PRELIMINARY STUDY ON FERMENTATION OF *Acremonium chrysogenum* AIMING AT PRODUCTION OF ANTIMICROBIAL SUBSTANCES

Kátia G. Corrêa[1,2], Cynthia Mantovani[2,3], Ingrid B. Pinto[2,3], Adilson Beatriz[2,3] & Janaína S. Ferreira*[1,2]

[1]*Curso de Engenharia Química/Universidade Federal de Mato Grosso do Sul, Instituto de Química. Campo Grande, MS, Brazil.*
[2]*Centro de Pesquisa e Inovação em Bioprospecção e Síntese de Produtos para a Saúde Humana e Animal – CIBSint, Instituto de Química, Universidade Federal de Mato Grosso do Sul. Campo Grande, MS, Brazil.*
[3]*Programa de Pós-Graduação em Biotecnologia, Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição – FACFAN, Universidade Federal de Mato Grosso do Sul. Campo Grande, MS, Brazil.*
** Corresponding author's email address: ferreira.janaina@ufms.br*

ABSTRACT

Antimicrobial resistance (AMR) poses risks to public health, driven by factors such as the inappropriate use of antibiotics. Endophytic fungi offer a potential solution by producing bioactive compounds, such as *Acremonium chrysogenum*. The aim of this study was to investigate antimicrobial activities of this fungus from fermentation. Over 21 days, *A. chrysogenum* exhibited growth with concurrent consumption of the culture medium throughout all days. Agar Spot tests revealed antimicrobial activity, and this was further emphasized by MIC assays demonstrating activity against both gram-positive and gram-negative bacteria. These results demonstrate the potential of *A. chrysogenum* in combating AMR and provide crucial insights for optimization the fermentation parameters with design of experiments aiming production in bioreactors (scale-up).

Keywords: *Acremonium chrysogenum*. Fermentation. MIC. Antimicrobial resistance.

1 INTRODUCTION

Antimicrobial resistance (AMR) has become an increasing threat to human and animal health. AMR arises when microorganisms (viruses, bacteria, fungi, and parasites) develop mechanisms to resist the effects of drugs used to treat them.[1] Several factors can cause AMR, including the excessive or incorrect use of drugs, the spread of resistant organisms among humans or animals, and genetic changes in individual organisms.[1,2,3]

Therefore, there is an urgent need for the development of new antibacterial agents. In this context, endophytic fungi are considered reservoirs of various bioactive compounds and may be a promising alternative in combating infections caused by resistant microorganisms.[4]

These natural compounds exhibit specific pharmacological characteristics, including antimicrobial, antifungal, anti-inflammatory, antioxidant, and anticancer activities.[5] Among antibiotic-producing fungi, the genera Penicillium and Acremonium are noteworthy, with species such as *Penicillium griseofulvum*, *Penicillium chrysogenum*, and *Acremonium chrysogenum* being particularly significant industrially for the production of antibiotics like griseofulvin, penicillins, and cephalosporins, respectively.[6] The cephalosporins used in clinical practice are semi-synthetic derivatives of 7-ACA, which is derived from cephalosporin C produced by *Acremonium chrysogenum*. Semi-synthetic penicillins and cephalosporins continue to be of interest to many research groups seeking to discover new antibiotics capable of combating super bacteria.[7]

Hence, this study aimed to investigate the fermentation and antimicrobial activity of the fungus *Acremonium chrysogenum* as starting points for future optimization of its fermentation a design of experiments in bioreactors, with the goal of producing compounds with antibiotic properties.

MATERIAL & METHODS

Fermentation: The fermentation of the fungus was carried out in three Erlenmeyer flasks containing 250mL of potato dextrose medium each at 140 rpm and 29°C on the shaker. Over 21 days, 3mL of fermentation from each Erlenmeyer flask were collected daily, divided into 3 tubes of 1.5mL each (previously weighed). The tubes were centrifuged for 10 minutes at 13,400rpm, and the supernatants were collected and transferred to new tubes. The masses of the remaining fungi in the tubes were washed with water to remove the medium, and then dried in ovens at 37°C for 24 hours. After drying the masses, the tubes were weighed again, and the weight of the empty tube was subtracted from this value to measure the dry mass of the fungus. The sugar concentration was monitored through Brix using a digital refractometer.

Agar Spot test: The Spot test was conducted following[8] with modifications. In brief, the spot tests were conducted on disposable petri dishes containing Mueller Hinton agar (MHA) medium. In the MHA medium, 10µL of the supernatant was spotted.

Inhibition was tested on two strains *of Escherichia coli bacteria* (one susceptible and one resistant) and two strains of *Staphylococcus epidermidis* bacteria (both resistant) at a dilution of 1:50 in 0.6% soft MHA medium, which was poured over the MHA MEDIUM to form a film containing the bacteria. The plates were then incubated in ovens at 37°C for 18 hours until the inhibition halos were observed.

MIC (Minimum Inhibitory Concentration): A minimum inhibitory concentration (MIC) against pathogenic bacteria was determined using the broth microdilution method according to the Clinical Laboratory Standards Institute (CLSI-2013) in 96-well microplates (polystyrene; Kasvi® model: K30-5096P). In summary, pathogenic bacteria were grown in 5 mL of Mueller-Hinton broth (MHB) with agitation at 200 rpm at 37 ºC overnight. Subsequently, the bacteria were diluted 1:50 (v/v) in MHB until the optical density (O.D.) reached half of the exponential phase. After reaching the required O.D., 50 µL aliquots of these bacteria at 5×10^5 CFU/mL^{-1} were added to the microplate containing cell-free supernatants from *A. chrysogenum* fungus at dilutions ranging from 1.56% to 50% or ciprofloxacin/ampicillin at concentrations from 4 to 128 µg/mL^{-1} and incubated at 37 °C for 18h. Following the incubation period, inhibition of bacterial growth was assessed in a microplate reader (Biochrom EZ Read 400) at 600 nm. Positive and negative controls were determined by bacterial growth and bacteria-free culture medium, respectively. MIC was defined as the lowest concentration of each supernatant capable of completely inhibiting bacterial growth. Experiments were performed in triplicate.

2 RESULTS & DISCUSSION

Fermentation: The fungus exhibited growth over the 21 days of cultivation, starting with a Brix of 2.5 and ending with a Brix of 0.5, indicating slow and continuous consumption of the medium. This consumption was accompanied by a steady increase in the dry mass of the fungus, which started with an average of 0.0004g and ended with 0.0084g (Figure 1).

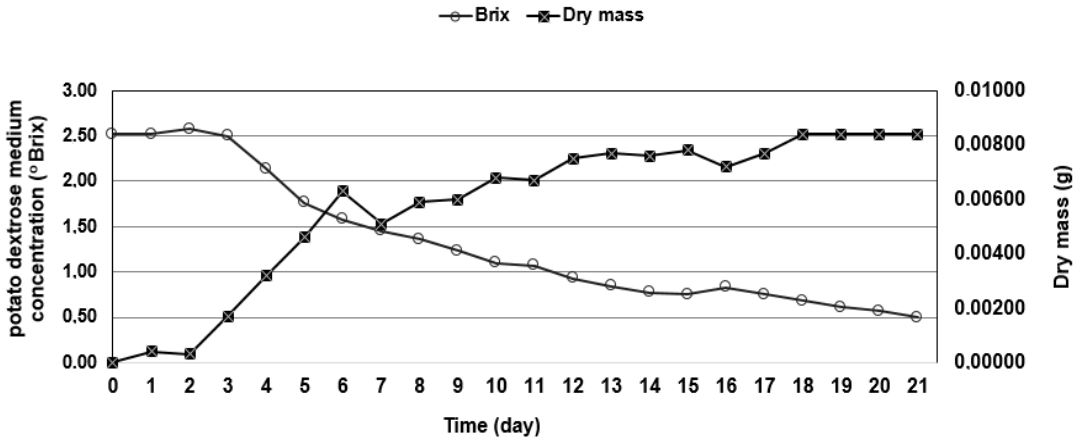

Figure 1 Graph demonstrating the continuous and slow consumption of the medium as measured daily by Brix and continuous increase in the dry mass of the fungus.

Spot test: In the spot tests, inhibition halos were observed in both strains of *S. epidermidis* from day 6 onwards. The halos intensified between days 8 and 12, which is why days 8, 10, and 12 were chosen for MIC determination. This result demonstrates that the fungus produces and releases molecule(s) that have strong activity against gram-positive bacteria (Figure 2)

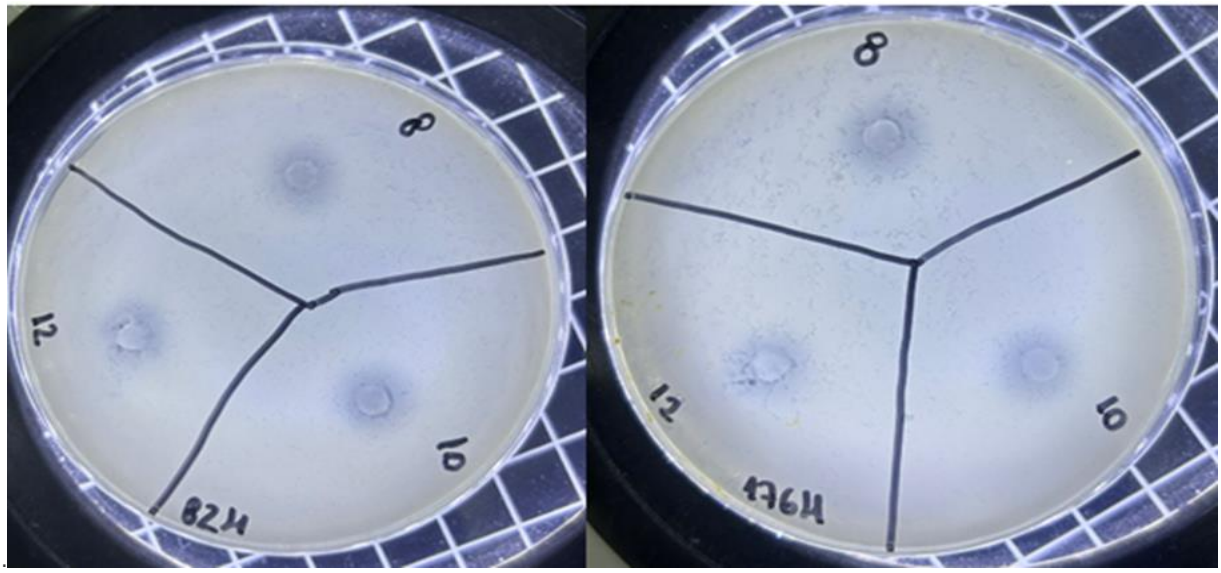

Figure 2 Image showing inhibition halos against two resistant strains of S. epidermidis in the supernatants from days 8, 10, and 12.

MIC: Despite no inhibition halo being observed in the spot test for gram-negative bacteria (E. coli), the MIC allowed the identification of inhibitory action in these bacteria as well, possibly due to the technique's increased sensitivity. The inhibition was most effective in E. coli 34 (resistant), with a stronger action observed with the supernatant from day 8. Regarding the results with gram-positive strains, the inhibitory action even surpassed that of the antibiotic (ampicillin) with the supernatant from all three days tested (Figure 3).

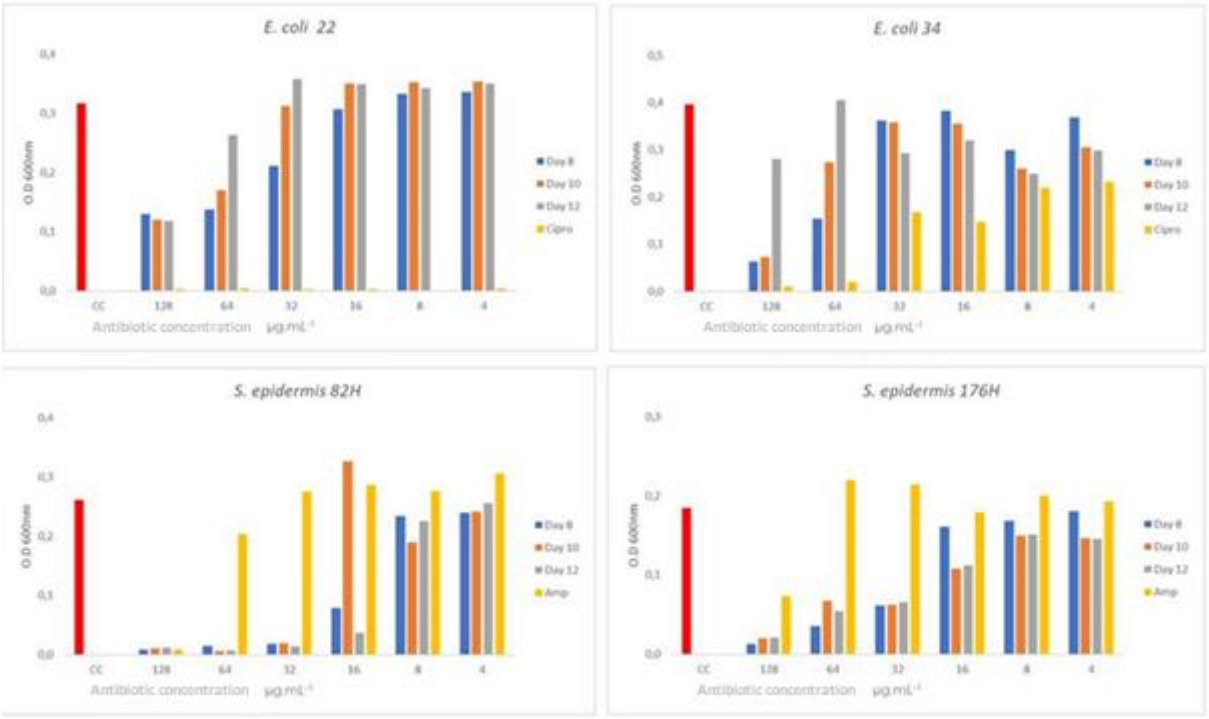

Figure 3 MIC results showing the inhibition of supernatants from days 8, 10, and 12 against the four bacterial strains tested in this study.

*CIPROFLOXACIN (CIPRO) / AMPICILLIN (AMP)

3 CONCLUSION

Through the fermentation of the fungus *A. chrysogenum*, it was observed that it showed slow and steady growth during the experimental period of 21 days. Regarding the inhibitory action tested in the four bacterial strains, the supernatants from the collections of days 8, 10 and 12 showed a strong action, demonstrating high antibacterial potential. These preliminary results show the capacity of using this fungus for the production of antibiotics. A more advanced study with the design of experiments for optimize the parameters of fermentation will confirm the investment for bioreactor (scale-up).

REFERENCES

1 Morrison, L., & Zembower, T. R. (2020). Antimicrobial resistance. Gastrointestinal Endoscopy Clinics, 30(4), 619-635.
2 Levy, S. B., & Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nature medicine, 10(Suppl 12), S122-S129
3 McEwen, S. A., & Collignon, P. J. (2018). Antimicrobial resistance: a one health perspective. Antimicrobial resistance in bacteria from livestock and companion animals, 521-547.
4 Mishra, V. K., Passari, A. K., Chandra, P., Leo, V. V., Kumar, B., Uthandi, S., ... & Singh, B. P. (2017). Determination and production of antimicrobial compounds by Aspergillus clavatonanicus strain MJ31, an endophytic fungus from Mirabilis jalapa L. using UPLC-ESI-MS/MS and TD-GC-MS analysis. PloS one, 12(10), e0186234.
5 Kusari, S., Hertweck, C., & Spiteller, M. (2012). Chemical ecology of endophytic fungi: origins of secondary metabolites. Chemistry & biology, 19(7), 792-798.
6 Li, M., Yu, R., Bai, X., Wang, H., & Zhang, H. (2020). Fusarium: a treasure trove of bioactive secondary metabolites. Natural product reports, 37(12), 1568-1588.
7 Beatriz, A., Mondino, M.G., de Lima, D.P. (2022). Lactams, Azetidines, Penicillins, and Cephalosporins: An Overview on the Synthesis and Their Antibacterial Activity. In: Ameta, K.L., Kant, R., Penoni, A., Maspero, A., Scapinello, L. (eds) N-Heterocycles. Springer, Singapore. https://doi.org/10.1007/978-981-19-0832-3_3
8 Gaudana, S. B., Dhanani, A. S., & Bagchi, T. (2010). Probiotic attributes of Lactobacillus strains isolated from food and of human origin. British Journal of Nutrition, 103(11), 1620-1628.

ACKNOWLEDGEMENTS

The authors acknowledge the Brazilian funding agencies FUNDECT (Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil) – Finance Code 001, as well as the Federal University of Mato Grosso do Sul, for financial support.