Journal de Mycologie Médicale (2016) 26, 160—169

Available online at
ScienceDirect
www.sciencedirect.com

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

ORIGINAL ARTICLE/ARTICLE ORIGINAL

Activity of 2,4-Di-tert-butylphenol produced by a strain of *Streptomyces mutabilis* isolated from a Saharan soil against *Candida albicans* and other pathogenic fungi

Activité du 2,4-Di-tert-butylphenol produit par une souche de Streptomyces mutabilis *isolée d'un sol Saharien contre* Candida albicans *et d'autres champignons pathogènes*

S. Belghit [a], E.H. Driche [a], C. Bijani [b], A. Zitouni [a], N. Sabaou [a], B. Badji [a,*], F. Mathieu [c]

[a] *Laboratoire de Biologie des Systèmes Microbiens (LBSM), École Normale Supérieure de Kouba, Alger, Algeria*
[b] *Laboratoire de chimie de coordination (LCC), CNRS, université de Toulouse, UPS, INPT, LCC, 205, 12, route de Narbonne, 31077 Toulouse, France*
[c] *Laboratoire de Génie Chimique, LGC, Université de Toulouse, UMR 5503 (CNRS/INPT/UPS), Toulouse, France*

Received 2 August 2015; received in revised form 4 March 2016; accepted 12 March 2016
Available online 20 April 2016

KEYWORDS
Streptomyces;
Taxonomy;
Antifungal activity;
Candida albicans;
2,4-Di-tert-butylphenol

Summary In a search for new antifungal antibiotics active against *Candida albicans* and others pathogenic fungi, a strain of actinobacteria, designated G61, was isolated from a Saharan soil and tested for its activity against these microorganisms. The analysis of its 16S rDNA sequence showed a similarity level of 100% with *Streptomyces mutabilis* NBRC 12800^T. The highest anticandidal activities produced by the strain G61 were obtained on Bennett medium in the fourth day of incubation. The active product, extracted by *n*-butanol, contained one bioactive spot detected on thin layer chromatography plates. It was purified by HPLC and its chemical structure was determined by spectroscopic analyses as 2,4-Di-tert-butylphenol. The minimum inhibitory concentrations (MIC) of this product against several strains of pathogenic micro-organisms are interesting.

* Corresponding author.
E-mail address: badji_boubekeur@yahoo.fr (B. Badji).

http://dx.doi.org/10.1016/j.mycmed.2016.03.001
1156-5233/

MOTS CLÉS
Streptomyces ;
Taxonomie ;
Activité antifongique ;
Candida albicans ;
2,4-Di-tert-butylphénol

Résumé Dans le cadre de la recherche de nouveaux antifongiques actifs contre *Candida albicans* et contre d'autres champignons pathogènes, une souche d'actinobactérie, nommée G61, a été isolée à partir d'un sol saharien et testée pour son activité contre ces microorganismes. L'analyse de sa séquence d'ADNr 16S a montré un taux de similarité de 100 % avec la souche-type *Streptomyces mutabilis* NBRC 12800^T. Les plus fortes activités anticandidosiques produites par la souche G61 ont été obtenues sur le milieu Bennett au quatrième jour d'incubation. Le produit actif, extrait par le *n*-butanol, contient une tache active détectée par chromatographie de couche mince. Elle a été purifiée par HPLC et sa structure chimique a été déterminée par analyses spectroscopiques en tant que 2,4-Di-tert-butylphénol. Les concentrations minimales inhibitrices (CMI) de ce produit contre diverses souches de microorganismes pathogènes sont intéressantes.

Introduction

Fungi are responsible for a large number of diseases in humans, animals and plants, and cause damage to wood products, fruits, vegetables and other foodstuff [3]. Diseases or mycoses are classified as fungal infections caused by eumycotic organisms such as opportunistic and also pathogenic *Candida* spp., *Aspergillus* spp., *Fusarium* spp., *Cryptococcus* and some other fungal species [22]. These invasive diseases have assumed a greater importance because of their increasing incidence in persons with haematological malignancies and in many other immunocompromised patients. Most infections are caused by *Candida* spp., but the emergence of *Aspergillus* spp. and other fungi as etiological agents is continuously changing the spectrum of diseases [39].

Candida albicans is the most important fungal opportunistic pathogen of humans [49]. It belongs to the normal microbiota of individual's mucosal oral cavity, gastrointestinal tract and vagina [44], and it is responsible for various clinical manifestations from mucocutaneous colonization to blood stream infections [36]. It alone accounts for 60% of nosocomial fungal infections [33]. In addition, *C. albicans* causes systemic candidiasis associated with high mortality despite the availability of treatment [4]. The high mortality rates occurring during invasive fungal infections cases may be due to delayed diagnosis and treatment, development of resistance or severity of illness [37].

The antifungal drugs currently used for the treatment include amphotericin B, nystatin, fluconazole, itraconazole, ketoconazole, voriconazole, 5-fluorocytosine and caspofungin [56]. However, the choice of suitable antifungal agents remains relatively limited due to the toxicity and low solubility of some of them, emergence of resistant fungal strains, delayed diagnosis of fungal infection, variable drug bioavailability in immunocompromised patients, lack of either oral or intravenous preparations and drug interaction infections [10]. There is a need to explore novel therapeutic approaches and to develop new antifungal compounds to treat fungal infections [45].

The antibiotics for treatment of infections are derived either directly from natural sources, semisynthesized from a natural product parent, or completely synthesized but modeled after a natural product lead compound [9]. It has been estimated that about two-third of the natural antibiotics have been isolated from actinobacteria, especially from the genus *Streptomyces* [6].

Previous studies have reported the abundance and diversity of actinobacteria in Algerian Saharan soils [40,58]. In our laboratory, it has been demonstrated that many strains of actinobacteria isolated from these soils were found to produce bioactive compounds with interesting activities [2,3,13,52].

In the present study, we describe the taxonomy of an actinobacteria strain isolated from a Saharan soil. In addition, we report on production and characterization of its antifungal compounds.

Materials and methods

Isolation of the actinobacteria strain

The strain G61 was isolated from soil sample collected from Metlili (Ghardaïa, 32°16′N, 3°37′E), province in Sahara of Algeria, by the dilution agar plating method using a chitin-vitamin agar medium [17] consisting of (per liter of distilled water): 2 g chitin, 0.35 g K_2HPO_4, 0.15 g KH_2PO_4, 0.2 g $MgSO_4$ $7H_2O$, 0.3 g NaCl, 0.02 g $CaCO_3$, 10 mg $FeSO_4$ $7H_2O$, 1 mg $ZnSO_4$ $7H_2O$, 1 mg $MnCl_2$ $4H_2O$ and 18 g agar. The medium was supplemented with penicillin (25 mg/L) and cycloheximide (50 mg/L) to inhibit the growth of undesirable bacteria and fungi, respectively. The plates were incubated at 30 °C for 3 weeks.

Morphological characteristics

The morphology of the strain G61, grown on various media at 30 °C for 10 days, was examined under light microscopy for the mycelia organization and sporulation.

Cultural characteristics observed on media from the International *Streptomyces* Project (ISP), ISP-1, ISP-2 and ISP-4 media [47], nutrient agar and Bennett agar [55] were recorded after 7—14 days incubation at 30 °C. Colors were determined according to the ISCC—NBS centroid color chart [23].

Chemotaxonomic study

For chemotaxonomic analyses, strain G61 was grown in ISP-2 broth medium at 30 °C for 4 days on a rotary shaker (250 rpm). The biomass was harvested by centrifugation at 6000 rpm and washed twice with distilled water [47]. The determination of diaminopimelic acid isomer and the presence or absence of