optical density at 600 nm of the aqueous phase.

Lipidomics analysis

Lipidomic studies were conducted using liquid chromatography-mass spectrometry (LC-MS) technology. The experimental workflow primarily included sample collection, lipid extraction, LC-MS/MS detection, and data analysis. Prior to injection, the first three Quality Control (QC) samples were used to monitor the instrument's status and equilibrate the chromatography-mass spectrometry system. The subsequent three QCs were used for segmented scanning. Together with the secondary spectra obtained from experimental samples, they facilitated qualitative analysis of lipid compounds. QC samples inserted during sample detection were used to evaluate the system's stability throughout the experiment and perform data quality control analysis. The raw files (.raw) obtained from mass spectrometry detection were imported into Lipidsearch software for spectral processing and database searching, yielding qualitative and quantitative results of lipid compounds. Subsequently, multivariate statistical analysis, including Principal

Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), was conducted on the lipid compounds to reveal metabolic pattern differences between different groups. Hierarchical Cluster Analysis (HCA) and correlation analysis of lipid compounds were used to reveal relationships among samples and between lipid compounds.

Results

Lignin promotes estradiol biodegradation and cell growth

In summary, lignin had a significant positive effect on the biodegradation of E2 by strain RCBS9.The addition of various concentrations of lignin correlates with an increase in the degradation rate of estrogen, up to a certain threshold. Beyond this concentration, specifically 0.25 mM of lignin, the degradation rate essentially remains unchanged. At this concentration, the degradation rate of estrogen reaches 94.27% at 5 days, representing a 1.4-fold increase (Fig. 1A). Throughout the process, the growth of Rhodococcus sp. RCBS9 increased (Fig. 1B). In MSM with lignin alone without E2, the OD600 values remained essentially unchanged, so they are not shown in the pictures.These results indicate that lignin promotes the biodegradation of E2, and Rhodococcus can utilize E2 to enhance its growth, thereby leading to increased E2 consumption.

Effect of lignin on salinity and pH tolerance

In the presence of 30 mg/L E2, sodium chloride was added to inorganic salt medium at final concentrations of 0 g/L, 5 g/L, 10 g/L, 20 g/L, and 40 g/L to assess the salt tolerance of Rhodococcus sp. RCBS9 following lignin addition. The results indicated that under varying salt conditions, strain RCBS9 continued to degrade E2, with all lignin-added groups exhibiting stronger degradation abilities compared to the control group. Even at a sodium chloride concentration of 10 mg/L, the experimental groups maintained degradation rates without significant decline. This demonstrates ability of lignin addition to enhance the salt tolerance of the strain to a certain extent (Fig. 2A). When evaluating the strain's tolerance to acidity and alkalinity with final pH values of 3, 5, 7, 9, and 11 after lignin addition, Rhodococcus sp. RCBS9 exhibited greater alkaline tolerance compared to acidity. The results indicated that at pH 3, the addition of lignin did not markedly alter the degradation rate of the

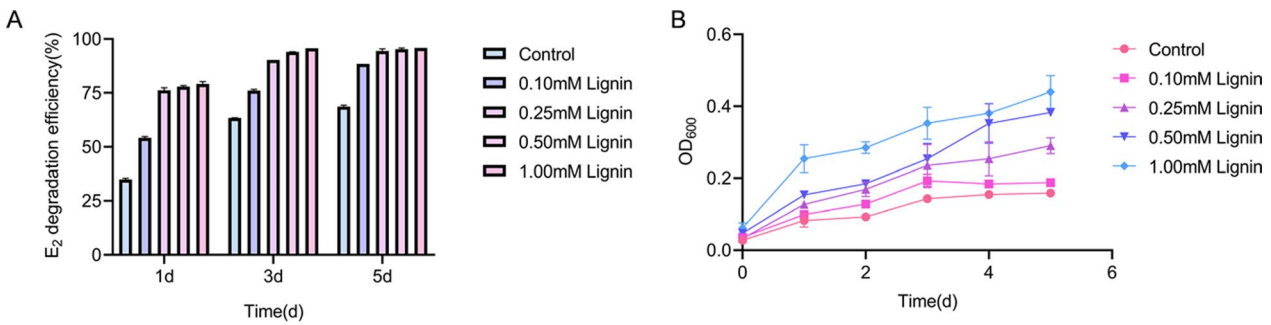

Fig. 1 Effect of different Lignin concentration (25 ℃,1% inoculum amount,30 mg/L E2). **A** Impact on E2 biodegradation and **B** Influence on cell growth

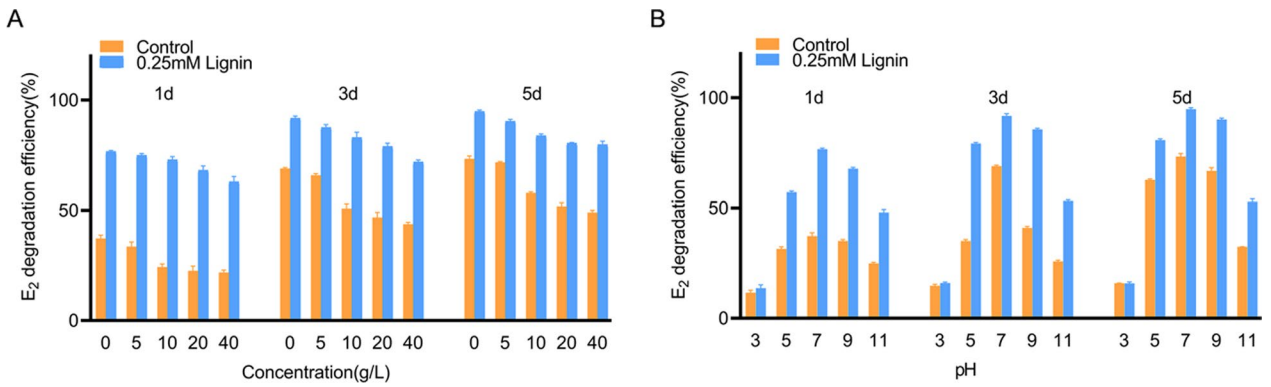

Fig. 2 The effect of lignin on the tolerance to salinity and pH. **A** The impact of different salinity levels (0 g/L,5 mg/L,10 g/L,20 g/L,40 g/L) on lignin tolerance, **B** The impact of different pH levels on lignin tolerance

bacterial strain. At pH 5 and 9, there was little difference in the degradation ability of the strain with and without lignin, although the group with lignin exhibited slightly higher degradation than the control group. However, at pH 11, the lignin-added group showed a obvious increase compared to the control group (Fig. 2B). This findings suggests that lignin addition can enhance the salt-alkali tolerance of the bacterial strain to a certain extent, thereby reducing the environmental impact on the strain and enabling it to better exert its degradation function.

The effect of lignin on strain cell characteristics

The regulation of functional groups on the bacterial surface is crucial for hydrocarbon biodegradation and can be analyzed through changes in the position and relative intensity of absorption peaks in infrared spectra. In the infrared spectrum of the control group, the peak at 3300 cm-1 is attributed to the hydroxyl -OH stretching vibration peak The double peaks at 2934-1 and 2866 cm-1 are assigned to the asymmetric and symmetric stretching vibrations of saturated hydrocarbon methylene and methyl C–H, respectively, which are characteristic peaks of lipids on the cell membrane (Fig. 3A). The peak at 1722 cm-1 is assigned to the C=O stretching vibration of –COOH, the peak at 1658 cm-1 corresponds to the amide I band C=O stretching vibration, and the peak at 1500 cm-1 is the C=C skeleton vibration of the benzene ring. The peak at 1577 cm-1 represents the amide II band N–H bending vibration, and the peaks at 1287 cm-1 and 1251 cm-1 are the amide III band C–N stretching vibrations. The peak at 1460 cm-1 is the C–H bending vibration in saturated hydrocarbons, which are characteristic of proteins. The peak at 1115 cm-1 is the C–O stretching vibration in polysaccharides, and the peaks at 875 cm-1, 821 cm-1, and 617 cm-1 are the in-plane bending vibrations of C–H on the benzene ring. After adding lignin, it can be seen that the positions of characteristic peaks of lipids, proteins, and polysaccharides remain essentially unchanged, indicating that the basic structure of the samples remains unchanged. Whereas, the increased intensity of the characteristic lipid peak at 1722 cm-1 may indicate some changes in lipids. Additionally, a new peak appears at 586 cm-1, which can be attributed to to different substituents on the aromatic ring.

Rhodococcus sp. RCBS9 is a Gram-positive bacterium, typically exhibiting a short rod-shaped morphology at 25 ℃. After culturing in inorganic salt medium containing estrogen, the surface shows minor damage along with some shrinkage and deformation (Fig. 3B). Upon the addition of lignin, the damage decreases, and the cells change from short rod-shaped to long rod-shaped, thereby increasing the membrane area to some extent (Fig. 3C). These results indicate that adding lignin can alleviate the stress experienced by RCBS9.

Lignin alleviates cellular oxidative stress

Bacteria exposed to toxic environments usually produce oxidative stress responses. Superoxide dismutase (SOD) and catalase (CAT) are essential antioxidants for microbes; SOD catalyzes the conversion of superoxide radicals (•O2-) into oxygen (O2) and hydrogen peroxide

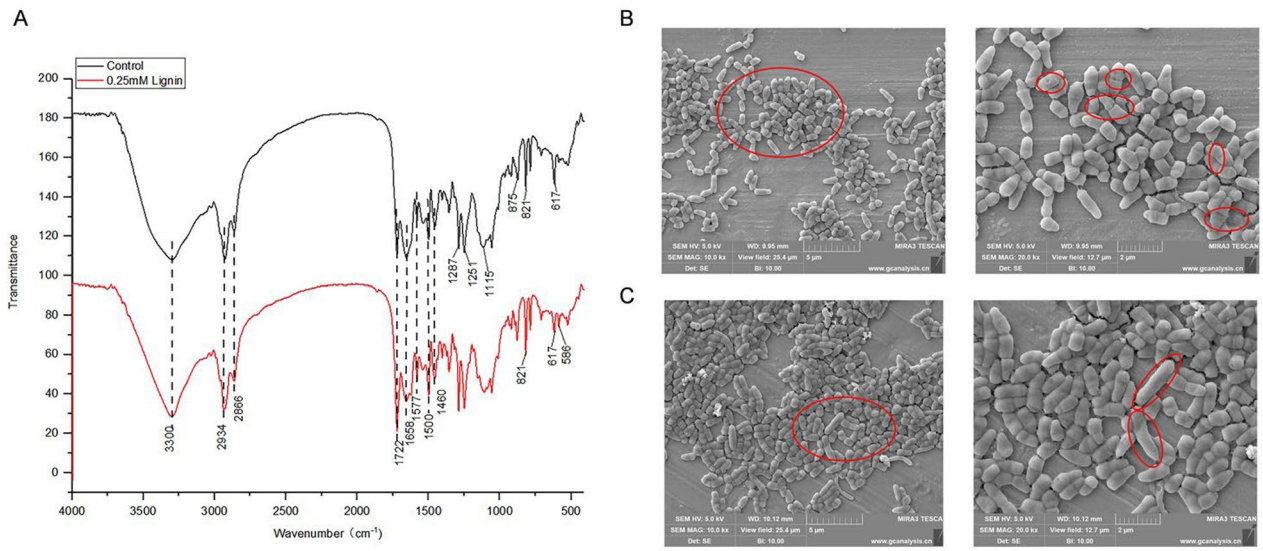

Fig. 3 Cell surface changes. **A** FTIR characteristics of FTIR of Rhodococcus sp.RCBS9 cells cultured in MSM, **B** SEM images of Rhodococcus sp. RCBS9 in 30 mg/L E2, **C** SEM images of Rhodococcus sp. RCBS9 in 30 mg/L E2 and 0.25mM lignin (the red circle represents regions of interest and highlights)

(H2O2), while CAT further reduces H2O2 into H2O. Compared to the control group, the activities of SOD and CAT in Rhodococcus sp. RCBS9 decrease with the addition of lignin. The results indicate that the presence of lignin reduces the production of free radicals induced by E2, thus alleviating oxidative damage to cells. This reduction in oxidative stress is beneficial for cellular functions and enhances biodegradation (Fig. 4A and B).

The body produces oxygen free radicals through enzymatic system and non-enzymatic system. The latter can attack polyunsaturated fatty acid (PUFA) in biological membranes, triggering lipid peroxidation and forming lipid peroxide malondialdehyde. Compared to the control group, the production of MDA decreases after adding lignin. The results show that the addition of lignin alleviates attack by free radicals on Rhodococcus sp. RCBS9 (Fig. 4C).

The substances that make up microbial cells—proteins, nucleic acids, lipids, and polysaccharides—all require ATP for energy synthesis. Compared to the control group, the ATP level significantly increases after adding lignin, indicating that the physiological state of Rhodococcus sp. RCBS9 improves with the addition of lignin (Fig. 4D).

Cell membrane permeability and hydrophobicity

Cell membrane permeability and hydrophobicity often undergo changes in response to environmental variations. In this study, the cell membrane permeability and hydrophobicity of Rhodococcus sp. RCBS9 were compared under conditions of 30 mg/L E2, both and without the addition of 0.25 mM lignin. The results showed that cell membrane permeability increased over time, with slightly higher permeability observed in the presence of lignin compared to the control group (Fig. 5A). Hydrophobicity exhibited a similar pattern, which is generally associated with lipid composition of the cell membrane surface (Fig. 5B). An increase in short-chain unsaturated fatty acids enhances hydrophobicity. Higher hydrophobicity can improve the adhesion capacity to pollutant surfaces, thereby accelerating biodegradation. This may explain why the addition of lignin enhances the degradation rate of E2.

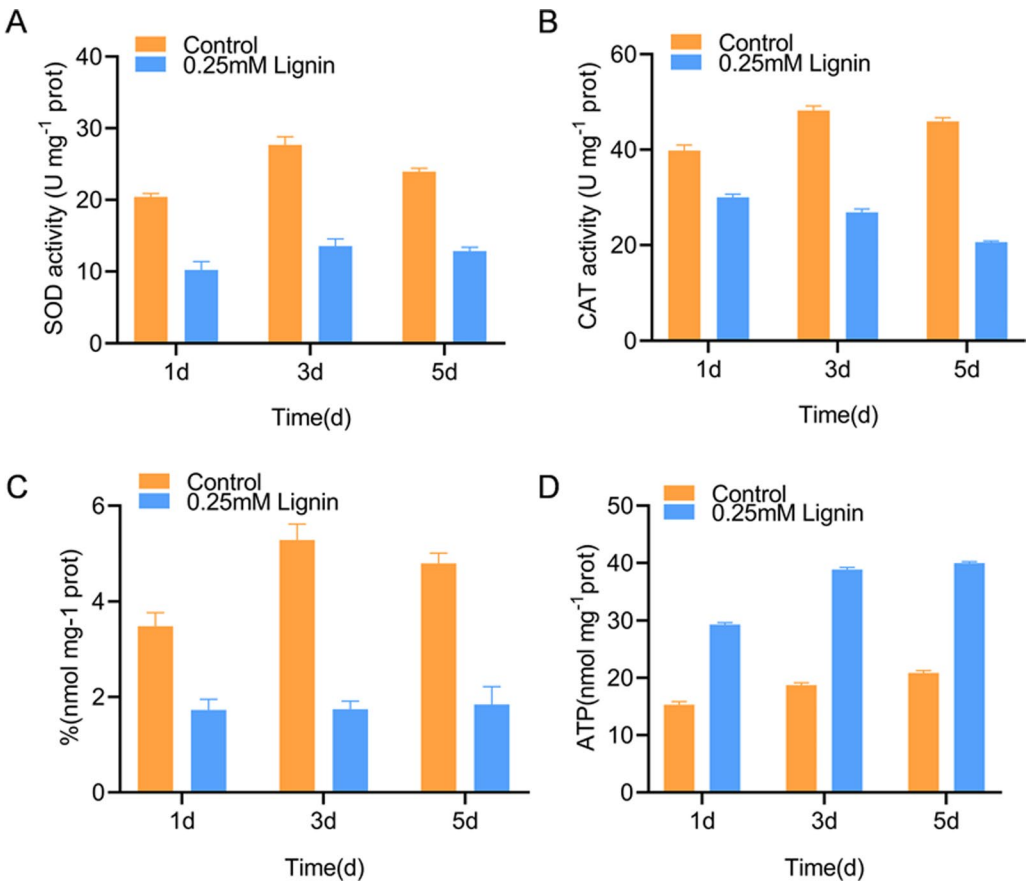

Fig. 4 Cellular oxidative stress indices. **A** SOD activity, **B** CAT activity, **C** MDA levels, and **D** ATP levels

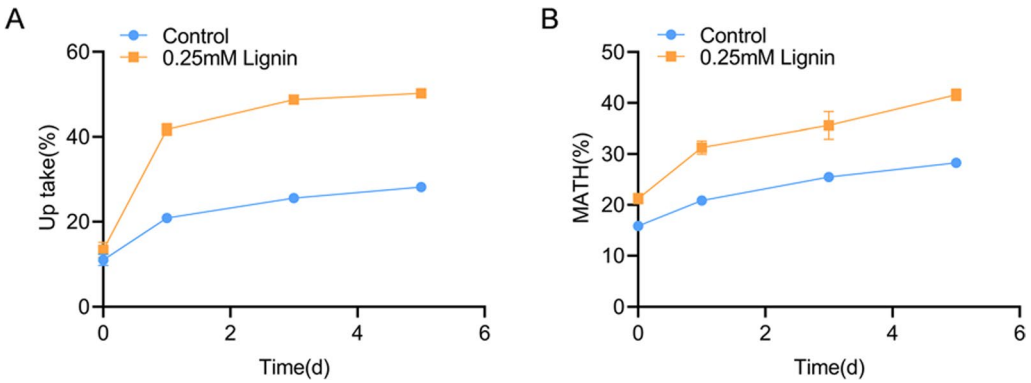

Fig. 5 Cell membrane permeability and hydrophobicity. **A** Crystal violet uptake of Rhodococcus sp.RCBS9 treated with control (30 mg/L E2) and experimental group (0.25mM Lignin and 30 mg/L E2), **B** Rhodococcus sp.RCBS9 membrane hydrophobicity. All the values are averages of three replicates from three independent experiments

Comparative analysis of lipids with/without lignin addition

Comparative lipidomics methods can elucidate differences in lipids in microbial cells grown on polycyclic aromatic hydrocarbons. As shown in (Fig. 6A), the correlation of three control samples exceeds 98%, as does the correlation among three samples with added lignin, indicating good repeatability of the samples. Figure 6B illustrates the positive (POS) and negative (NEG) ion modes during data collection. In the positive ion mode, the group with added lignin and the control group exhibited 828 and 407 differentially expressed lipids (VIP > 1.0, FC > 1.5), respectively. In the negative ion mode, they had 482 and 256 differentially expressed lipids (VIP > 1.0, FC > 1.5), respectively. The volcano plot visually displays the overall distribution of differentially expressed lipid compounds as shown in Fig. C. The results indicate that in the positive ion mode, the comparison between the lignin group and the control group significantly

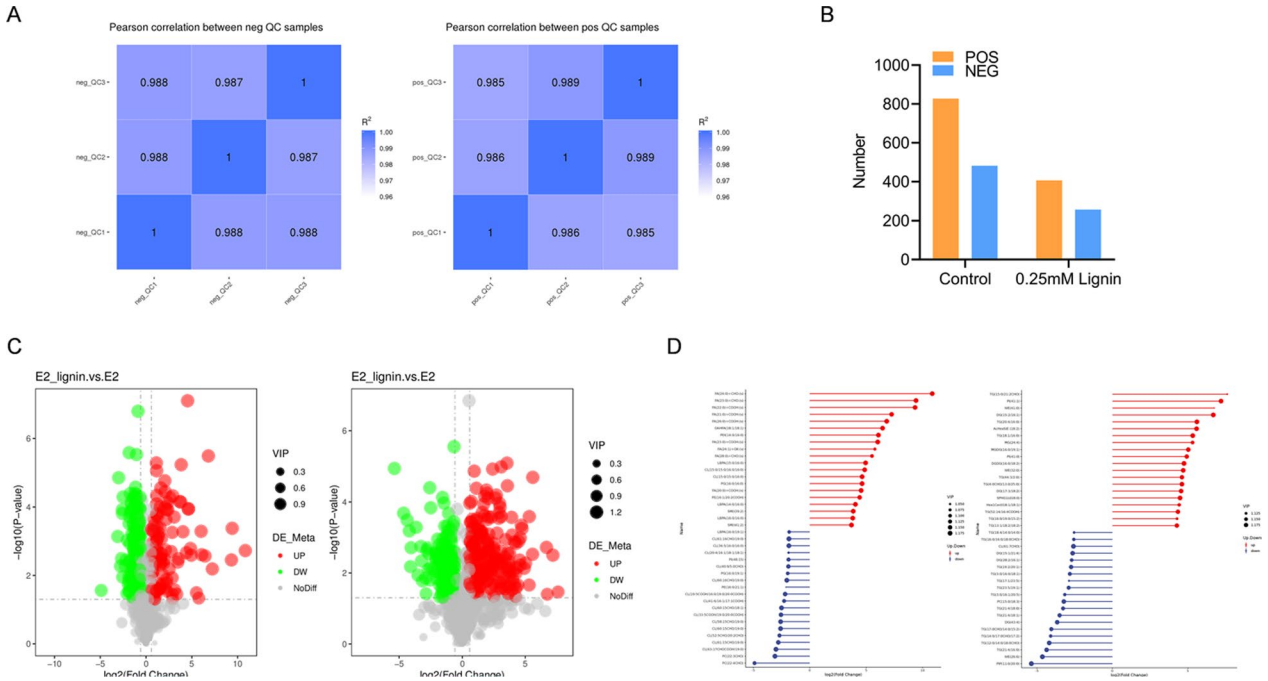

Fig. 6 Comparative analysis of lipids with/without lignin addition. **A** Correlation heatmap (POS represents positive ion direction detection, NEG represents negative ion direction detection), **B** Screening results of differential lipid compounds (VIP > 1.0, FC > 1.5), **C** Volcano diagram of differential lipid compounds, **D** Matchstick plots illustrating the upregulation and downregulation of lipid compounds with significant fold changes

up-regulated a total of 220 lipid compounds and significantly down-regulated a total of 187 lipid compounds. In the negative ion mode, the comparison significantly up-regulated a total of 108 lipid compounds and significantly down-regulated a total of 148 lipid compounds (Fig. 6C). Matchstick plots drawn from the differential lipid compounds obtained from each group's differential comparison clearly represent the up-regulation and down-regulation of lipid compounds and substances with significant fold changes (Fig. 6D). The results show that in the cation mode, the mainly up-regulated and significant substances include Sphingomyelin (SM), Fatty acid (FA), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and Cardiolipin (CL). In the anion mode, the mainly up-regulated and significant substances include triglycerides (TG), Phosphatidylinositol (PI), diacylglycerol (DG), and Monogalactosyl diglyceride (MGDG).

Discussion

Steroid chemicals, as major constituents of endocrine-disrupting chemicals (EDCs) pollutants, have garnered widespread public attention in recent years. Among them, environmental estrogens (EEs) pollution has become a concern due to its broad distribution, prolonged persistence, and significant adverse effects [46]. Human activities and livestock breeding are the primary sources of natural estrogen entering ecosystems, where environmental estrogens pose risks to the health of humans, animals, and plants [58]. Therefore, the degradation of environmental estrogens is urgent, and biodegradation represents a green and eco-friendly method for this purpose [56]. Some studies have demonstrated that microorganisms are capable of degrading estrogens, with a variety of estrogen-degrading bacteria being isolated from different environmental habitats, such as Pseudomonas, Sphingomonas, Comamonas, Rhodococcus, and Acinetobacter [16, 22, 26, 34, 39, 40]. However, biodegradation is significantly influenced by external factors, making the exploration of methods to overcome these limitations an important topic in the bioremediation of estrogens.

In this study, we selected Rhodococcus, which is widely distributed in the environment, to degrade estrogen and added lignin, a common organic substance in the environment of the Northeast region, to promote the estrogen degradation [2, 8]. Environmental temperature affects the microbial metabolic activities, inevitably influencing their microbial metabolic activities [13]. Therefore, we conducted degradation experiments at the optimal temperature for the estrogen-degrading strain RCBS9, which is 25 °C. Our experiments revealed that at the optimal degradation temperature, lignin addition still markedly enhanced the degradation rate, thereby confirming its efficacy in enhancing degradation (Fig. 1A). An increased cell count per unit volume helps estradiol consumption, and the results also indicated that cells could utilize estradiol to stimulate their growth, resulting increased biomass after lignin addition, thereby accelerating the degradation rate (Fig. 1B). Under Scanning Electron Microscope (SEM) observation, Rhodococcus sp. RCBS9 cells exhibited slight damage to the cell membrane in a mineral salt medium containing 30 mg/L estradiol (Fig. 3B). In contrast, lignin addition maintained membrane integrity, with cells more often assuming elongated rod shapes, thereby increasing the surface area (Fig. 3C). This observation further demonstrates that lignin addition promote strain's growth and mitigate the impact of stress caused by estradiol. Generally, microbial cell membrane performance is related to cell characteristics and plays a crucial role in adapting to harmful substances [15, 21, 50]. The regulation of bacterial surface functional groups is critical for hydrocarbon biodegradation. Therefore, we performed a detailed assessment of the functional groups and appearance of the cell membranes and observed an increase in peak intensity at 1722 cm-1, suggesting that the addition of lignin may favor high-quality lipid synthesis. Microorganisms can adjust their membrane permeability by altering fatty acid composition to cope with environmental toxic stressors [24, 28, 42]. Lipids constitute the primary components of prokaryotic cell membranes, and alterations in lipid composition can influence the fluidity and permeability of the cell membrane (Fig. 5A). Increasing membrane fluidity is beneficial for the uptake of estradiol, thereby accelerating the estradiol degradation efficiency. The addition of easily metabolized carbon sources can also change the composition and content of lipids, potentially reducing membrane stress responses, enhancing cell vitality, and proliferative capacity [53]. Moreover, lipids are involved in regulating various life processes, including energy conversion, substance transport, information recognition and transmission, cell development and differentiation, and apoptosis [49].

Lipidomics analyses have predominantly been conducted in eukaryotic systems, but with the increasing exploration of bacterial lipidomics, it has been discovered that lipids in bacteria play a significant role in maintaining bacterial structure and providing protection against the surrounding environment [4]. In this study, nine significantly up-regulated lipids that play crucial roles were identified. The discussion focusing on these nine up-regulated lipids, most of which are important components of the cell membrane structure, participating in the construction of the cell membrane's bilayer structure [19]. The bacterial cell membrane serves as a barrier between the internal and external

environments of the bacterial cell, protecting the internal structures of the bacteria, controlling the entry and exit of substances, and determining the shape and function of bacterial cells. These nine important lipids not only constitute the cell membrane but also play significant roles in other physiological functions. Among them, Monogalactosyl diglyceride plays an important role in organisms that tolerate high salt environments, helping to maintain normal growth and metabolism in high salt conditions [36, 38]. The increase of monogalactosyl diglyceride after the lignin addition promotes bacterial growth. Some bacteria living in frigid environments synthesize a large amount of monogalactosyl diglyceride to help cells withstand the effects of low temperatures and freezing. This will provide great help for the subsequent strain RCBS9 to degrade estrogen in complex real-world environments, and may still maintain an efficient degradation ability under cold conditions. Cardiolipin has antioxidant properties that can help bacteria resist oxidative stress and protect the cells from oxidative damage. This is validated by previous experiments where the lignin addition resulted in a decrease both in catalase and superoxide dismutase activities, indicating that the lignin addition can reduce oxidative damage to a certain extent (Fig. 4A and B). MDA is a natural product of lipid oxidation in organisms. The determination of MDA often complements that of SOD, where the SOD activity level indirectly reflects the body's ability to eliminate oxygen free radicals, while the MDA level indirectly reflects the severity of cellular damage caused by free radicals [35].(Fig. 4C) Phosphatidylglycerol can affect the fluidity and permeability of the cell membrane. Increased membrane fluidity allows the membrane to remain in a liquid state under low-temperature conditions, thus increasing cold resistance and affecting the adaptability of bacteria to the environment [17, 20, 30]. This also confirms the change in cell membrane fluidity after lignin addition in the experiment (Fig. 5A). Phosphatidylglycerol, with its negative charge on the cell membrane, helps maintain the electrostatic balance inside and outside bacterial cells, influencing the transport and balance of various ions and molecules within the cell to maintain cellular homeostasis [3, 23]. Sphingolipids may enhance bacterial tolerance to adversity such as high temperature and pH changes, aiding bacteria in survival and proliferation under harsh conditions. This also verifies the experimental results that lignin can still maintain the high efficiency of estradiol interpretation in alkaline environment (Fig. 2B). Many lipids act as signaling molecules in cells, regulating the physicochemical properties of cells [41]. For instance, sphingolipids serve as signaling molecules within bacterial cells and participate in intercellular signaling pathways, regulating bacterial growth, metabolism, and adaptability, impacting bacterial responses to the external environment. Phosphatidylethanolamine acts as a signaling molecule involved in intracellular and extracellular signal transduction processes, playing a crucial role in bacterial growth, development, and environmental adaptation by interacting with other molecules to regulate bacterial metabolism, growth, and environmental adaptation capabilities. Phosphatidylinositol is a key molecule in intracellular signal transduction, allowing bacteria to regulate various signal transduction pathways in the cell through its different phosphorylation states, participating in biological processes such as cell growth, differentiation, movement, and apoptosis. Diacylglycerol serves as a second messenger within bacterial cells, participating in signal transduction pathways. Changes in diacylglycerol levels triggered by external stimuli lead to intracellular signal transduction reactions that affect bacterial growth, metabolism, and adaptability. Certain fatty acids (FAs) act as signaling molecules in bacterial signal transduction processes, where some long-chain fatty acids regulate gene expression by binding to specific transcription factors. Most lipids can also participate in energy storage and metabolism processes. Bacteria can utilize fatty acids (FAs) for energy storage and metabolism. When bacteria grow in environments rich in carbon sources, they can convert excess carbon into fatty acids through the process of fatty acid biosynthesis and store them as triglycerides (TAGs) or other forms of fatty acid storage bodies. In situations of energy deficiency, bacteria can release stored energy by breaking down fatty acids. Lignin is also a kind of carbon source, therefore, following lignin addition, the strain RCBS9 may store the excess carbon source as reserve energy under a condition of abundant carbon source (Fig. 4D). Phosphatidylethanolamine is also involved in bacterial energy metabolism processes, playing a crucial role in lipid metabolism pathways and participating in bacteria's utilization of different carbon sources and energy synthesis processes. Triglycerides are typically used as a form of energy reserve, accumulating within bacterial cells as a way to store energy when nutrients are abundant. When bacteria face energy shortages, triglycerides can be hydrolyzed into glycerol and free fatty acids, releasing stored energy to sustain bacterial survival and metabolic activities. Diglycerides can exist as a form of energy reserve similar to triglycerides. When energy demands are high or nutrients are scarce, bacteria can hydrolyze diglycerides into free fatty acids and glycerol, releasing energy to maintain their survival activities. Some lipids also participate in

the localization, modification, and regulation of certain proteins [7]. Phosphatidylinositol plays a role in positioning proteins to the cell membrane or specific subcellular regions in bacteria. By interacting with specific structural domains, phosphatidylinositol can regulate the function and activity of internal bacterial proteins. Cardiolipin can interact with proteins, influencing protein structure and function, and regulating various biological processes within bacteria, such as enzyme activity and signal transduction [44]. Phosphatidylethanolamine is associated with antibiotic resistance in bacteria [6, 12]. Some studies suggest that the PE content is associated with the sensitivity or resistance of bacteria to antibiotics, potentially influencing bacterial responses to antibiotics by affecting the structure and properties of the cell membrane [27].

Conclusions

This study demonstrates the effectiveness of lignin supplementation in enhancing the estrogen degradation capability of bacterial strain RCBS9, and promoting biomass production. Lipidomic analysis indicates that lignin addition fosters the synthesis of diverse lipids, primarily comprising key constituents of cell membranes, energy storage metabolism, protein function regulation, and cell signal transduction. Additionally, these lipids enable tolerance to adverse environmental conditions such as high salinity and low temperature, thereby positively influencing the survival and biological functions of strain RCBS9. These findings provide some insights into the mechanism underlying lignin-induced promotion of estradiol degradation. Future research endeavors may entail the application of these findings in real-world environmental settings for a more judicious approach to addressing environmental pollutants.

Author contributions

Conceptualization, H.P. and Y.G.; Data curation, H.P. and P.H.;Formal analysis, H.P. and Y.G.; Funding acquisition, Y.G.; Investigation, H.P., P.H, Q.L., Z.L.,and K.G.; Methodology, H.P., P.H., Q.L.and K.G.; Project administration, Y.G.; Resources, D.W.; Software, H.P.and K.G.; Supervision, H.P., D.W., X.L. and Y.G.; Validation, P.H. and H.P.; Visualization, H.P. and Q.L.; Writing—original draft, H.P.; Writing—review & editing, H.P. and Y.G.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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