Journal de Mycologie Médicale (2016) **26**, 193—200

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal activity of a Saharan strain of *Actinomadura* sp. ACD1 against toxigenic fungi and other pathogenic microorganisms

*Activité antifongique d'une souche saharienne d'*Actinomadura *sp. ACD1 contre des champignons toxinogènes et autres microorganismes pathogènes*

A. Lahoum [a], A. Aouiche [a], N. Bouras [a,b], C. Verheecke [c], H.-P. Klenk [d], N. Sabaou [a,*], F. Mathieu [c]

[a] *Laboratoire de Biologie des Systèmes Microbiens (LBSM), École Normale Supérieure de Kouba, Alger, Algeria*
[b] *Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, BP 455, 47000 Ghardaïa, Algeria*
[c] *Laboratoire de Génie Chimique, LGC, Université de Toulouse, UMR 5503 (CNRS/INPT/UPS), Toulouse, France*
[d] *School of Biology, Newcastle University, Ridley Building, Newcastle upon Tyne, NE1 7RU, United Kingdom*

Received 8 November 2015; received in revised form 7 February 2016; accepted 13 February 2016
Available online 17 March 2016

KEYWORDS
Actinobacterium;
Actinomadura;
Pathogenic microorganisms;
Antimicrobial activity

Summary A new strain of actinobacteria, designated ACD1, was isolated from a Saharan soil sample in the Hoggar region (Algeria). Morphological study led to this strain being classified as a member of the *Actinomadura* genus. Phylogenetic analysis based on the 16S rRNA gene showed that the strain is closely related to *Actinomadura sediminis* DSM 45500^T (98.5% sequence similarity). Furthermore, strain ACD1 presented a strong activity against mycotoxigenic and phytopathogenic fungi, including *Aspergillus* and *Fusarium* strains, and other pathogenic microorganisms. The kinetics of antimicrobial activity were investigated on ISP-2, Bennett and TSB media. Four solvents (*n*-hexane, dichloromethane, ethyl acetate and *n*-butanol) were used for the extraction of the produced antibiotic. The highest antimicrobial activity was obtained using the butanolic extract from the ISP-2 medium after seven days of fermentation culture. The active antibiotic was purified by reverse-phase HPLC using a C18 column. The UV-visible and mass spectra were determined. The minimum inhibitory concentrations (MIC) of this antibiotic were determined against pathogenic microorganisms.

* Corresponding author.
E-mail address: sabaou@yahoo.fr (N. Sabaou).

http://dx.doi.org/10.1016/j.mycmed.2016.02.017
1156-5233/

MOTS CLÉS
Actinobactérie ;
Actinomadura ;
Microorganismes pathogènes ;
Activité antimicrobienne

Résumé Une nouvelle souche d'actinobactérie, désignée ACD1, a été isolée à partir d'un échantillon de sol provenant de la région du Hoggar (Algérie). L'étude morphologique de cette souche a permis de la rapprocher du genre *Actinomadura*. L'analyse phylogénétique basée sur le gène codant pour l'ARNr 16S a montré que la souche-type la plus proche phylogénétiquement est *Actinomadura sediminis* DSM 45500^T (98,5 % de similarité). De plus, la souche ACD1 a présenté une forte activité contre des champignons phytopathogènes et mycotoxinogènes, y compris les souches des genres *Aspergillus* et *Fusarium* et d'autres microorganismes pathogènes. Les cinétiques de l'activité antimicrobienne sont réalisées sur les milieux ISP-2, Bennett et TSB. Quatre solvants (le *n*-hexane, le dichlorométhane, l'acétate d'éthyle et le *n*-butanol) ont été utilisés pour l'extraction de l'antibiotique produit. La meilleure activité antimicrobienne a été obtenue en utilisant l'extrait butanolique du milieu ISP-2 après sept jours de fermentation. L'antibiotique actif est purifié par HPLC en utilisant une colonne C18. Le spectre UV-visible et le spectre de masse sont déterminés. Les concentrations minimales inhibitrices (CMI) sont réalisées contre des microorganismes pathogènes.

Introduction

Filamentous fungi and yeasts are the causal agents of several of the most serious diseases of humans and plants. Many fungi, especially species from the genera *Aspergillus*, *Fusarium* and *Penicillium* are capable of producing mycotoxins that cause a toxic response when ingested by humans and animals [13]. Among these mycotoxins, ochratoxin A (OTA), known to induce nephropathies and urothelial tumors, is produced by *Aspergillus westerdijkiae*, *Aspergillus carbonarius* and *Aspergillus niger* [1]; aflatoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are known to cause cancer [16]; and deoxynyvalenol (DON), produced by *Fusarium* spp., is an inhibitor of protein synthesis [31].

The deficiency of antifungal antibiotics and the increased resistance of fungal species to these molecules are real problems for public health. Therefore, many researchers have focused on the isolation of new antifungal compounds. Actinobacteria represent an excellent resource for the discovery of new interesting antibiotics [6]. The genus *Streptomyces* produces about 80% of all the known actinobacteria antibiotics [11]. In recent years, the rate of discovery of new antibiotics from the genus *Streptomyces* has decreased considerably [7]. Thus, many laboratories around the world are focusing their search for new microbial derived antibiotics on non-*Streptomyces* actinobacteria.

The Algerian Saharan soils, exposed to an arid climate, constitute one of the most attractive sources for several rare actinobacteria genera, such as *Actinomadura*, *Actinopolyspora*, *Amycolatopsis*, *Saccharopolyspora* and *Saccharothrix* [32]. In these extreme conditions, the microorganisms developed a specialized metabolism including many interesting antibiotics, such as dithiolopyrrolones [10,21,25] and anthracyclines [39].

In this context, we isolated from a Saharan soil an actinobacterial strain other than the genus *Streptomyces*. The antimicrobial activity of the extract produced by this strain was studied against several toxigenic fungi and against other human pathogenic microorganisms.

Materials and methods

Isolation and features of the actinobacteria strain

The strain ACD1 was isolated from Saharan soil collected in the Hoggar region, Tamanrasset province (southern Algeria). One gram of dry soil was suspended in 9 mL of sterile deionized water. Serially diluted samples were prepared and aliquots (0.1 mL) of each dilution were plated on chitin-vitamin-agar medium, recommended for the isolation of rare actinobacteria [15]. The medium was supplemented with actidione (80 mg/L) to prevent growth of fungi. The plates were incubated at 30 °C for three weeks.

The morphological and cultural characteristics of strain ACD1 were determined on the International *Streptomyces* Project media: yeast extract-malt extract agar (ISP-2), oatmeal agar (ISP-3) and inorganic salts-starch agar (ISP-4) [35], and also on the Bennett medium [38]. After incubation at 30 °C for 14 days, morphological characteristics were recorded by the naked eye and by using a light microscope (Motic, B1 Series, Hong Kong). The ISCC-NBS color name chart [18] was used to determine the color of the aerial mycelium, the substrate mycelium and diffusible pigments.

For molecular characterization, strain ACD1 was grown in ISP-2 broth, and genomic DNA was extracted with a DNA extraction kit (MasterPure™ Gram Positive DNA Purification Kit, Epicentre® Biotechnologies, Madison). PCR amplification of the 16S rRNA gene sequence of strain ACD1 was performed as described by Rainey et al. [30] by using two universal primers: 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3'). The PCR products were sequenced using the same primers as above and an automated sequencer. The sequence obtained was compared for similarity with the reference species in the public sequence databases and with the EzTaxon-e server [20].

Target microorganisms

Except some bacteria and yeasts pathogenic to humans, most target microorganisms used were filamentous toxigenic fungi. These toxigenic fungi were:

- four OTA producing strains (*A. carbonarius* M333 and Ac2, *A. niger* An1 and *A. westerdijkiae* ATCC 3174);
- three aflatoxin producing strains (*A. flavus* Af3 and E73 and *Aspergillus parasiticus* CBS 100926);
- two DON producing strains (*Fusarium culmorum* Fc1 and *Fusarium graminearum* Fg1);
- one patulin producing strain (*Penicillium expansum* Pe1).