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SECONDARY METABOLITES FROM *DIPLODIA MAYDIS* AND *SCLEROTIUM ROLFSII* WITH ANTIBIOTIC ACTIVITY

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SHORT COMMUNICATION

ABSTRACT

Ethyl acetate extracts of the phytopathogenic fungi *Diplodia maydis* and *Sclerotium rolfsii* presented antibacterial activity against multidrug resistant bacteria isolated from patients in the University Hospital (HUOP-Unioeste, Brazil).

Key words: *Diplodia maydis*, *Sclerotium rolfsii*, multidrug resistance, phytopathogenic fungi, secondary metabolites

Biotechnology consists in the use of cellular systems for the development of processes and products holding economical and social interest. Within these systems, the fungi are of great biotechnological interest in fermentative processes that culminate in the production of secondary metabolites that are important for search of new antibiotics. The discovery and development of antibiotics was one of the most significant advances in medicine in the 20th century. Nevertheless, many antibacterial agents that were used to treat a variety of human infectious diseases are now ineffective. Therefore, to ensure that effective drugs will be available in the future, it is necessary to improve the antibacterial use patterns and to devise strategies to identify new antibiotics through previously unexplored targets (5). As part of an ongoing research for biological active secondary metabolites from phytopathogenic fungi (1,2) we have detected antibacterial activity in fermentation ethyl acetate extracts of *Diplodia maydis* and *Sclerotium rolfsii* fungi that were isolated from culture of mays and soya, respectively. They were evaluated against twelve Gram-negative bacteria and four Gram-positive bacteria using the bioautography assay (3,4). The fungi were obtained at the Phytopathology Department of the Universidade Estadual do Oeste do Paraná, PR, Brazil. The production was carried out by inoculating 10^8 spore/mL in BDA medium at 28ºC in a shaking incubator at 150 rpm for 168h. The cultures were filtered and submitted to the process of liquid-liquid partition furnishing the ethyl acetate extract that were c oncentrated by rotaevaporation. For the antibacterial assay 2.0 µg/mL of the extracts were applied to pre-coated TLC plates, without elution of the samples. The inoculum was prepared by culturing each organism on tryptone soya agar medium (TSA, Oxoid) at 37ºC to turbidity equivalent to McFarland 0.5 standard (1.5 x 10^8 CFU/mL). One microliter of each diluted inoculum (10^4 - 10^6 CFU) was applied onto Mueller Hinton Agar medium (MHA-DIFCO), and distributed over TLC plates (2x4 cm). After solidification of the media, the TLC plates were incubated overnight at 37ºC. Ciclopirox olamine was used as positive control. The experiments were repeated twice. The extracts presented activity on all tested bacteria with the strongest activity on the multidrug resistant bacteria *Acinetobacter baumannii, Enterococcus cloacae, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococcus aureus* (Table 1 and 2).

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Table1. Antibacterial activity of *Diplodia maydis* and *Sclerotium rolfsii* fungi.

Bacteria	Control 10 µL/mL	Diplodia maydis 2 µg/mL	*Sclerotium rolfsii* 2 µg/mL
	Zone of inhibition (mm)		
*Acinetobacter baumannii**	13	19	20
Enterobacter aerogenes	5	7	10
*Enterococcus cloacae**	8	10	13
Enterococcus faecalis	14	5	15
Escherichia coli	8	6	17
*Klebsiella pneumoniae**	5	18	20
*Proteus mirabilis**	4	8	15
Pseudomonas aeruginosa	5	6	20
Salmonella sp.	10	9	10
Shigella sp.	6	13	18
Bacillus cereus	5	7	15
*Staphylococcus aureus**	7	4	17
Staphylococcus coagulase negative*	12	12	16
Staphylococcus epidermidis	5	NT	15
Plesiomonas	5	NT	18
Citrobacter diversus	5	NT	12

*= Multidrug resistant bacteria; Control= Ciclopirox olamine; NT= not tested.

Table 2. Multidrug resistant bacteria.

Bacteria	Antibiotics
Acinetobacter baumannii	amikacin, ampicillin, aztreonam, cefotaxime, ceftazidime, cefepime, ciprofloxacin, chloranphenicol, gentamicin, imipenem and sulfazotrin.
Enterococcus cloacae	ampicillin, cephalothin, cefotaxime, chloranphenicol, gentamicin and sulfazotrin.
Klebsiella pneumoniae	nalidixic acid, pipemidine acid, ampicillin, cephalothin, cefepime, ceftazidime, gentamicin, nitrofurantoin and sulfazotrin.
Proteus mirabilis	nalidixic acid, pipemidine acid, ampicillin, cephalothin, cefepime, ceotaxime, cefoxitin, ceftazidime, nitrofurantoin and sulfazotrin.
Staphylococcus aureus	azithromycin, ciprofloxacin, clindamycin, chloranphenicol, erythromycin, oxacillin, penicillin and sulfazotrin.
Staphylococcus coagulase negative	azithromycin, ciprofloxacin, clindamycin, chloranphenicol, erythromycin, oxacillin, penicillin, sulfazotrin, tetracycline and vancomycin.

The results showed a variable effect of fungi extracts on the microorganisms. The fungus *S. rolfsii* was more active showing greatest values of zone of inhibition than *D. maydis* and antibiotic used as positive control. Screening for antibacterial activity of phythopatogenic fungi has not been described yet. This study demonstrated that ethyl acetate extracts of *D. maydis* and *S. rolfsii* fungi have a broader spectrum of inhibiting action to growth of all the bacteria tested showing that the fungi produced secondary metabolites that may be used as antibiotic agents or as prototypes of them. Results indicate that the potential of these microorganisms to produce antibacterial compounds is great and must be better explored, stimulating us to investigate antibacterial chemical constituents that will be reported in due course.

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RESUMO

Metabólitos secundários de *Diplodia maydis* and *Sclerotium rolfsii* com atividade antibiótica

Extratos de acetato de etila obtidos dos fungos fitopatogênicos *Diplodia maydis* e *Sclerotium rolfsii* mostraram atividade bactericida contra bactérias multi droga resistentes isoladas de pacientes de Hospital Brasileiro.

Palavras-chave: *Diplodia maydis*, *Sclerotium rolfsii*, multi droga resistência, fungos fitopatogênicos, metabólitos secundários

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