SUMMARY

of the doctoral thesis entitled:

SELECTION AND CHARACTERIZATION OF MICROBIAL STRAINS PRODUCING METABOLITES USEFUL IN BIOTECHNOLOGICAL PROCESSES

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KEYWORDS: L-asparaginase, plant protection, antifungal activity, mycotoxins, biofilm

Most microorganisms play an important role in the environment, contributing to various industries such as food, medical, and agriculture. Many microorganisms are capable of producing valuable biologically active compounds, including enzymes.

Enzymes are protein structures found in the human and animal body, in plants, and in microorganisms. These compounds can catalyze reactions that underpin normal metabolic functions.

L-asparaginase plays an important role in medicine. It is considered one of the most studied enzymes, with the current focus on new sources that can produce it in the purest form possible.

Theoretical research is outlined in two chapters addressing topics related to L-asparaginase and obtaining biologically active compounds important in agriculture.

In the first chapter, this paper focuses on the theoretical and practical aspects of L-asparaginase. This enzyme is particularly important for the medical field as it helps treat various neoplastic diseases and is successfully used in chemotherapy regimens. Its utility is very high, and global demand is continuously increasing.

In practice, L-asparaginase is produced by the bacterium *Escherichia coli*, but a major disadvantage of this method is that the resulting L-asparaginase enzyme produces both beneficial effects and serious side reactions. These adverse reactions are due to the enzyme not being produced in a pure form, resulting in contamination with other similar enzymes.

In addition to its great utility in medicine, L-asparaginase is also used in the food industry. This enzyme helps reduce Maillard compounds formed in starchy food products. It reduces acrylamide, a toxic compound that forms in carbohydrate-rich foods cooked at high temperatures. The enzyme's importance has been highlighted in various heat-treated food products. In some trials, the enzyme maintained its activity after several cycles of use, demonstrating its potential for wide-scale industrial applications.

Theoretical research highlights that L-asparaginase is produced mainly by bacteria but also by some plants. This enzyme, isolated from different sources, has specific physicochemical and kinetic parameters, with the one closest to the form secreted by *Escherichia coli* being selected. These theoretical studies strengthen the idea that microbial sources have promising characteristics for obtaining a purer enzyme.

The second chapter of theoretical research addresses the importance of different microorganisms producing L-asparaginase in agriculture. This field faces several issues mainly due to climate change.

One of the important aspects helping agriculture is obtaining antimicrobial compounds with the help of microorganisms. These compounds aim to reduce or even eliminate plant contaminants.

A series of studied compounds have demonstrated strong antifungal and antibacterial activities over time, suggesting their potential for use in plant biocontrol. These activities are crucial for developing eco-friendly alternatives to traditional chemical pesticides.

Personal contributions are outlined in two chapters based on all theoretical research results. The first chapter's research objectives included: 1) Isolating new microorganisms capable of producing L-asparaginase free from other contaminating compounds; 2) Evaluating the isolated microorganisms' ability to produce the enzyme of interest using both qualitative and quantitative methods.

The first part of the work provides a foundation for understanding the action mode, obtaining methods, and importance of L-asparaginase, while the practical part validates the potential of the tested microorganisms to produce this enzyme.

Specific objectives included evaluating the possibilities of obtaining L-asparaginase in the highest possible quantity from new biological sources without containing other contaminating enzymes.

Tested microorganisms were from both the Biotechnology Faculty's collection and new isolates from soil. They were cultivated on specific culture media to assess their potential to produce the enzyme of interest. Experiments were complemented by quantifying enzyme production using a spectrophotometric method.

During practical experiments in chapter three, bacteria from the *Streptomyces* sp. genus, rhizobacteria, endophytic bacteria, yeasts, and fungi were tested for obtaining L-asparaginase free from glutaminase, urease, or $NaNO_3$. The selected microorganisms ability to produce the enzyme of interest was evaluated using both rapid qualitative and quantitative methods. These methods allowed a more detailed assessment of each microbial strain's production potential. Results were standardized following dynamic enzymatic quantification over several days, showing that the highest enzymatic quantity was produced by the *Streptomyces* (2 strains), *Bacillus* (4 strains), endophytic bacteria (2 strains), and new soil bacterial isolates GM1, GM3, and GM18.

The second part of the research started from the first practical chapter's results, focusing on evaluating the potential applications of microbial strains producing biomedical enzymes in plant protection. This led to two secondary objectives: 1) Determining the selected bacterial strains' ability to produce metabolites inhibiting

phytopathogen development; 2) Testing the plant growth and development stimulation properties of the selected strains.

In addition to producing L-asparaginase, chapter four evaluated the selected strains for their ability to produce compounds inhibiting phytopathogen development and aiding plant growth stimulation. These studies pave the way for using these microorganisms in agriculture, contributing to healthier and more productive crops.

Selected bacterial strains were tested for their antimicrobial activity against common plant pathogens. Results showed that certain strains, especially those from the *Bacillus* sp. genus, exhibited significant antimicrobial activity. These strains inhibited the growth of pathogens such as *Fusarium* graminearum, a wheat pathogen, by producing biologically active compounds with antifungal effects. *Bacillus* sp. GM3 showed strong antifungal activity, inhibiting the growth of all tested fungi, with effects lasting throughout the incubation period.

Microorganisms antifungal activity is attributed to their natural properties that enable the production of various compounds. These compounds act on fungi through different mechanisms, including nutrient competition, antibiosis (biosurfactant and/or antibiotic production), and hydrolytic enzyme synthesis. Additionally, some microorganisms can stimulate plant growth by synthesizing phytohormones, producing siderophores, fixing nitrogen, solubilizing phosphate, and producing secondary metabolites with complex effects. The genetic evaluation of the GM3 bacterial strain's ability to produce biosurfactants showed that it possesses the *ituD*, *fen*, *bmyA*, and *ituA* genes responsible for synthesizing valuable agricultural compounds.

Determining the bacterial strains ability to produce antifungal metabolites suggested that the selected strains could decompose fungal cell walls by secreting hydrolytic compounds (proteases, chitinases, cellulases), with good production observed in the GM3, GM12, and GM21 strains. Additionally, the impact of the GM3 bacterial strain on mycotoxin production was studied, revealing that it significantly reduces zearalenone and ochratoxin levels.

Chapter four also focused on the selected bacteria's biofilm formation, highlighting their ability to colonize plant roots and protect them against stress. Experiments showed that the tested bacteria could form strong biofilms, enhancing their colonization and plant protection capacity. This ability is crucial for the biotechnological application of these bacteria in agriculture, ensuring an efficient and durable symbiosis between bacteria and plants.

The tested bacterial strains not only contributed to plant protection but also improved plant germination and development rates. Another important research aspect was evaluating the bacterial treatment's impact on plant germination and development. Wheat seeds treated with the GM3 bacterial strain germinated quickly (95% rate) and produced vigorous plants, while those treated with the *Fusarium gram*in*earum* pathogen showed a lower germination rate (61.6%) and weak plants. These results underline the selected bacteria's potential to stimulate plant growth and protect them against pathogens. The *Bacillus* sp. GM3 strain can be considered a good option for

fungal pathogen biocontrol and plant growth stimulation, offering a sustainable alternative to chemical fungicides and promoting ecological agriculture.

Based on the obtained results, the *Bacillus* sp. GM3 strain is considered a promising candidate for plant protection applications. However, further information is needed on the mechanisms involved in its antifungal activity and biostimulation potential.

All research focused on highlighting the potential of microbial strains to produce high-purity L-asparaginase with promising applications in the medical field and using these strains in plant protection. These results emphasize the importance of isolating and characterizing microorganisms in detail to maximize biotechnological benefits.

At the end of the work, general conclusions from all chapters are presented, summarizing the novelty elements and a series of recommendations for future research. Future research can focus on optimizing the production and purification processes of L-asparaginase, leading to a high-yield and efficient product.

Overall, the recommendations highlight the biotechnological potential of the selected bacterial strains producing L-asparaginase for plant protection and growth stimulation. The need for continued research is explicit to better understand the involved mechanisms and optimize the application of these microorganisms in agriculture.