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Search of Antibiotic Metabolites from Phytopathogenic Fungi

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SUMMARY. Ethyl acetate and dichloromethane extracts from culture broths of *Fusarium solani, Colletotrichum musae, Cladosporium fulvum, Septoria lycopersici, Rhizoctonia solani* and *Alternaria solani* phytopathogenic fungi showed antibacterial activity by bioautography methods against bacteria isolated from patient of University Hospital (HUOP-Unioeste), some of them multi-drug resistant.
RESUMEN. "Estudio de Sustancias com Actividad Antibiótica en Extractos de Hongos Fitopatógenos" . Los extractos de acetato de etilo y diclorometano de cultivos de los hongos fitopatógenos *Fusarium solani, Colletotrichum musae, Cladosporium fulvum, Septoria lycopersici, Rhizoctonia solani* y *Alternaria solani* mostraron actividad antibacteriana utilizando técnicas de bioautografia contra algunas bacterias multi-droga resistentes aisladas de pacientes del hospital universitario (HUOP-Unioeste).

INTRODUCTION

Microorganisms have been traditionally used to produce a variety of important substances for the pharmaceutical and food industries. Hence, primary and secondary metabolites, such as peptides, enzymes, organic acids and antibiotics produced by filamentous fungi are used for these purposes [1,2]. The discovery and development of antibiotics was one of the most significant advances in medicine in the 20th century. Nevertheless, many antimicrobial 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 antimicrobial use patterns and to devise strategies to identify new antibiotics through previously unexplored targets [3]. The fermentation process is an important tool for production of secondary metabolites from fungi because of the ease of increasing production by environmental and genetic manipulation [2]. As part of an ongoing research for biological active secondary metabolites from phytopathogenic fungi [4] we have detected antibacterial activity in fermentation ethyl acetate and dichloromethane extracts of *Fusarium solani, Colletotrichum musae, Cladosporium fulvum, Septoria lycopersici, Rhizoctonia solani* and *Alternaria solani* using the bioautography assay methods [5,6].

MATERIAL AND METHODS

Fungi material

The fungi *Fusarium solani, Colletotrichum musae, Cladosporium fulvum, Septoria lycopersici, Rhizoctonia solani* and *Alternaria solani* were obtained at the Phytopathology Department of the Universidade Estadual do Oeste do Paraná, PR, Brazil.

Fermentation

The fermentation was carried out by inoculating 10^8 spore/mL of each fungus in medium containing 150 mL BDA, flasks were incubated in an orbital shaker adjusted to 150 rpm for 168 h at 28 °C.

KEY WORDS: Antibiotics, Bioautography, Phytopathogenic Fungi.
PALABRAS CLAVE: Antibióticos, Bioautografia, Hongos Fitopatógenos.

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Partition of the culture broth with organic solvents

The culture broths were separated by filtration, followed by three times partition with dichloromethane and ethyl acetate in sequence. All the resulting organic solvents were evaporated under vacuum.

Antibacterial assay

The crude extracts were investigated for antibacterial activity by using bioautography methods [6]. For the antibacterial assay 2.0 µg/mL of each extract were applied to pre-coated TLC plates, without elution of the samples. Previous to test, the bacterial cultures were activated by subculture on tryptone soy agar during 24 h at 37 °C. After subculture, the bacterial inoculum was prepared so that the turbidity of the suspension was similar to 0.5 Mc Farland standard (1,5 x 10^8 cfu/mL). Immediately one microliter of each diluted inoculum (1 x 10^7 cfu/mL) was applied onto Mueller Hinton Agar medium (MHA-DIFCO), and distributed over TLC plates (2 x 4 cm). After solidification of the media, the TLC plates were incubated overnight at 37 °C. Ciclopirox olamine was used as positive control. The experiments were repeated twice.

RESULTS

The antibacterial activities of the fungi extracts are shown in Tables 1 and 2. The extracts presented activity on same tested bacteria with the strongest activity on the multi-drug resistant bacteria *Staphylococcus aureus* for *F. solani* extracts and *Escherichia coli* for *R. solani* extracts. The results showed a variable effect of fungi extracts on the microorganisms. The *A. solani* fungus was more active showing more zone of inhibition than the other fungi. This study demonstrated that ethyl acetate extracts of fungi tested have a broader spectrum of inhibiting action to growth of same 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.

Bacteria	Fungi										
	C	*A. solani* [a]		*C. musae* [a]	*C. fulvum* [a]	*S. lycopersici* [a]		*R. solani* [a]		*F. solani* [a]	
	Zone of inhibition (mm) [C]										
		A	D	A	A	D	A	D	A	D	A
Escherichia coli	9	12	n/a	n/a	n/a	n/a	n/a	19	15	n/a	n/a
Klebsiella pneumoniae *	13	7	3	3	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pseudomonas aeruginosa *	5	8	n/a	n/a	10	n/a	n/a	n/a	7	n/a	n/a
Enterobacter aerogenes	8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	14
Acinetobacter baumannii *	14	9	n/a	n/a	4	n/a	n/a	9	n/a	n/a	n/a
Salmonella sp	8	n/a	n/a	n/a	n/a	n/a	8	n/a	8	n/a	n/a
Shigella sp	11	n/a	n/a	12	n/a	7	11	n/a	n/a	n/a	n/a
Proteus mirabilis *	8	7	n/a	3	4	5	8	n/a	n/a	n/a	8
Plesiomonas sp	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Citrobacter diversus	5	8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Bacillus cereus	10	n/a	n/a	n/a	3	n/a	8	8	21	n/a	n/a
Bacillus subtilis	10	10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	23
Staphylococcus epidermidis	6	10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
*Enterococcus cloacae**	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Enterococcus faecalis	7	10	n/a	n/a	n/a	n/a	n/a	n/a	10	21	23
*Staphyococcus aureus**	12	12	n/a	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Staphylococcus intermedius	10	11	n/a	n/a	10	n/a	n/a	8	23	n/a	n/a
Streptococcus mutans	12	12	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Staphylococcus coagulase negativa *	5	10	n/a	5	n/a	12	5	n/a	n/a	n/a	n/a

Table 1. Antibacterial activity of phythopatogenic fungi. * = Multi-drug resistant bacteria; Control = Ciclopirox olamine 5 µg/mL; Extracts D = CH_2Cl_2 extracts; A = AcOEt extracts 2 µg/mL n/a= not active.

Bacteria	Antibiotics
Acinetobacter baumannii	amikacin, ampicillin, aztreonam, cefotaxime, ceftazidime, cefepime, ciprofloxacin, chloramphenicol, gentamicin, imipenem and sulfazotrin.
Enterococcus cloacae	ampicillin, cephalothin, cefotaxime, chloramphenicol, 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, chloramphenicol, erythromycin, oxacillin, penicillin and sulfazotrin.
Staphylococcus coagulase negative	azithromycin, ciprofloxacin, clindamycin, chloramphenicol, erythromycin, oxacillin, penicillin, sulfazotrin, tetracycline and vancomycin.
Pseudomonas aeruginosa	ampicillin, cephalothin, cefotaxime, chloranphenicol, and sulfazotrin.

Table 2. Multi-drug resistant bacteria.

CONCLUSION

Microbial natural products still appear as the most promising source of the future antibiotics that society is expecting. The arguments supporting this idea are the unparalleled structural diversity that can be found in nature, in this regard, the phytopathogenic fungi can be considers a new source of substances that may be used as antibiotic agents or as prototypes of them. The potential of these microorganisms to produce antibacterial compounds is great and must be better explored for antibiotic therapy.

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