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RESEARCHES OF SOIL LACCASES IN THE 21ST CENTURY: MAIN DIRECTIONS AND PROSPECTS

Laccases (benzodiol: oxygen oxidoreductases, EC 1.10.3.2) belong to the so-called blue-copper oxidase family and are copper-containing enzymes that are involved in oxidative processes by catalyzing the oxidation of various compounds with molecular oxygen, including o- and w-diphenols, aminophenols, polyphenols, polyamines, aryl diamines, phenolic substructures of lignin, and also some inorganic ions. The physiological functions of laccases are diverse: participation in the formation of pigments and the formation of fruiting bodies of fungi, detoxification of phenols, catalysis of the oxidation of non-phenolic lignin units (C_4-esterified) to radicals.

Laccase activity increases due to the introduction of Cu^{2+}, Mg^{2+} and Na^{+}, but is strongly inhibited by Fe^{2+}, Ag^{+}, l-cysteine, dithiothreitol and NaN_3. In the lower soil layers, the activity of laccase shows a significant increase when supplied with mineral N, the addition of compost leads to increased activity in the surface layer.

The prospects for the practical use of oxidases increased after the discovery of the possibility of enhancing their action using redox mediators, which are substrates of these enzymes, during the oxidation of which highly redox potential and chemically active products are formed. Biocatalytic systems created by nano-technologies (bacterial nanocellulose, carbon nanotubes, magnetic nanoflowers etc.) increase the reaction efficiency by increasing the surface area and loading capacity, and reducing the mass transfer resistance. The effectiveness of immobilization is highly dependent on the process conditions, the properties of the enzyme and the material of the carrier. In particular, a clear correlation was established between the redox potential of the substrate and the efficiency of homogeneous catalysis.

Of particular note is the effect of laccase on soil emissions of CO_2 and other greenhouse gases. Participating in the polymerization of soluble phenols, they thereby contribute to humification, forming stable humic fractions that bind soil carbon.

The data presented indicate that soil laccase is an important factor in the functionality of soil, but they need to be studied in more detail in order to understand the mechanisms that regulate their activity.

Key words: laccase, enzymatic activity, immobilization, greenhouse gas emissions..

Laccases (benzodiol: oxygen oxidoreductases, EC 1.10.3.2) belong to the so-called blue-copper oxidase family and are copper-containing enzymes that are involved in oxidative processes by catalyzing the oxidation of various compounds with molecular oxygen, including o- and w-diphenols, aminophenols, polyphenols, polyamines, aryl diamines, phenolic substructures of lignin, and also some inorganic ions. [8, 15, 20]. Due to the coordinated interaction of four copper ions of three different types that make up the active center of laccases, the enzyme is able to directly bioelectrocatalyst the molecular oxygen reduction reaction by the mechanism of direct mediatorless electron transfer from the electrode to the active center, followed by oxygen reduction directly to water, bypassing the formation stage intermediate highly reactive toxic oxygen intermediates such as superoxide anion radical l (Og")", hydroxyl radical (OH") and hydrogen peroxide (H_2O_2) [3]. Qi Luo, who described an effective method for the degradation of perfluorooctanoic acid through a laccase-catalyzed oxidative humification reaction, concludes that the degradation mechanism involves the chain reaction of free radicals initiated by their direct attacks on the CC bond of this perfluoroalkyl acid [31]. A number of works have shown the high potential of basidiomycetes as effective destructors of xenobiotics, including pesticides [18, 24, 26]. Laccases are found in many xylotrophic and phytopathogenic fungi, as well as soil saprophytes.

Laccase of basidiomycetes of white rot of wood is able to catalyze the oxidation of non-phenolic lignin units (C_4-esterified) to radicals, whereby during this reaction laccase acts in the presence of radical mediators that are formed during the conjugated oxidation of thiols or unsaturated lipids [15, 17]. Laccase presence in cultural filtrates, it has been proven for most lignin-destroying fungi, including: *Coriolus (Trametes) versicolor, C. hirsutus, C. zonatus, Phanerochaete chrysosporium, Pleurotus eryngii, Panus tigrinus, Fomes sp., Cerrena maxima, Rigidoporis sp., Phellinus sp .*, *Lentinus tigrinus*, *Clitocybula dusenii*, *Nematoloma forwardii*, *Pholiota mutabilis*, *Collybia sp.*, *Armillariella sp.*, *Coprinus cinereus*, *Phlebia brevispora*, *Poria cinerescens*, *Bjerkandera adusta*, *Ganoderma lucidum*, *Irpex lacteum* etc. In *Phanerochaete chrysosporium* marked by a rather low level of laccase activity [9].

In the literature, there is evidence that copper ions in the active center of laccases may, in the process of biosynthesis, appear to be partially replaced by ions of other metals. For example, two forms of laccase are found in oyster mushrooms. One of them, like most other laccases, is induced by an excess of copper ions, has an absorption maximum in the blue part of the visible spectrum and contains four copper ions. The second contains one copper ion, two zinc ions and one iron ion, and does not have an absorption maximum of about 600 nm. Instead, it has a broad absorption maximum at 400 nm. The only difference between the two enzymes from the other laccases is the lack of activity towards guaiacol. Leontievsky described the yellow laccases of the species *Partus tigrinus*, *Phlebia radiata*, *Phlebia tremellosa* and *Agaricus bisporus*, isolated from solid-phase cultures and not having typical spectral and catalytic properties, unlike blue laccases from submerged cultures. It is assumed that yellow laccases are formed as a result of the modification of ordinary blue laccases by decomposition products of lignin. In this case, the secondary structure and microenvironment of copper atoms in the active center change, and yellow laccase acquires the ability to oxidize stable lignin substructures [2]. In addition, laccase secretion has been described in a number of bacteria of such species as *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* etc [21].

Patricia Luis, using the example of brown forest soils, describes the specific distribution of laccase genes and the corresponding fungal species in different soil horizons (A_0, Ad, A_1): forest litter showed the highest diversity of genes. In this saprophytic fungi were less common in researched horizons and demonstrated a higher diversity in laccase genes than mycorrhizal [23].

This class of enzymes has many functions, both at the organism level and at the ecosystem level, and can initiate both positive and negative feedback loops between soil organisms and soil organic matter. The physiological functions of laccases are diverse: participation in the processes of formation of pigments and the formation of fruit bodies of fungi, biodegradation of lignin and detoxification of phenols. In connection with the above features of this family of enzymes, they are intensively used in various branches of biotechnology.

It was reported that laccase with a higher redox potential tends to have a higher oxidation rate [5, 13]. Gorbachev showed that the efficiency of catalysis of high- and low-potential laccases in relation to substrates donor electrons of similar structure linearly depends on the "driving force of the reaction", i.e. from the difference between the redox potentials of the T1 center of the enzyme and the substrate. She experimentally proved that chelated ions of divalent manganese are natural substrates of highly redox-potential fungal laccases and the chelated ions of trivalent manganese formed as a result of the enzymatic reaction are capable of non-enzymatic oxidation of the model compound of lignin — veratric alcohol to veratric acid. Low redox potential wood laccase does not catalyze this reaction [1].

Of great interest, both in fundamental and applied terms, is the substrate specificity of these enzymes, which can oxidize a wide range of organic as well as inorganic compounds. There is evidence that the secretion of laccase depends on the cultivation conditions and may be caused by metallic, phenolic or aromatic compounds. In particular, Thiago Santana using the example of *Lentinus crinitus* laccase shows that the interaction of guaiacol or veratril alcohol and copper (250 μM) added to

the culture medium causes synergistic effects leading to an increase in the activity of laccase [32]. Ranjit Das, who studied the activity of laccase from spore cells of *Bacillus sp*. GZB in the process of degradation of bisphenol A, claims that the activity of laccase was increased due to the introduction of Cu^{2+}, Mg^{2+} and Na^{+}, but was strongly inhibited by Fe^{2+}, Ag^{+}, l-cysteine, dithiothreitol and NaN_3 [10]. Adeline Vigno at al. reported that the laccase activity essentially inhibiting with enological tannins [34]. In order to avoid secondary contamination with heavy metals, Yun Zeng suggests for the oxidation of polycyclic aromatic hydrocarbons to use Cu-independent bacterial laccase CotA from *Bacillus subtilis*, which also has a relatively high redox potential (525 mV compared to 440 mV in CueO from *Escherichia coli*) [38]. Martina Mazzon testifies that in the lower soil layers, laccase activity showed a significant increase in the supply of mineral N, whereas the addition of compost led to increased activity in the surface layer [25].

Laccases can quickly oxidize benzo[a]pyrene. It is believed that the metabolites with increasing solubility in water caused by the oxidation of benzo[a]pyrene, can stimulate the subsequent mineralization. Jun Zeng suggests that the soil contaminated with benzo[a]pyrene can be detoxified by laccase mainly by forming a bound residue for the organic matter of the soil by covalent binding. Laccase contributed to the dissociation of benzo[a] pyrene (15.6 %) from the soil, followed by trace mineralization (<0.58 ± 0.02 %) and the formation of a substantial bound residue (~ 80 %). Increase ~ 15 % in the related residual fraction was observed when the action of laccase, which was mainly due to covalent binding residues humic fraction. In contrast, benzo[a]pyrene, treated with laccase, led to a smaller shift in the composition of the bacterial community, which indicates a decrease in the disturbance of soil microbial communities [38]. Navada reports that the addition of mediators (syringaldehyde, vanillin, ABTS and α-naphthol) accelerated the decomposition of chloramphenicol from 10 % to 100 % within 48 hours [28].

Despite the fact that enzymes have a unique and unprecedented catalytic activity and selectivity over a wide range of substances, problems related to their stability often hinder their use in real environmental conditions. Interest in the practical use of oxidases increased in the mid-1990s, after the discovery of the possibility of enhancing the action of these enzymes using various redox mediators [7], which made it possible to significantly expand the scope of their practical application. MSO mediators are substrates of these enzymes, in the process of oxidation of which highly redox-potential and chemically active products are formed. The latter can react with compounds that are not subjected to oxidation by oxide alone or participate in electron transfer in electrochemical reactions, accelerating electrochemical processes involving these enzymes. In addition, during the oxidation of organic substrates, free radicals are formed, which can modify other compounds [19].

Biocatalytic systems created with the help of nanotechnology have attracted attention for many applications, since nanoscale carriers for immobilizing enzymes can improve the factors that determine efficiency, for example, increasing surface area and loading capacity and reducing mass transfer resistance. Laccases, which play an important role in the degradation of soil phenol or phenol-like substances, can be potentially used to restore the soil through immobilization through physical adsorption or covalent binding. So Mitra Naghdi found that immobilized laccase has a higher stability with respect to temperature and pH changes. compared to free laccase. In addition, the immobilized laccase retained its catalytic characteristics for up to seven recycling cycles and shows more than 50 % of the initial activity after two months of storage at room temperature [27]. There is evidence that Fe- and Al-containing minerals can adsorb extracellular enzymes in the soil environment [35]. This is consistent with the results of Wendy Hernandez-Moniaras, which suggests that laccase activity in the intracellular fraction of *Fusarium oxysporum* f. sp. *lycopersici* wild-type and mutant strains increases with the addition of iron chelator (53.4 and 114.32 %, respectively) [16].

However, the efficiency of immobilization strongly depends on the conditions of immobilization and the properties of the enzyme and the material of the carrier. So, on the basis of a comprehensive

study of the biochemical, spectral and electrochemical characteristics of blue copper-containing oxidases with different values of the redox potential of T1 centers, Shleev established a clear correlation between the substrate redox potential and the effectiveness of homogeneous catalysis and suggested the presence of an endodermic stage in the process of intramolecular electron transfer with T1 center on T2/TZ copper cluster of highly redox-potential copper-containing oxidases. According to the model proposed by him, the mechanism and efficiency of bioelectrocatalysis depend on the orientation of enzyme molecules on the electrode surface. The orientation of the T1 enzymes center to the electrode surface determines the effective bioelectrocatalytic reduction of molecular oxygen by the mechanism of direct electron transfer [4].

Haibin Yuan, who conducted a comparative analysis of the process of immobilizing laccase on bacterial nanocellulose (BNC), produced by four different strains, showed that different types of BNC-immobilized laccase had different affinity for the substrate, while all of them showed high operational stability after ten consecutive biocatalytic reaction cycles. The results show that the structural diversity of BNC from different strains can directly lead to different efficiencies in the immobilization of laccase, with the white fiber network in the BNC with high porosity particularly effectively promotes the immobilization of the enzyme [36]. Monica Bansal found that the activity of laccase immobilized on nanocellulose fibers remained at 60.5 % even after 15 repeated uses, while the enzyme remained immobilized stable with a relative activity of 75 % after 45 days [6].

Among nanomaterials, carbon nanotubes (CNTs) have unique features as support for the immobilization of the enzyme, that is, with a high surface to volume ratio, a porous structure, and the presence of functional groups on its surface. Linson Lonappan in his research, shows that laccase immobilized on CNTs has a shelf life of three times higher than that of the free enzyme, and notes that regardless of the origin of the substrate, when the initial concentration of laccase in the raw solution increased, the binding capacity and the result, the immobilization efficiency also increased. The same author proposes, in order to increase the efficiency of immobilization, the preliminary functionalization of the substrate with citric acid [22]. Everton Skoronski, using the example of CNT-immobilized laccase from *Aspergillus oryzae*, demonstrated that under stable conditions, the enzyme quickly loses its activity after the second reaction cycle during immobilization using physical adsorption, while using the covalent bond method, about 80 % of the activity remains after six cycles [33].

Meihua Fu, who studied the issues of biodegradation of bisphenol A (BPA), proposes to use the so-called immobilization substrate. Magnetic nanoflowers (MNF) — spherical, porous and hierarchical structures with an average diameter of 15 μm, filled with laccase, by attaching amino functional magnetic nanoparticles to a hybrid laccase-inorganic base. He reports that under optimal conditions in the presence of ABTS, MNF reached 100 % BPA degradation in just 5 minutes. In addition, after 60 days of storage at 4 °C, more than 92 % of the initial activity of the laccase remained. After processing the MNF and their reuse for 5 cycles, only a 5 % decrease in the efficiency of degradation of BFA was observed [11]. Significant results in the field of bisphenol biodegradation are reported by Jakub Zdart, who used the new material based on the sponge *Hippospongia communis* as a biopolymer basis for immobilizing laccase from *Trametes versicolor*. He has shown that under optimal conditions, almost 100 % of BPA and BPF and more than 40 % of BPS are removed from the solution at a concentration of 2 mg/ml. Laccase immobilized in this way has a high reusability and storage stability, retaining more than 80 % of its initial activity after 50 days of storage. In addition, they identified the main biodegradation products BPA and BPF. It was shown that after the oxidation of bisphenols by immobilized laccase, mainly dimers and trimers are formed [37]. Osikoya reports that the adsorption capacity increases significantly with doping of graphene nano-sheets with O, N and Cl atoms. [29] .

Special attention should be paid to the participation of laccase in the soil emission of CO_2 and other greenhouse gases. To mitigate climate change, it is

necessary to reduce or slow down the accumulation of greenhouse gases in the atmosphere by increasing sequestration and storing C in the soil. Carbon sequestration usually refers to medium and long-term (15—50 years) storage of C in terrestrial ecosystems, in underground conditions, mainly in the form of carbonates or in the oceans. The net amount of sequestered C is a long-term balance between absorption and release of C.

Soils have the ability to adapt to the addition of significant amounts of C from the atmosphere through photosynthesis and to isolate it for a sufficiently long time to substantially reduce the accumulation of atmospheric CO_2.

Unlike theories of humic substances (HS) as high molecular weight polymers, recent theories have suggested that HS are supramolecules consisting of associations of small heterogeneous molecules held together not by covalent bonds, but by weak forces, such as dispersive hydrophobic interactions (Van der Waals, π-π, CH-π-binding) and hydrogen bonds in the adjacent hydrophilic and hydrophobic domains, apparently, of high molecular size. This unstable conformation is stabilized by an increase in intermolecular covalent bonds by oxidation enzymes, such as phenol oxidase. It was found that the copper-containing phenol oxidase enzyme, laccase, is produced by soil fungi and mycorrhiza. Laccases are probably the largest class of ligninolytic enzymes in the soil and perform various oxidative and polymeric functions. The enzymes of the first group are mainly involved in the breakdown of lignin, while the latter are mainly involved in the polymerization of soluble phenols, thereby promoting humification and [12].

The data collected in this study suggest a relationship between the amount and expression of the bacterial LMCO (laccase-like multicopper oxidases) genes on the one hand, and the amount and stability of HA with the other. The soils under the vegetation cover are processed by mechanical methods, where, after 30 years of experiments, the highest levels of HA were obtained, showed the maximum population of bacteria rich in laccase genes. In addition, environmental conditions contributed to a corresponding higher level of gene expression in these soils compared to other modes. The structure of the bacterial community based on the LMCO genes also indicates a phylogenetic difference in the SM soils because of the farming system used.

Kwan Meng Go suggests that hydrophilic components, released from the microbial degradation of plant tissues or formed as a result of microbial synthesis, should be gradually sequestered in the hydrophobic humus domains to protect against further degradation. Persistent humic fractions contain mainly aliphatic or alkyl (lipid structures) compounds. Hydrophobic protection is most effective for fractions of silt and clay. However, hydrophobic C sequestration can also occur with larger soil particles.

The stability of the soil as a whole increased and was maintained with time by hydrophobic, but not by hydrophilic components of organic matter. This implies that the total soil stability or stabilization of C can be improved by increasing the hydrophobicity of the native humus or by adding materials, such as organic waste or lignite, with high hydrophobic components.

Several biological mechanisms and processes have also been proposed, but the extent and relative significance of these mechanisms are still unclear. These include the classical model of the formation and organization of aggregates, in which microaggregates are interconnected by roots and fungal hyphae and temporary (polysaccharides) agents, the role of residues of roots and rhizomes of plants, the production of laccase enzymes by white rot and mycorrhiza, a variety of microbial communities and the formation of organic refractory compounds microbiota soil anthropodes. Most of these proposals are at the experimental stage, and there is currently insufficient data to verify and confirm the proposed mechanisms [14].

Asrin Partavian, based on the fact that laccases are central to the decomposition of an inaccessible SOM, suggested that plants and elevated levels of CO_2 stimulate laccase activity. Increased CO_2 levels have amplified the yield of *Deschampsia flexuosa* and underground respiration. Plants stimulated microbial soil biomass, respiration underground and laccase activity, and laccase stimulation caused by plants was particularly noticeable in the soil subjected to prolonged exposure to increased CO_2 in the field, while laccase activity did

not affect the short-term increase in CO_2. Therefore, actively growing plants can stimulate laccase activity, but the potential for plant-induced laccase production seems to depend on the potential for laccase production in the soil. In addition, the initial differences in laccase production potential prevailed during the six-month experimental period regardless of the current level of CO_2, although during this period the productivity of plants increased with an increased level of CO_2. Thus, although laccase activity depends on the presence of a plant, the potential for laccase production does not respond quickly to an increase in plant production [30].

The given data show that the soil laccase — important factor of soil functionality, but they should be investigated in more detail to understand the mechanisms that regulate their activities.

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ИССЛЕДОВАНИЯ ПОЧВЕННЫХ ЛАККАЗ В XXI в.: ОСНОВНЫЕ НАПРАВЛЕНИЯ И ПЕРСПЕКТИВЫ

Лакказы относятся к сине-медным оксидазам, являясь Cu-содержащими ферментами, катализирующими окисление соединений молекулярным кислородом, включая о- и w-дифенолы, аминофенолы, полифенолы, полиамины, арилдиамины, фенольные подструктуры лигнина и некоторые неорганические ионы. Физиологические функции лакказ разнообразны: участие в формировании пигментов и образовании плодовых тел грибов, детоксификация фенолов, катализ окисления нефенольных лигниновых единиц (C_4-этерифицированных) до радикалов.

Активность лакказы возрастает за счет введения Cu^{2+}, Mg^{2+} и Na^+, но сильно ингибируется Fe^{2+}, Ag^+, l-цистеином, дитиотреитолом и NaN_3. В нижних слоях почвы активность лакказы значительное увеличивается при снабжении минеральным азотом. Добавление компоста приводит к повышенной активности в поверхностном слое.

Перспективы практического использования оксидаз расширились после открытия возможности усиления их действия с использованием редокс-медиаторов, представляющих собой субстраты этих ферментов, в процессе окисления которых образуются высоко-редокс-потенциальные и химически активные продукты. Биокаталитические системы, создаваемые путем нанотехнологий (бактериальная наноцеллюлоза, углеродные нанотрубки, магнитные нанобукеты и др.), повышают эффективность реакции за счет увеличения площади поверхности и загрузочной способности и уменьшения сопротивления массо-переноса. Эффективность иммобилизации в значительной степени зависит от условий процесса, свойств фермента и материала носителя. В частности, установлена четкая корреляция между редокс-потенциалом субстрата и эффективностью гомогенного катализа.

Отдельного внимания заслуживает влияние лакказы на почвенную эмиссию CO_2 и других парниковых газов. Участвуя в полимеризации растворимых фенолов, они способствуют гумификации, образуя стойкие гуминовые фракции, связывающие почвенный углерод.

Приведенные данные свидетельствуют о том, что почвенные лакказы — важный фактор функциональности почвы, но необходимо провести дополнительные исследования, чтобы понять механизмы, регулирующие их деятельность.

Ключевые слова: лакказа, ферментативная активность, иммобилизация, эмиссия парниковых газов.

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ДОСЛІДЖЕННЯ ҐРУНТОВИХ ЛАКАЗ У XXI ст. : ОСНОВНІ НАПРЯМИ ТА ПЕРСПЕКТИВИ

Лакази належать до синьо-мідних оксидаз, будучи Cu-вмісними ферментами, котрі каталізують окиснення сполук молекулярним киснем, зокрема о- і w-дифеноли, амінофеноли, поліфеноли, поліаміни, арилдіаміни, фенольні підструктури лігніну та деякі неорганічні іони. Фізіологічні функції лаказ різноманітні: участь у формуванні пігментів і створенні плодових тіл грибів, детоксикація фенолів, каталіз окиснення нефенольних лігнінових одиниць (C_4-етерифікованих) до радикалів.

Активність лаказ значно зростає за рахунок введення Cu^{2+}, Mg^{2+} і Na^+, але сильно інгібується Fe^{2+}, Ag^+, l-цистеїном, дітіотреїтолом та $NaNO_3$. У нижніх шарах ґрунту активність лакази значно збільшується при постачанні мінерального азоту. Додавання компосту спричиняє підвищену активність у поверхневому шарі.

Перспективи практичного застосування оксидаз розширилися після відкриття можливості посилення їх дії з використанням редокс-медіаторів, котрі являють собою субстрати цих ферментів, у процесі окиснення яких утворюються високо-редокс-потенційні та хімічно активні продукти. Біокаталітичні системи, створені шляхом нанотехнологій (бактеріальна наноцелюлоза, вуглецеві нанотрубки, магнітні нанобукети тощо), підвищують ефективність реакції завдяки збільшенню поверхні та завантажувальної здатності та зменшенню опору масо-переносу. Ефективність іммобілізації значною мірою залежить від умов процесу, властивостей ферменту та матеріалу носія. Зокрема встановлено чітку кореляцію між редокс-потенціалом субстрату та ефективністю гомогенного каталізу.

На окрему увагу заслуговує вплив лакази на ґрунтову емісію CO_2 та інших парникових газів. Беручи участь у полімеризації розчинних фенолів, вони сприяють гуміфікації, створюючи стійкі гумінові фракції, які зв'язують ґрунтовий вуглець.

Наведені дані свідчать про те, що ґрунтові лакази — важливий чинник функціональності ґрунту, але необхідно провести додаткові дослідження, щоб зрозуміти механізми, котрі регулюють їх діяльність.

Ключові слова: лаказа, ферментативна активність, імобілізація, емісія парникових газів.