JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov. 2013. Vol. **7**(Spl. Edn.), p. 23-34

Isolation and Characterization of a *Streptomyces* Strain Endowed with Antifungal Activity

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(Received: 30 September 2013; accepted: 27 October 2013)

An actinomycete strain, named H3, was recovered from a deteriorated wood sample of an old house located in the Medina of Fez, Morocco. Molecular identification via 16S rDNA amplification and sequencing showed that it belongs to the genus *Streptomyces*. Screening of this strain for its antimicrobial activity against different Gram positive and negative bacteria, yeasts and fungi showed that it was active against yeasts and fungi and had no activity against bacteria. The antifungal activity of the organic extract of strain H3 wasn't eliminated upon treatment by Proteinase K and heat, revealing its non-protein nature. Moreover, this organic extract was able to release nucleic acids of *Candida tropicalis* indicating that it acts probably on the yeast envelope. In addition, the study of the production kinetics of strain H3 in ISP2 broth showed that the maximum of production was obtained at the 9th day of fermentation. Otherwise, thin layer chromatography followed by bioautography using *C. tropicalis* as test organism, allowed localizing the active substance of strain H3. Furthermore, the phytochemical tests carried out showed the presence of flavonoids and polyphenols in the organic extract of strain H3.

Keys words: Actinomycetes, *Streptomyces*, antimicrobial activity, molecular identification.

The demands from the industries of leather, wood, textiles and food of active compounds against mouldiness and little toxic for human beings and animals are increasing (Larpent and Sanglier, 1989). On the other hand, the frequency of fungal infections has increased due to increasing number of immunodepressed patients and the extended use of large spectra antibiotics (Yashuda, 2001). Thus, the need for novel antifungal compounds is greater now than ever before.

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In recent years, microorganisms have become important in highlighting of novel microbial products exhibiting antimicrobial, antiviral, antitumoral as well as anticoagulant and cardioactive properties (Bharti *et al.*, 2011).

Among these microorganisms, actinomycetes have occupied a prominent position for their capacity to produce secondary metabolites including antibiotics and other compounds of biotechnological importance (Cross, 1982; Jain and Jain, 2005; Petrova *et al.*, 2006; Janardhan *et al*., 2012).

Member of the genus *Streptomyces*have been shown to have characteristics which include the production of different kinds of secondary metabolites and biologically active substances of high commercial value such as enzymes which degrade the fungal cell wall, or antifungal

compounds (El-Tarabily *et al*., 2000; Errakhi *et al*., 2007; Getha *et al*., 2005; Goodfellow and Williams, 1983). The antifungal potential of extracellular metabolites from *Streptomyces* against some fungi was previously reported (Prapagdee *et al*., 2008; Joo, 2005; Fguira *et al*., 2005; Chamberlain and Crawford 1999; El-Abyad *et al*, 1993; Rothrock and Gottlieb 1984).

There is a high degree of similarity between fungi and mammalian cells making identification of safe and effective antifungal drugs a difficult task (Xu *et al*., 2013). Despite its serious side effects, amphotericin B is still widely used in treatment for deep-seated mycoses (Gallis *et al*., 1990). To overcome such a problem the search for the new antifungal metabolites and from new sources remains crucial.

During our study we found that the strain H3 isolated from deteriorated wood sample had the property of inhibiting fungi. The antifungal substance produced by strain H3 was partially purified and characterized and the strain was identified as *Streptomyces* sp.

MATERIALS AND METHODS

Sample collection and used strains

Actinomycetes were isolated from an old house located in the oldest of Morocco's imperial cities, Fez. This house, built 450 years ago, is located in the former DerbLamté in the Medina of Fez and is currently in an advanced state of deterioration. The wood samples (cedar wood) were cut, fragmented by a sterile scalpel and transported aseptically in sterile containers to the laboratory.

The target strains used for screening antimicrobial activity were: Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*and *Bacillus cereus*), Gram negative bacteria (*Escherichia coli* DH5±, *Escherichia coli* CIP 7624,*Pseudomonas aeruginosa*27853 and *Pseudomonas aeruginosa*A22), Mycobacteria (*Mycobacterium smegmatis* and *Mycobacterium aurum*), yeasts (*Candida albican s*and *Candida tropicalis*) and fungi (*Penicillium granulatum*, *Cladosporium cladosporioides*, *Penicillium commune* and *Aspergillus niger*).

Isolation of actinomycetes

Ten gram of wood sample were suspended in physiological water and incubated at room temperature for two hours with agitation. After decantation, the supernatant was diluted (10^{-1} to 10^{-7}) and 100 µl of each dilution was spread on the surface of Actinomycete Isolation Agar (AIA) plates and incubated at 30°C. After two to four weeks of incubation, the plates were examined for the presence of actinomycetes colony. Colonies of actinomycetes were picked on the basis of their morphological characteristics and purified on ISP2 agar.

Characterization of the isolate

Actinomycetes cultures were spot inoculated. The ability to grow at pH 5, 6, 7, 8, 9 and 10 was determined on ISP2 medium plates and the temperature range for growth was tested (at 25, 30, 37, 42 and 45°C) on ISP2 medium plates at pH 7.2.The plates were checked for growth after seven days of incubation at 30°C

The production of melanoid pigments was carried out on peptone-yeast extract-iron agar (ISP6) (peptone 20 g; ferric citrate ammoniacal 0.5 g; sodium thiosulfate 0.08 g; yeast extract 1 g; K_2HPO_4 1 g; agar 15 g; H_2O 1000 ml; pH 7.2) and tyrosine agar (ISP7) (glycerol 15 g; L-tyrosin 0.5 g; L-asparagine 1 g; K_2HPO_4 0.5 g; $MgSO_4 7H_2O$ 0.5 g; NaCl 0.5 g; $FeSO_4 7H_2O$ 0.01 g; standard saline solution 1 ml; agar 18 g; H_2O 1000 ml; pH 7.2) (Shirling and Gottlieb, 1966).

DNA extraction, PCR amplification and 16S rRNA sequencing

The genomic DNA used for PCR amplification was prepared from single colonies grown on the ISP2 medium. The colony was suspended in 50 µL of sterile distilled water, mixed on a vortex and then heated in boiling water for 10 min, allowed to cool for 20 min (this thermolysis was repeated 4 times) and centrifuged at 7500 rpm for 5 min. The supernatant was transferred to a clean tube. The 16S rRNA gene fragment was amplified using universal primers fD1 (5'AGAGTTTGATCCTGGCTCAG3') and Rs16 (5'TACGGCTACCTTGTTACGACTT3') (Weisberg *et al*., 1991). Conditions of the PCR were the following: denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1min 30 s. The final extension was at 72°C for 5 min.

PCR products were analysed by

electrophoresis in 1% agarose gel and visualized by ethidium bromide (10 mg/ml) staining. The sequencing was carried out by using an automatic sequencer of the innovation center of Fez. The same primers as above were used for this purpose. The resulting sequences were analyzed using the data base of the National Center for Biotechnology Information (NCBI) and the BLAST N program.

Screening of activity against bacteria and yeasts on different culture media

To determine the optimal medium, which ensures maximum antimicrobial activity, strain H3 was grown on different agar media GLM (yeast extract 3 g; malt extract 3 g; peptone 5 g; glucose 10 g; agar 16 g; H_2O 1000 ml; pH 7.2) (Kitouni *et al*., 2005), GYEA (yeast extract 10 g; glucose 10 g ; agar 16 g; H_2O 1000 ml; pH 7.2) (Athalye *et al*. 1985), ISP2 (yeast extract 4 g ; malt extract 10 g ; glucose 4 g; agar 16 g; H_2O 1000 ml; pH 7.2) (Shirling and Gottlieb, 1966), Bennett (peptone 2 g; beef extract 1 g; yeast extract 1 g; glucose 10 g; agar 16 g; H_2O 1000 ml; pH 7.2) (Badji *et al*., 2005) and ISP1 (tryptone 5 g; yeast extract 3 g; agar 16 g; H_2O 1000 ml; pH 7.2) (Shirling and Gottlieb, 1966). After incubation for 7 days at 30°C, agar cylinders were cut out (Petrosyan *et al*., 2003; Saadoun *et al* moumani, 1997; Shomura *et al*., 1979) and placed on the surface of the Mueller Hinton media, which had previously been seeded with each test microorganisms (10^5-10^6 CFU/ml). Plates were kept at 4°C for at least 2h to allow the diffusion of produced antimicrobial metabolites then the diameters were measured after 24h to 48 h of incubation at 30°C for yeasts and at 37°C for bacteria.

Detection of antifungal activity

Antifungal activity was evaluated by the double layer method against pathogenic fungi (*Penicillium granulatum*, *Cladosporium cladosporioides*, *Penicillium commune* and *Aspergillus niger*). The strain H3 was sown by touch in the center of ISP2 agar plates. After incubation at 30°C during 7days, the culture was covered by 8ml of ISP2 medium containing 10g of agar, already sowed by the test germ. It was then incubated at 30°C after which the diameter of inhibition was determined after 48h of incubation (Boughachiche *et al*., 2005).

Extraction of antimicrobial metabolites

The isolate H3 was cultivated in 100 ml of ISP2 broth medium and incubated at 30°C for 7 days under constant agitation of 250 rpm. The production medium was centrifuged for 20 min at 8000 rpm to remove the mycelium. Ethyl acetate was added to the supernatant in the ration 1:1 (v/v) and shaken vigorously for 2 h at room temperature. The organic extract was evaporated to dryness using a Rotavapor (Zitouni *et al*. 2005). The resulting dry extract was recuperated in 1 ml of methanol or 1 ml of physiological water to evaluate its activity against *C. tropicalis* or its effect on cell wall of this yeast (see below), respectively.

Kinetics of antimicrobial activity of the isolate H3

Pre-culture of the actinomycete isolate was tested in 250 ml Erlenmeyer flask containing 50 ml of the ISP2 medium. After incubation for 48h at 30°C under constant agitation at 250 rpm, the flask was homogenized and 5 ml of pre-culture was used to inoculate a 500 ml flask containing 100 ml of ISP2 medium. The flask was then incubated under the same conditions as above. Immediately after inoculation and at 24 h intervals, samples of 5 ml were taken and used for antimicrobial activity against *C. Tropicalis* by the agar well diffusion method (Boudjella *et al*., 2006). The experiment was repeated twice.

Partial characterization of the antimicrobial products

The sensitivity to heat was examined by boiling the supernatant to 60°C, 80°C and 100°C for 15 min then the treated supernatant was tested against *C. tropicalis* to determine its antimicrobial activity using the protocol described above. After incubation for 24h at 30°C, the inhibition zone was measured. In addition, the sensitivity of organic extract to proteinase K was tested as follow: 40µl of a Proteinase K solution (1 mg/ml) was mixed with 100 µl of the extract and incubated for 3 h at 37°C (Wu *et al*., 2005). Then, the effect of the Proteinase K treated extract was tested against *C. tropicalis* using the protocol described above. The control was a solution of Proteinase K at the same concentration (the experiment wasrepeated twice).

Extraction of nucleic acids of *C. Tropicalis* by the organic extract of the isolate H3

1 ml of an overnight culture of *C. tropicalis* was centrifuged at 5000 rpm during 5 min. The microbial pellet was re-suspended in 360 µl of the organic extract (recuperated in 1 ml of

physiological water). This microbial suspension was incubated at 37°C during 24h and then centrifuged at 5000 rpm during 5 min. DNA was precipitated with two volumes of cool 95% (v/v) ethanol and 40 µl of NaCl 5 M. After incubation at -20°C during 30 min and centrifugation at 10000 rpm during 20 min, the pellet was dried and re-suspended in 20 µl of sterile distilled water. The control used corresponded to the same protocol as above, except for the use of 360 µl of physiological water instead of the organic extract (Houssaini-Iraqui *et al*., 1991).

The nucleic acids obtained were detected by electrophoresis on 1% of agarose gel. The experiment was repeated three times.

Detection of flavonoids and polyphenol

In order to identify the molecules responsibles for microbial growth inhibition, we determined the total polyphenols and flavonoids contents in the crude extracts.

These principal chemical constituents were characterized in the extracts by colorimetric assays. To detect the chemical entities the cyanidin reagent was used for the flavonoïds (karumi *et al*., 2004) and the Folin-Ciocalteu reagent for the polyphenols (Singleton *et al*., 1999). The experimental protocols used were as follows:

Detection of flavonoids: The test consisted in the addition of 5 ml of HCl, zinc dust and 5 ml of isoamylic alcohol to 5 ml of alcoholic extract. After three minutes, the appearance of a red-orange color indicated the presence of flavonoids.

Detection of polyphenols: 500 µl of a 10^{-1} dilution of the Folin-Ciocalteu reagent and 400 µl of a Na_2CO_3 solution (75 mg/ml), was added to l00 µl of the extract, and incubated for 2 h at room temperature. The appearance of a dark blue color indicated the presence of polyphenols. For all these tests the control consisted of a sample, without the extract, subject to the same conditions.

Thin layer chromatography and bioautography

The concentrated ethyl acetate extract was analysed by TLC. Chromatograms were observed under UV light. Direct bioautography was used to localize the antimicrobial compounds in the extract. The plate was developed using ethyl acetate-methanol (9:1, v/v).

After migration and once dried, the plate was covered with a thinlayer of LB agar mixed with a liquid culture of *C. tropicalis*. After 24 h of incubation at 30°C, the inhibition zone was measured.The sterile zone on the media proved the presence of active antimicrobial compounds (Wu *et al*., 1995). The Rf value of antimicrobial compounds were determined.

RESULTS AND DISCUSSION

Isolation and characterization of the isolate H3

In this study, an actinomycete isolate named H3 was isolated from deteriorated wood in an old house located in the former Derb Lamté in the Medina of Fez. The isolated strain was Gram positive and formed colored tough and filamentous colonies that were hard to pick from the culture media as a characteristic of actinomycetes.

The isolate H3 was obtained in the AIA medium. Uzel *et al*. (2011) found that AIA was useful for isolation because it yielded a great number of actinomycetes colonies compared to other media. Thakur *et al*., (2007) reported that the majority of their isolates were collected from the AIA medium. Indeed, this medium seems to be the most specific and sensitive for actinomycetes (Thakur *et al*., 2007; Fguira *et al*., 2012) because it contains glycerol that most actinomycetes use as a carbon source and sodium propionate which acts as an antifungal agent.

The growth of the isolate was tested on ISP2 media at different pH and temperatures. We found that the isolate grew at pH and temperature ranged from 6 to 10 and from 25 to 42°C, respectively. Goodfellow and Williams (1983) reported that most actinomycetes behave as mesophiles in the laboratory, with optimum growth temperature at 25 to 30°C. Thermophilic, psychrophilic and psychrotolerant strains have also been found.

Concerning pH, Thakur *et al*. (2007) reported that some *Streptomyces* can adapt to wide pH range whereas Kutzner(1986) and Locci(1989) reported that most streptomycetes prefer neutral to alkaline pH (optimal growth pH range between pH 6.5 to 8.0). Acidophilic and alkalophilic strains have also been found.

Some actinomycetes are capables of producing dark pigments melanin or melanoid, which are considered a useful criterion in taxonomic studies (Dastager *et al*., 2006). In our

study, no melanin was observed on peptone yeast extract iron agar or tyrosine agar which means that the isolate H3 didn't produce melanin pigments.

Molecular identification of the isolate H3

Identification of actinomycetes using molecular tools proved to be faster and least difficult compared to classical biochemical methods (Boudemagh *et al*., 2005). Among the used methodologies, PCR is widely practiced. The gene 16S rRNA is the tool mainly used for molecular identification of bacteria (Boudemagh *et al*., 2005). It is a chromo sonic gene present in all bacteria species (universal gene) whose sequence is specific to each species and whose ends 52 and 32 (15 first and 15 last bases) are conserved in all bacteria species (Valli *et al*., 2012).

In this study, the comparison of the sequence obtained (375 bp) from the amplified fragment of the 16S rDNA of H3 with sequences contained in the data banks showed that the isolate H3 was closely related to Streptomyces sp. with 100% sequence similarity. This is consistent with literature because the genus *Streptomyces* largely predominates among strains of actinomycetes (Sabaou *et al*., 1998; Lechevalier and Lechevalier, 1967).

Analysis of 16S rDNA generally allows identifying the organisms up to the genus level only. As alternatives to 16S rRNA sequences, protein-coding genes (e.g. rpoB, gyrB,nifD, recA, atpD) have been tested (Palmisano *et al*., 2001; Qi *et al*., 2001; Ko *et al*., 2003; Blackwood *et al*., 2004; La Duc *et al*., 2004). Of them, rpoB provide an acute and convenient tool for the phylogenetic analysis of the *Streptomyces* (Kim *et al*., 2004).

McTaggart *et al*. (2010) reported identiûcation of *Nocardia* species through multilocus sequence analysis (MLSA).

Table 1. Sensitivity of the active substance from the isolate H3 to Proteinase K

	Inhibition diameter against *C. tropicalis* (cm)		
	Without treatment with Proteinase K	After treatment with Proteinase K	Control
Organic extract of the isolate H3	1,9 ± 0,14	1,85 ± 0,21	0

Values are means of two replicates ± standard deviation. The control used was a solution of proteinase K

Table 2. Sensitivity of the active substance from the isolate H3 to temperature

Inhibition diameter against *C. tropicalis* (cm)				
Supernatant of the isolate H3	Supernatant not subject to heat treatment	Supernatant subject to heat treatment		
		60°C	80°C	100°C
	1.9 ± 0,14	1,95±0,07	1,95±0,07	1,9±0.00

Values are means of two replicates ± standard deviation

Screening of activity against bacteria and yeasts on different culture media

The screening of antimicrobial activity of *Streptomyces* strains usually involves a search for optimal media for their production. In fact, it's known that nutritional sources like carbon and nitrogen have profound effects on antibiotic production by theses bacteria (Himabindu and Jetty, 2006)

To determine the best production medium of antimicrobial substances by the isolate H3, the following culture medium were used: GLM, GYEA, ISP2, ISP1 and Bennett (Figure 1). The microorganisms tested were *E. coli* CIP 7624, *P. aeruginosa*27853, *P.aeruginosa*A22, *B.* subtilis, *S.* aureus, *C.albicans* and *C. tropicalis*.

First, we note that no activity was observed against all test bacteria independently of the production medium used. In addition, the isolate H3 was not active against other bacteria (*B.*

cereus, *M. smegmatis* and *M. aurum*) on ISP2 medium (data not shown). However, the use of other test bacteria and other production medium is required prior to conclude on to the possession of antibacterial activity by isolate H3.

Second, the isolate H3 exhibited antifungal activity toward the yeasts *C. albicans* and *C. tropicalis*. The activity was different depending on the production medium used. Thus, the activity was observed on Bennett, ISP2, GYEA and GLM media whereas no activity was observed on ISP1 medium. Furthermore, the best activities were obtained on ISP2 and Bennett. ISP2 medium is recognized in the literature as a good production medium (Boughachiche *et al*., 2005; Badji *et al*.,2006; Badji *et al*., 2007). So, in the following, ISP2 medium was used as the production medium.

Antifungal activity of the isolate H3 on ISP2 medium

The isolate H3 was active against *P. granulatum*, *C. cladosporioides*, *P. commune* and *A. niger* indicating that the isolate H3 possesses an antifungal activity (Figure 2). Several researchers have already reported antifungal activity of *Streptomyces* (Otani *et al*, 1998; Sakai *et al*., 2004; Sajid *et al*., 2008; Oskay, 2009). Many species of actinomycetes, particularly those belonging to the genus *Streptomyces*, are well known as antifungal biocontrol agents (El-Tarabily *et al*., 2000; Errakhi *et al*., 2007; Joo, 2005; Xiao *et al*., 2002). The antagonistic activity of Streptomyces to fungal pathogens is usually related to the production of antifungal compounds (Getha and Vikineswary, 2002; Fguira *et al*., 2005; Taechowisan *et al*., 2005) and extracellular hydrolytic enzymes (Trejo-Estrada *et al*., 1998; Mukherjee and Sen, 2006).

The activity of the isolate H3 against yeasts and fungi makes it more interesting to study because of the problems posed by fungi such as dermatophytoses, onychomycoses, *candidiasis* and *aspergillosis*.

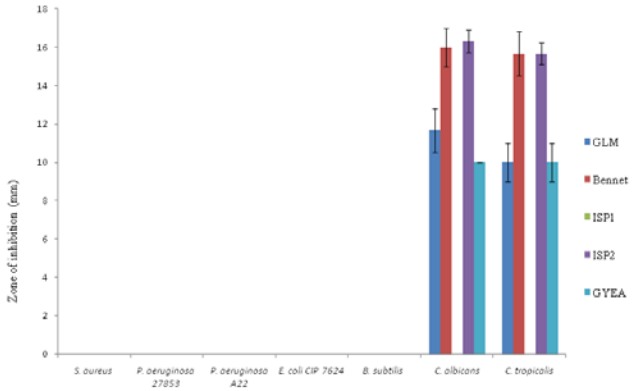


Fig. 1. Antimicrobial activity of the isolate H3 on different agar media

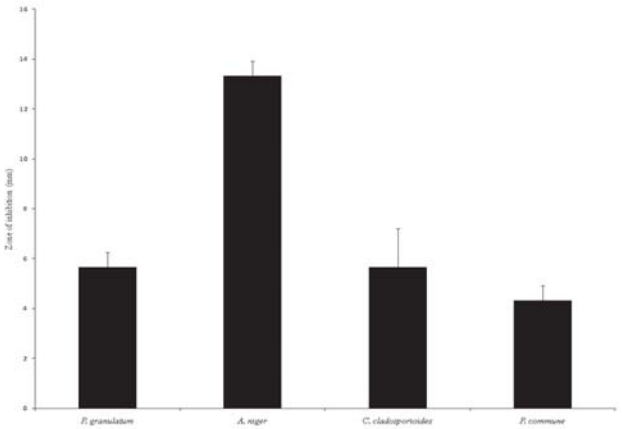


Fig. 2. Antifungal activity of the isolate H3 on ISP2 medium

Kinetics of the antimicrobial production of the isolate H3

Production kinetics was studied on ISP2 broth and the antimicrobial activity was evaluated against *C. tropicalis* (Figure 3). The antimicrobial activity appeared at the 5th day of incubation and increased to reach its maximum with the 9thday.

Yepes *et al*. (2011) reported that the onset of antibiotic production depends on the growth stage of the microorganism and usually takes place with differentiation of the aerial mycelium into spores. Whereas Osman *et al*. (2011) found that the antibiotic biosynthesis takes place in the stationary phase or may be growth associated and maximum production can be attain in the log phase.

Badji *et al*. (2006) found that the antifungal activity of Actinomadura sp. AC104 was detected after 4 days of culture and reached a maximum after 7 days. In addition, Kojiri *et al.* (1992) reported that the optimum production by *Streptomyces* sp. was reached at 6th day of incubation whereas Bouras *et al*. (2013) reported that the time of maximal antimicrobial activity by *Streptomyces* sp.PP14 was obtained always after 7, 8 or 9 day of fermentation.

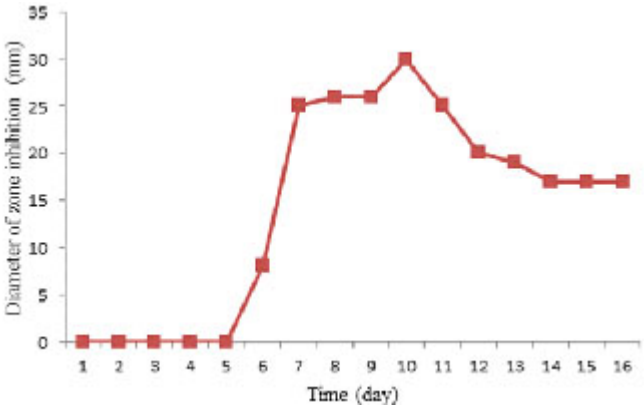
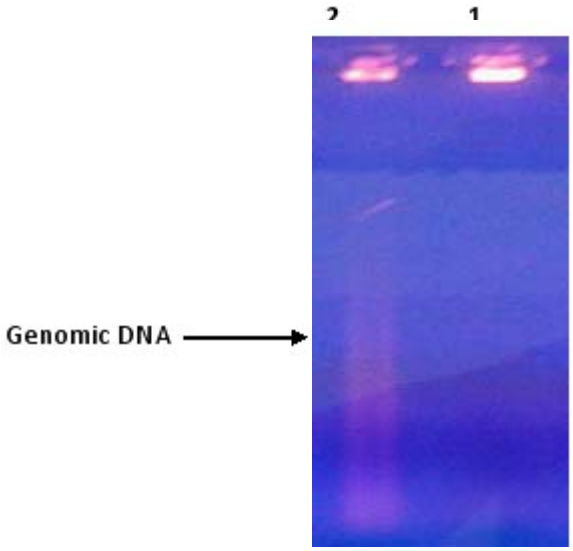


Fig. 3. Kinetics of antimicrobial activity of the isolate H3 against *C. tropicalis*



1: Control corresponding to DNA extraction with physiological water
2: DNA extraction of *C. tropicalis* with the active substance of the isolate H3

Fig. 4.Agarose electrophoresis analysis of *C. tropicalis* genomic DNA extracted with the active substance produced by the isolate H3

Partial characterization of the antimicrobial product

The active substance of the isolate H3 inhibited *C. tropicalis* growth creating an inhibition zone around the wells. This activity wasn't eliminated upon treatment by Proteinase K (Table 1) or temperature (60°C, 80°C and 100°C) (Table 2). Thus, the active substance would be of non protein nature.

Extraction of nucleic acids of *C. tropicalis* by the organic extract of the isolate H3

The active substance from the isolate H3 strain was extracted by ethyl acetate, recuperated in physiological water (previously explained in methods) and then tested for its capacity to lyse *C. tropicalis*. The figure 4 shows that the active substance was able to extract nucleic acids from *C. tropicalis* in contrast to the control.This extraction was carried out in the absence of conventional agents of lysis, such as lysozyme, SDS or proteinase K, indicating that the active ingredient acts at the *C. tropicalis* wall level. The use of more important concentration of the purified active substance would make it possible to increase the output of candida genome extraction. Due to its action on the candida wall, this active substance would be of great interest. For example, yeast-lysing glucanases have an enormous field of applications like their use for nucleic acid extraction from yeast and transformation (Salazar and Asenjo, 2007). Amphotericin B, a polyene with high affinity for sterol binding, is one of the most potent antifungal drugs; its mechanism produces pores in the membrane surface of the yeast, resulting in leakage of the cell contents (Odds, *et al*., 2003). Nystatin obtained from *Streptomyces noursei* binds to ergosterol in the fungal membrane, producing membrane permeability changes which allow the release of K+, sugars and metabolites (Carrillo-Muñoz *et al*., 2001; Carrillo-Muñoz *et al*., 2004; Zotchev, 2003). Disruption of the cell membrane is believed to be responsible for fungal death. Igarashi *et al*. (2003) found that Yatakemycin, an antifungal antibiotic produced by *Streptomyces* sp. TP-A0356, inhibited the growth of pathogenic fungi such as *Aspergillus fumigatus* and *C. albicans*.

Detection of flavonoids and polyphenol

Flavonoids are important plant specific secondary metabolites that are synthesized from the general phenylpropanoid pathway and have

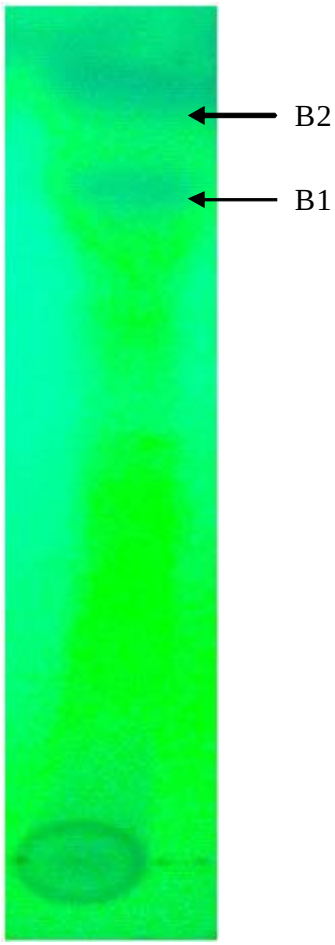


Fig. 5. TLC analysis of the crude extract of Isolate H3 visualized under UV

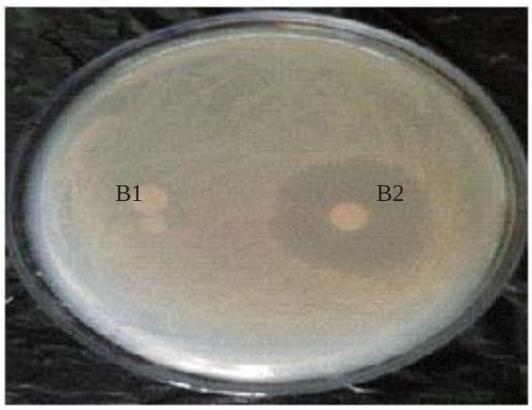


Fig. 6. Effect of the two products (B1 and B2) on *C. tropicalis* growth

many pharmaceutical and nutraceutical functions (Du *et al*., 2011). Separation of pure flavonoids from plants material is very difficult and chemical syntheses of flavonoids require extreme reaction conditions and toxic chemicals. However, microorganisms are becoming increasingly important as flavonoids producer.

Results of the phytochemical showed the presence of flavonoids and total polyphenols in the crude extract of the strain H3.

Previous studies have shown that the secondary metabolites flavonoids produced by *Streptomyces* sp. were toxic to mosquitoes (Rao *et al*., 1990). Maskeya *et al*. (2003) described the structure elucidation of genistein-4'-(6"-methyl)-salicylate and 4',7-bis-(² -cymaropyranosyl)-genistein, two isoflavoinoids isolated from terrestrial actinomycetes.

Thin layer chromatography and bioautography

The antifungal effect of the ethyl acetate extract of strain H3 was visualized using TLC to locate the active substances responsibles for this biological activity. TLC silica gel analysis of the crude extract of H3 showed two bands B1 and B2 having respectively the following Rf values 0.66 and 0.76 (Figure 5). After elution and evaporation of the silica gel around the inhibition zone, the product was recuperated in 20 µl of sterile distilled water and tested using the disc method. Figure 6 shows that the active spots was B2.

CONCLUSION

A *Streptomyces* strain producing antifungal substances was isolated from detriorated wood. The active substance was partially characterized and purified. This substance might act on the cell wall of *C. albicans*. However, additional research is needed to clarify its chemical structure and elucidate its mode of action. In addition, the study of its toxicity is requiered to demonstrate its importance for possible clinical use to overcome the problems posed by fungal infections.

ACKNOWLEDGMENTS

We thank the CNRST Morocco for financial support; we also thank the Innovation Center University Sidi Mohamed Ben Abdellah, Fez, Morocco for BIOSYSTEME 3130 automated gene sequencer analysis.

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