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THE EFFECT OF BISPHENOL A ON THE LIGNIN PEROXIDASE ACTIVITY OF CORYNEBACTERIUM GLUTAMICUM AND MICROCOCCUS LUTEUS

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Bisphenol A (BPA) is a significant industrial component used in the production of plastics, particularly polycarbonates. This study emerges against the backdrop of increasing interest in understanding the environmental risks associated with BPA-induced pollution. BPA has been detected in various environmental media and within the tissues of living organisms. The pollutant causes damage to reproductive organs, the thyroid gland, and brain tissues, particularly during early developmental stages in animals and humans.

It is known that the molecular basis of BPA's destructive effects lies in the suppression of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), as well as in the stimulation of lipid peroxidation and the accumulation of reactive oxygen species (ROS), which adversely affect the antioxidant system and impair mitochondrial function (Coppola, 2023). These processes lead to disruptions in the reproductive, metabolic, and immune systems, as well as neurodevelopmental processes.

Microbial degradation is an effective method for environmental remediation. The key enzymes involved in the transformation of bisphenol A are ligninolytic enzymes, including lignin peroxidase, laccase, and manganese peroxidase (Amaro Bittencourt, 2023).

However, BPA is also biotoxic to microorganisms. It has been established that BPA exerts toxic effects on microorganisms by inhibiting their growth and disrupting metabolic processes. Its suppressive action, like most toxic substances, intensifies with increasing concentration (Park, 2023).

This study used Gram-positive microorganisms C. glutamicum and M. luteus to analyze changes in protein content in the culture medium and lignin peroxidase activity upon the addition of Bisphenol A at a concentration of 7.5 mg/mL. Experimental data show that the presence of BPA in the cultivation medium leads to an increase in lignin peroxidase activity, indicating the adaptive mechanisms of the bacteria to break down the toxic compound. The research also demonstrates that the increase in protein content in microbial cells, caused by the aggressive action of the xenobiotic, may result from the activation of metabolic pathways responsible for detoxification. The obtained results emphasize the importance of further investigation of microbial bioremediation mechanisms, which could serve as the basis for developing effective technologies for the restoration of environments contaminated with Bisphenol A.

Keywords: Bisphenol A (BPA), BPA exposure, chemical pollutants, bioremediation, lignin peroxidase (LiP), xenobiotic.

Introduction. Bisphenol A (BPA), 2,2-bis (4-hydroxyphenyl) propane, is widely used in the production of polycarbonate plastics and epoxy resins, which are utilized in the manufacturing of various consumer goods, such as food containers and water bottles. Additionally, BPA is a component of the inner lining of many canned foods to prevent corrosion. Due to the widespread use of BPA and products containing it, this compound accumulates extensively in the environment, contaminating water, soil, and air. Because of its xenoestrogenic activity, Bisphenol A is classified as an endocrine disruptor. Its presence poses a threat to flora and fauna, as well as to human and animal health, as they are exposed to this substance on a daily basis. Bisphenol A (BPA) can affect the functions of the endocrine system, leading to disruptions in metabolism, the reproductive system, and increasing the risk of cardiovascular diseases and certain types of cancer (Costa, 2024). Pollution hotspots are found in countries with developed electronics industries, where uncontrolled burning and disposal of household and electronic waste contribute to the release of Bisphenol A into the environment (Vasiljevic, 2021). The recorded concentration of Bisphenol A in soil ranges from 0.01 to 1000 μg/kg, in surface waters from 0 to 56 μg/L, and in the atmosphere from 0.004 to 17 ng/m³ (Corrales, 2015). Although Bisphenol A has a relatively short half-life (approximately 4.5 days), it can sometimes degrade for up to 38 days. The degradation process is influenced by factors such as temperature, water composition, pH level, microbiota, and BPA concentration, leading to its pervasive presence due

to ongoing release from plastics and packaging materials (Santos, 2023). Therefore, the issue of pollutant removal from the environment is becoming more significant on a global scale. Among all methods, bioremediation stands out as an effective and environmentally friendly approach to eliminating BPA. Although some microorganisms are capable of biodegrading Bisphenol A, most of the isolated bacteria belong to the genera *Pseudomonas, Sphingomonas,* and *Bacillus* (de Morais Farias, 2022). However, this xenobiotic exerts a suppressive effect on the growth and development of other microorganisms. Microbial extracellular enzymes, such as laccase (EC 1.10.3.2), manganese-dependent peroxidase (EC 1.11.1.13), and lignin peroxidase (EC 1.11.1.14), are crucial components in the degradation of BPA. While there is a significant body of literature on the effects of bisphenol A dissolved in polar solvents on microorganisms, this study focuses on assessing the impact of its undissolved form, applied in powdered form.

Materials and Methods. The subjects of the study were pure cultures of Gram-positive bacteria *Corynebacterium glutamicum*, a rod-shaped bacterium known for its ability to synthesize amino acids, and *Micrococcus luteus*, a spherical bacterium that inhabits water, soil, and air. The inoculum was prepared using a liquid nutrient medium, with bacterial cultures grown in test tubes in a thermostat for 24 hours at 37°C. The primary fermentation was conducted in 250 ml Erlenmeyer flasks with a working volume of 50 ml under identical conditions, supplemented with a 10% inoculum. BPA was introduced into the medium at a concentration of 7.5 mg/mL, which corresponded to local contamination conditions and significantly exceeded the established pollutant levels in the soil. Subsequently, the cultures were centrifuged for 15 minutes at 3000 rpm to separate the cellular biomass and obtain the culture supernatant. The protein content was measured using the Lowry method (Lowry, 1951). The lignin peroxidase activity was determined using a spectrophotometric method based on the oxidation of methylthioninium chloride by the enzyme. The reaction mixture consisted of 2.2 mL of supernatant, 0.1 mL of methylene blue solution with a concentration of 1.2 mM, 0.6 mL of sodium acetate buffer solution (0.5 M, pH 4), and 0.1 mL of 2.7 mM H_2O_2 (Ingale, 2021). The enzymatic activity was assessed by monitoring changes in optical density at a wavelength of 664 nm over time. All experimental procedures were conducted in six replicates, and the resulting data were subjected to statistical analysis using Microsoft Excel software.

Results and Discussion. The presence of Bisphenol A (BPA) in the microbial environment significantly disrupts the growth and development processes of most prokaryotic strains (Tian, 2022). To further understand the impact of the pollutant, we performed an analysis of the protein content in the culture medium and assessed the activity of lignin peroxidase. Based on bioinformatics resources, both *Corynebacterium glutamicum* and *Micrococcus luteus* are known to possess the ability to synthesize and secrete lignin peroxidases, which may play a crucial role in their interaction with xenobiotics such as Bisphenol A. The obtained results demonstrated that both protein content and lignin peroxidase activity increased after the addition of the insoluble form of Bisphenol A in the form of powder at a concentration of 7.5 mg/mL to the liquid nutrient medium on the 5th day of cultivation. The conducted studies revealed a significant increase in the total protein content in the medium for both microorganisms under the influence of Bisphenol A. In particular, in *Corynebacterium glutamicum*, the protein content increased by 60% compared to the control values. In *Micrococcus luteus*, an increase in total protein content was also observed, rising by 51% compared to the control level. These results can be explained by the lipophilicity of Bisphenol A (logP = 3.6). This compound is capable of penetrating lipid membranes of cells, accumulating within them, and forming clusters. This leads to the disruption of molecular interactions at the phase boundaries, causing membrane fluidization. As a consequence of these alterations in membrane structure, lipid extraction may occur, leading to the formation of pores that compromise the integrity of cellular structures (Hąc-Wydro, 2019). These modifications can increase membrane permeability and trigger protective mechanisms, including the upregulation of membrane protein synthesis, stress proteins, and exoenzymes. This, in turn, facilitates an increase in the overall protein content within the culture medium. Since a key aspect of pollutant biodegradation involves the reduction or complete elimination of toxic by-products, the activity of one of the key enzymes involved in bisphenol A (BPA) degradation was examined. Lignin peroxidase (LiP), which is produced extracellularly, plays a vital role in the breakdown of lignin and other environmental contaminants.

As shown in the data presented in Figure 1, a marked increase in lignin peroxidase (LiP) activity was observed in the culture medium of both bacterial strains. Specifically, in *Corynebacterium glutamicum*, enzyme activity increased by 3.5-fold compared to the control, whereas in *Micrococcus luteus*, the increase did not exceed three times the control values. This suggests a differential enzymatic response between the two strains, likely related to their inherent metabolic pathways and

adaptation mechanisms to BPA-induced stress. Although most studies focus on fungi, bacteria are also capable of producing lignin peroxidase (LiP) and participating in the degradation process of bisphenol A (BPA) (Grgas, 2023). The high enzyme activity observed in the medium can be attributed to several factors. BPA, being a phenolic compound, provides an ideal substrate for lignin peroxidase (Lee, 2019). This enzymatic activity, utilized by prokaryotes, serves as an adaptive mechanism to cope with hostile environmental conditions and prevent the formation of reactive oxygen species. From a bioremediation perspective, this phenomenon can aid in the detoxification and environmental cleanup processes, particularly in ecosystems contaminated by pollutants like BPA.

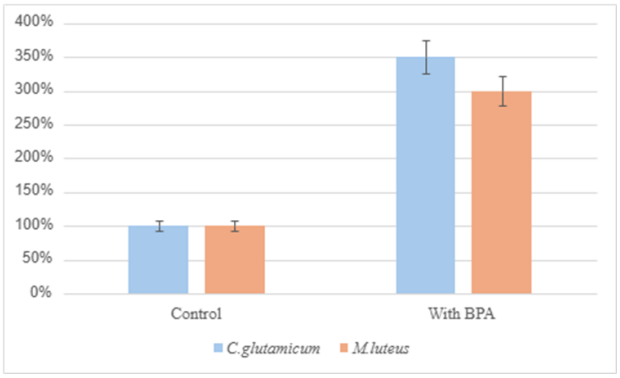

Fig. 1. Lignin peroxidase activity of Micrococcus luteus and Corynebacterium glutamicum in the presence of BPA (7.5 mg/ml) in the culture medium

Conclusions. This study revealed that bisphenol A (BPA) exerts toxic effects on the microorganisms *Corynebacterium glutamicum* and *Micrococcus luteus*. The pollutant induces cell wall disruption, upregulates protein synthesis, and stimulates the production of lignin peroxidase, a key enzyme involved in BPA degradation and mitigation of its harmful properties. These findings underscore the potential of the investigated bacterial strains in bioremediation processes aimed at mitigating BPA contamination.

Conflict of Interest: *The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.*

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ВПЛИВ БІСФЕНОЛУ А НА ЛІГНІНПЕРОКСИДАЗНУ АКТИВНІСТЬ CORYNEBACTERIUM GLUTAMICUM ТА MICROCOCCUS LUTEUS

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Бісфенол А (BPA) є важливим індустріальним компонентом, що використовується у виробництві пластмас, зокрема полікарбонатів. Це дослідження розгортається на тлі зростаючого інтересу до розуміння екологічних ризиків, пов'язаних із забрудненням навколишнього середовища, викликаним BPA. BPA було виявлено в різних середовищах довкілля та тканинах організмів. Полютант зумовлює пошкодження репродуктивних органів, щитоподібної залози та мозкових тканин, особливо на ранніх етапах розвитку тварин та людини. Відомо, що молекулярна основа руйнівної дії бісфенолу А полягає у зниженні активності антиоксидантних ферментів, таких як супероксиддисмутаза (SOD), каталаза та глутатіонпероксидаза (GSH-Px), а також у стимулюванні пероксидного окислення ліпідів і накопиченні активних форм кисню (АФК), що негативно впливає на антиоксидантну систему і погіршує функціонування мітохондрій (Coppola, 2023). Це спричиняє порушення функціонування репродуктивної, метаболічної та імунної систем, а також процеси нейророзвитку. Мікробна деградація є ефективним методом рекультивації навколишнього середовища. Найважливішими ензимами перетворення бісфенолу А є лігнінолітичні ферменти такі як лігнін пероксидаза, лакказа та манганпероксидаза (Amaro Bittencourt, 2023). Однак BPA також є біотоксичним для мікроорганізмів. Було встановлено, що BPA чинить токсичний вплив на мікроорганізми, інгібуючи їхній ріст і порушуючи метаболічні процеси. Його пригнічувальна дія, як і у більшості токсичних речовин, посилюється зі збільшенням концентрації (Park, 2023).

У цьому дослідженні використовували грампозитивні мікроорганізми C.glutamicum і M.luteus з метою аналізу зміни вмісту білка в культуральному середовищі та активності лігніннероксидази за умови внесення в середовище Бісфенол А в концентрації 7,5 мг/мл. Експериметальні дані показують, що присутність BPA в середовищі культивування спричиняє підвищення активності лігніннероксидази, що свідчить про адаптаційні механізми бактерій для розщеплення токсичної сполуки. Це дослідження також демонструє, що збільшення вмісту білка в клітинах мікроорганізмів, обумовлене агресивним впливом ксенобіотика, може бути результатом активізації метаболічних шляхів, що відповідають за детоксикацію. Отримані результати підкреслюють необхідність подальшого вивчення мікробних механізмів біоремедіації, що може стати основою для розробки ефективних технологій очищення навколишнього середовища контамінованого Бісфенолом А.

Ключові слова: Бісфенол А (BPA), вплив BPA, хімічні забруднювачі, біоремедіація, лігніннероксидаза (LiP), ксенобіотик.

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