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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal activity of silver ion on ultrastructure and production of aflatoxin B1 and patulin by two mycotoxigenic strains, *Aspergillus flavus* OC1 and *Penicillium vulpinum* CM1

*Activité antifongique des ions argent sur l'ultrastructure et la production d'aflatoxine B1 et de patuline par deux souches toxigéniques d'*Aspergillus flavus *OC1 et* Penicillium vulpinum *CM1*

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Patulin (PAT);
Silver ion;
Antifungal;
Ultrastructure

Summary
Objective. — The antifungal activity of silver ion from silver nitrate solution was tested against two pathogenic and toxigenic fungal strains. The first was *Aspergillus flavus* OC1, a clinical aflatoxigenic strain that causes fungal keratitis and the second was *Penicillium vulpinum* CM1, a maize-pathogenic strain that is positive for patulin (PAT) producing ability.
Materials and methods. — Agar well diffusion assays on yeast sucrose (YES) agar were applied for determination of the antifungal activity of silver ions either filter- or autoclaved-sterilized. Transmission electron microscopy was used to analyze the cellular effects of silver ion. The mycotoxins AFB1 and PAT were analyzed in the fungal strains cultures treated with silver ion.
Results. — Filter-sterilized ions have a greater potential for growth inhibition of both fungal strains than autoclaved-sterilized ions. The minimal inhibitory concentration of the filter-sterilized ions against *A. flavus* OC1 was 70 μg mL^{-1} and against *P. vulpinum* CM1 was 60 μg mL^{-1} and that the minimum fungicidal concentration was 120 μg mL^{-1} against the first strain and

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80 μg mL^{-1} against the second strain. Hyphal cells treated with silver ion showed considerable changes in the nature of cell membranes and cytoplasmic organelles. Silver applied to YES broth inhibited mycelial growth and AFB1 and PAT formation of both strains. Growth and mycotoxin production appeared to be correlated processes.
Conclusion. — These findings indicate the future possibility to use silver ion as substitute for synthetic fungicides to control the growth of pathogenic fungi and their mycotoxin production.

MOTS CLÉS
Aspergillus flavus ;
Penicillium vulpinum ;
Mycotoxines ;
Aflatoxine B1 (AFB1) ;
Patuline (PAT) ;
Ions d'argent ;
Antifongique ;
Ultrastructure

Résumé
Objectif. — L'activité antifongique ions argent de la solution de nitrate d'argent a été testée contre deux souches fongiques pathogènes et toxigènes. Le premier était *Aspergillus flavus* OC1, une souche clinique aflatoxigène qui provoque une kératite fongique et la seconde était *Penicillium vulpinum* CM1, une souche pathogène pour le maïs, qui est positive pour la capacité de production de patuline (PAT).
Matériels et méthodes. — Des méthodes par diffusion à partir de puits en yeast sucrose (YES) agar ont été appliquées pour la détermination de l'activité antifongique des ions argent, stérilisés par filtration ou par autoclavage. La microscopie électronique à transmission a été utilisée pour analyser les effets cellulaires des ions argent. Les mycotoxines AFB1 et PAT ont été analysées dans les cultures traitées avec l'ion argent.
Résultats. — Les ions stérilisés par filtration ont un potentiel plus grand d'inhibition de la croissance des deux souches fongiques que les ions stérilisés en autoclave. La concentration minimale inhibitrice (CMI) des ions filtrés contre *A. flavus* OC1 était de 70 μg mL^{-1}et contre *P. vulpinum* de 60 μg mL^{-1}. La concentration fongicide minimale a été de 120 μg mL^{-1} contre la première souche et 80 μg mL^{-1}contre la seconde souche. Les cellules des hyphes traitées avec l'ion argent ont montré des changements considérables dans la nature des membranes cellulaires et des organites cytoplasmiques. L'argent appliqué au YES en milieu liquide a inhibé la croissance du mycélium et la production de AFB1 et de PAT pour les deux souches. La croissance et la production de mycotoxines semblaient être des processus corrélés.
Conclusion. — Ces résultats indiquent la possibilité future d'utiliser des ions argent comme substitut aux fongicides synthétiques pour contrôler la croissance des champignons pathogènes et leur production de mycotoxine.

Introduction

Aflatoxins are a group of structurally related difuranocoumarin derivatives produced by *Aspergillus flavus* and *A. parasiticus* [42]. *A. flavus* was reported to cause damage to grains in storage and rotting of the seeds [13,28]. Additionally, it has been emerged as predominant pathogens in patients' sinusitis and fungal keratitis [23]. Among 18 different types of aflatoxins produced by *A. flavus* strains, aflatoxin B1 (AFB1) is the most extremely toxic, mutagenic and carcinogenic type [6,36].

Patulin (4-hydroxy-4H-furo[3,2-c] pyran-2(6H)-one) (PAT) is another secondary metabolite produced by several species of filamentous fungi belonging to the genera of *Aspergillus*, *Byssochlamys*, *Gymnoascus*, *Paecilomyces*, and *Penicillium* [10]. Among *Penicillium* species, *P. vulpinum* (Cooke & Massee) Seifert & Samson (formerly *P. claviforme*) was found to form coremia (sheaf-like aggregations of conidiophores) and has been reported to be more abundant due to urbanization of the environment. Pollution from traffic has been reported to alter the species composition of *Penicillium* fungi in soils along roadsides and coremial fungi are more resistant to extreme environmental conditions than those lacking coremia [26]. PAT is an especially dangerous polyketide that has been detected in apples and apple products and occasionally in other fruits such as pears, apricots, peaches and grapes and it is mainly produced in rotten parts of the fruits after infestation with PAT-producing fungus that can occur at 0 °C during storage or develops rapidly when fruits are returned to room temperatures [39]. Short-term and sub-chronic exposure to PAT causes immunotoxic, neurotoxic, and teratogenic effects [5,12].

Elimination or inactivation of mycotoxins by physical and chemical methods bears many drawbacks [25,33]. Physical approaches involving treatment with heat, UV light or ionizing radiation are not entirely effective. Chemical methods using chlorinating, oxidizing or hydrolytic agents are not widely accepted because they may be impractical or potentially harmful due to generation of toxic byproducts and/or significant alteration of product quality.

Silver ions (Ag^+) are one of the most widely used metal ions, most notably serving as an antimicrobial agent for management of plant diseases, in addition to multiple modes of inhibitory action against microorganisms [34]; therefore, it may be used with relative safety for control of various plant pathogens, compared to synthetic fungicides [18]. Ag^+ from silver-based solution is a long lasting biocide with high temperature stability and low volatility and is known to attack a broad range of biological processes in microorganisms including cell membrane structure and functions [30].