# UNIVERSIDADE DE SÃO PAULO

## Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto

Departamento de Química

Programa de Pós-Graduação em Química


**“Identificação e isolamento de fitotoxinas produzidas por actinobactérias isoladas da Caatinga”**


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# RESUMO

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Palavras chave: Espectrometria de massas; Produtos naturais; Fitotoxina; Albociclina; Actinobactéria; Caatinga.

As actinobactérias são uma reconhecida fonte de metabólitos secundários bioativos com grande diversidade estrutural e funcional. Dentre elas, o gênero *Streptomyces* é sem dúvida o mais explorado, correspondendo a cerca de 75% dos metabólitos secundários estudados, e por dois terços dos antibióticos conhecidos. Dessa forma, ainda é crescente a necessidade de novos herbicidas com perfil de baixa toxicidade ambiental e novos mecanismos de ação, para a substituição de produtos que são utilizados extensivamente há anos. Grandes avanços tecnológicos têm sido alcançados em termos de instrumentação analítica nas últimas décadas, tornando a espectrometria de massas a técnica mais eficiente e robusta na identificação de metabólitos secundários, principalmente quando acoplada a técnicas cromatográficas de ultra eficiência (UHPLC-MS). Nessa dissertação foi explorada a diversidade metabólica de actinobactérias isoladas do bioma Caatinga para a identificação de metabólitos secundários com atividade fitotóxica. Foram produzidos 166 extratos brutos de actinobactérias, das quais, 27 apresentaram algum nível de atividade fitotóxica contra *Lemna minor*. Devido pronunciada bioatividade (MIC = 6,25 µg) o extrato bruto da actinobactéria, CAAT 7-52, foi selecionado para desreplicação, caracterização e fracionamento guiado por bioensaios, e, ao mesmo tempo, um estudo taxonômico foi realizado para determinar o posicionamento filogenético do novo isolado. A análise da sequência do gene 16S rRNA suportou a classificação da linhagem no gênero *Streptomyces* e mostrou que CAAT 7-52 formou uma linha filética distinta junto com a linhagem tipo de *Streptomyces actinomycinicus*, com um valor de identidade da sequência de 99,0%. No extrato bruto de CAAT 7-52 foi identificada a Albociclina como responsável pela atividade fitotóxica (MIC = 3,12 µg), assim como alguns compostos análogos. O monitoramento da massa micelial seca e produção de Albociclina mostrou uma maior produção do composto ativo aos 21 dias de crescimento. Esse extrato foi submetido a ensaios de inibição de germinação, onde apresentou atividade contra sementes de buva (*Conyza canadensis*) (MIC = 12,5 µg), alface (*Lactuca sativa*) e rúcula (*Eruca sativa*). Variações no meio de cultivo mostraram uma produção seletiva de Albociclina nos meios de cultivo PMB e Czapeck-GL. Ensaios de fitotoxicidade contra *Lemna minor*, utilizando o fermentado do meio PMB sem extração com solventes orgânicos, mostrou atividade em uma diluição de 20%. Portanto, foi possível demostrar através desse estudo o grande potencial das actinobactérias na produção de metabólitos secundários bioativos e da espectrometria de massas como técnica analítica na identificação dos compostos ativos.

## ABSTRACT

FERREIRA JUNIOR, Osvaldo Luiz.: **Identification and isolation of phytotoxins produced by actinobacteria isolated from Caatinga.** 2018, 109 f. Dissertação de mestrado. Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2018.



Keywords: Mass spectrometry; Natural products; Phytotoxin; Albocycline; Actinobacteria; Caatinga.



Actinobacterias are a well know source of bioactive secondary metabolites with great structural and functional diversity. Among them, the *Streptomyces* genus is certainly the most explored, corresponding to about 75% of studied secondary metabolites, and for two thirds of known antibiotics. Thus, there is still a growing need for new herbicides with low environmental toxicity e new modes of action, for the replacement of products that have been broadly used for years. Great technological progress has been achieved in analytical instrumentation in the last few decades, making mass spectrometry the most efficient and robust technique to identify secondary metabolites, mainly when accoupled to ultra-performance chromatographic techniques (UHPLC-MS). In this essay, was explored the metabolic diversity of the actinobacterias isolated from the Caatinga biome to identification of secondary metabolites with phytotoxic activity. Were produced 166 crude extracts from actinobacterias, from this, 27 showed some phytotoxic activity against *Lemna minor*. Due to noticeable bioactivity (MIC = 6,25 µg) the crude extract from actinobacteria CAAT 7-52, was selected to dereplication, characterization and bioassay guided fractionation, and, at the same time, a taxonomic study was carried out to determinate the phylogenetic location of the new isolated. The analysis of 16S rRNA gene sequence supported the lineage classification on the genus *Streptomyces* and established that CAAT 7-52 assembled a distinct phyletic line together with the lineage type of *Streptomyces actinomycinicus*, with an identity sequence value of 99,0%. In the crude extract CAAT 7-52, Albocycline was identified as the responsible for the phytotoxic activity (MIC = 3,12 µg), so as some analogous compounds. The mycelial dry mass and Albocycline production monitoring revealed a major production of the active compound at 21 days of growth. This extract was subjected to germination inhibition assays, where revealed activity against horseweed (*Conyza canadensis*) (MIC = 12,5 µg), lettuce (*Lactuca sativa*) and arugula (*Eruca sativa*) seeds. Variations in the culture media showed a selective production of Albocycline in the PMB and Czapeck-GL culture media. Phytotoxicity assays against *Lemna minor*, applying the fermented PMB media without organic solvents extractions, revealed activity with a 20% dilution. Therefore, through this study was able to demonstrate the great potential of the actinobacterias in the production of bioactive secondary metabolites and the mass spectrometry as analytical technique to identification of the active compounds.

## CONCLUSÃO

De um total de 166 extratos brutos obtidos, 27 (16%) apresentaram algum nível de atividade fitotóxica com *Lemna minor*. Dentre eles, o extrato CAAT 7-52 (MIC = 6,25 µg) apresentou atividade fitotóxica mais pronunciada, o qual foi submetido ao fracionamento guiado por bioensaio para desreplicação dos compostos ativos por espectrometria de massas, onde foi observada a presença de dois compostos ativos os quais foram caracterizados como sendo o ácido 3-hidroxibenzóico presente na fração 15, e a Albociclina presente na fração 30 (MIC = 3,12 µg). Ambos os compostos não possuem relatos na literatura sobre possuírem atividade fitotóxica. O extrato CAAT 7-52 também apresentou atividade de inibição de germinação contra sementes de alface, rúcula e buva, todas dicotiledôneas, esta última sendo a mais promissora em razão de seu difícil controle. A actinobactéria CAAT 7-52 foi identificada através do sequenciamento do gene 16S rRNA apresentando um valor de identidade de sequência de 99% com *Streptomyces actinomycinicus*. Também foi verificada a atividade do meio de cultivo fermentado, sem extração com solventes orgânicos, o qual apresentou atividade fitotóxica até uma proporção de 20%, sendo de interesse em razão de possível utilização em teste para controle direto de plantas-daninhas. Ocorre a produção seletiva da albociclina quando utilizado o meio de cultivo PMB e Czapeck com xarope de glicose e a produção seletiva dos análogos da Albociclina em meio de cultivo ISP-2, sendo que ambos os extratos apresentaram atividade fitotóxica significativa. A partir do monitoramento da massa micelial e da produção de Albociclina, foi apresentado um máximo de produção de Albociclina em torno de 21 dias. Foram identificados os compostos indol-3-carboxaldeído, ácido benzacético e benzacetamida nas frações 14, 15 e 22 do extrato AM 33-22, os quais apresentaram atividade fitotóxica em grau moderado contra *Lemna minor*. Ainda que mais experimentos e otimizações necessitem ser realizados, tais resultados mostram o enorme potencial de aplicação biotecnológica das actinobactérias e de seus metabólitos secundários, como os obtidos nesse trabalho.

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