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Full Length Research Paper

Isolation and screening of *Streptomyces* from soil of Tunisian oases ecosystem for nonpolyenic antifungal metabolites

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The purpose of this study was to screen isolates of *Streptomyces* producing nonpolyenic antifungals. This choice was made to limit the problem of rediscovery of well-known antifungal families, especially polyenic antifungals. 68 *Streptomyces* strains were isolated from the soil sample collected from Tunisian oases ecosystem. These strains were tested for their capacity to produce active compounds using the diffusion method against two bacteria: *Escherichia coli* ATCC 8739, *Micrococcus luteus* LB 14110; two filamentous fungi: *Verticillium dahliae* and *Fusarium* sp. and two yeasts: *Candida tropicalis* R2 CIP203 and *Candida albicans* ATCC 2019. Among these isolates, 40 strains (58.82%) showed antibacterial activity, 18 strains (26.47%) showed antifungal activity, while 12 strains (17.64%) exhibited a broad-spectrum activity against all tested indicator cells. The production of nonpolyenic antifungal metabolites by promising isolates was investigated using their antibacterial activity and ergosterol inhibition as well as the UV-vis spectra of their corresponding active extracts. The obtained results showed that 13 isolates (19.11%) produced nonpolyenic antifungal activity. These data indicate the richness of the Tunisian oases ecosystem in actinomycetes bacteria producing active compounds. This fact, may partly explain the resistance phenomena of the Tunisian oasis palms against some phyto-pathogen fungus such as *Fusarium oxysporum* sp. *albidenis* (bayoud).

Key words: Soil of Tunisian oases ecosystem, *Streptomyces,* nonpolyenic antifungal activity.

INTRODUCTION

Despite the long list of currently available antibiotics in the market, antifungal antibiotics are a very small but significant group of drugs that have important roles in several fields: human and animal therapy, agriculture for protection of plant, food industries and treatment of wood. Only a limited number of antifungal agents are currently available for the treatment of life-threatening fungal infections (Vicente et al., 2003). The need for new, safe and more effective antifungals is a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immunocom-promised host. However, many compounds (polyenes in particular) cannot be used because of their toxicity, while most of them are of interest in animal therapy, agricultural industry. These antifungal agents show some limitations, such as the significant nephro-toxicity of amphotericin B (Georgopapadakou and Walsh, 1994). The toxicity of present antifungal therapy is due to the biochemical similarity between fungal pathogens and infected hosts (all eukaryotes).

The search for a new, safer, broad-spectrum antifungal antibiotic with greater potency has been progressing slowly (Gupte et al., 2002). The development of new antifungal agents, preferably naturally occurring with novel mechanisms of action, is an urgent medical need. Soil, in particular, is an intensively exploited ecological niche, the inhabitant of which produces many useful biologically active natural products, including clinically important antibiotics. The species belonging to the genus *Streptomyces* constitute 50% of the total population of soil actinomycetes (Williams et al; 1983), and 75 to 80%

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of the commercially and medicinally useful antibiotics have been derived from this genus (Miyadoh, 1993). In the course of screening for new antifungal antibiotics, several studies are oriented towards isolation of new *Streptomyces* species from different habitats (Ouhdouch et al., 2001; Hilali et al., 2002; Lemriss et al., 2003; Thakur et al., 2007).

Fusarium oxysporum sp. *albidenis* (Foa) fungus (Fernandez et al., 1998; El Hadrami et al., 1997) has caused destruction of a large number of palms in the oases of Algeria and Morocco but not for those in Tunisia. This surprising fact could be due to the physico-chemical characteristics of Tunisian oases soil, and/or to the presence of antagonistic microorganisms which might inhibit Foa development and dissemination. Hence, the screening of *Streptomyces* from rhizospheric soil of Tunisian oases ecosystem, presented a special attention to explore the potentialities of the diverse micro flora of this region. The present research was undertaken with an aim of highlighting the presence of actinomycetes, especially the genus *Streptomyces*, from different protected Tunisian oases soil, and express results obtained in the search for nonpolyenic antifungal metabolites produced by these strains.

MATERIALS AND METHODS

Sample selection and microbial strains

Sample was collected from the Tunisian oases ecosystem: rhizosphere soil of date palm ("Deglet Nour"). This sample was taken after removing approximately 20 cm of the soil surface and it was placed in sterile polyethylene bags, closed tightly and stored in the refrigerator at 4°C until use.

The target strains used for screening antimicrobial activity were procured from microbial type culture collection: Bacterial strains *Escherichia coli* ATCC 8739 (Gram-negative bacteria) and *Micrococcus luteus* LB 14110 (Gram-positive bacteria), were used as indicator microorganisms for antibacterial activity assays. Antifungal activity was determined against *Verticillium dahliae*, *Fusarium* sp., *Candida albicans* ATCC 2091 and *Candida tropicalis* R2 CIP203 and amphotericin B-nystatin resistant (amphotericin B and nystatin are polyenic antifungal).

Isolation of *Streptomyces*

One gram of the sample soil was suspended in 100 ml of physiological water (NaCl 9 g/L) then incubated in an orbital shaker incubation at 30°C with shaking at 200 rpm for 30 min. Mixture was allowed to settle, and serial dilutions up to 10^{-5} were prepared using sterile physiological water and agitated with the vortex at maximum speed. An aliquot of 0.1 ml of each dilution was taken and spread evenly over the surface of *Streptomyces* isolation agar (5 g/L glucose; 4 g/L sodium propionate; 2 g/L casein; 0.5 g/L K_2HPO_4; 0.5 g/L $MgSO_4.7H_2O$; 200 ml sterile soil extract (equal volumes of soil and distilled water were mixed overnight and filtered after sterilization at 120°C for 15 min), pH 7.2 and 20 g/L agar). The medium was then added to 5 µg/ml ampicillin and 50 µg/ml nystatin or cycloheximid to inhibit bacteria and fungal contamination, respectively. Plates were incubated at 30°C and monitored after 3, 5 and 7 days. *Streptomyces* colonies were recognized on the basis of morphology by height microscopy (G × 10). The isolates were identified morphologically as genus *Streptomyces* by comparing the morphology of spore bearing hyphae with entire spore chain as described in Bergey's Manuel (Holt et al., 1994). Finally, representative colonies were selected and streaked on new plates of *Streptomyces* isolation agar medium. Agar plates were then inoculated with the strains and incubated at 30°C until good growth was observed. The isolated strains were conserved at 4°C for two months, and in a freezer at -80°C in the presence glycerol (15%, v/v) for a longer period.

Submerge culture conditions

Isolates that showed activity against test organisms in agar medium were grown in the submerged culture in 250 ml flasks containing 50 ml of Bennett medium (1 g/L beef extract, 10 glucose, 2 g/L peptone, 1 g/L yeast extract). A 2 cm^2 piece of agar from each seven-day-old culture grown on *Streptomyces* isolation agar medium was used to inoculate the flasks. These cultures were grown in rotary shaker at 200 rpm, 30°C, for seven days. The resulting culture broths (approximately 50 ml), obtained following growth of each isolate in the culture media were separated from the mycelium by centrifugation at 900 rpm for 15 min. The supernatant, sterilized by filtration, was used for extracellular antimicrobial activity by agar well diffusion with one of the test organisms.

More also, for the determination of antibacterial activities, indicator microorganisms were grown overnight in LB medium at 30°C for *M. luteus* LB 14110 and at 37°C for *E. coli* ATCC 8739, then diluted 1:100 in LB medium and incubated for 5 h under constant agitation of 200 rpm at the appropriate temperature. Furthermore, for antifungal activity determination, *C. tropicalis* R2 CIP203 and *C. albicans* ATCC 2091 were grown in YP10 medium (10 g/L yeast extract, 10 g/L peptone, 100 g/L glucose, 15 ml of 2 g/L adenine solution) at 30°C for 24 h in an orbital incubator with shaking at 200 rpm. In addition, *V. dahliae* and *Fusarium* sp. were grown in potato dextrose agar (PDA) for 7 days at 30°C. Spores were collected in sterile distilled water then adjusted to a spore density of approximately 10^4 spores/ml.

Biological assay of antimicrobial activity

To isolate new actinomycete strains producing antimicrobial activities, we used the solid media bioassay test against *M. luteus* LB 14110, *E. coli* ATCC 8739, *V. dahliae, Fusarium* sp., *C. albicans* ATCC 2091 and *C. tropicalis* R2 CIP203. In solid medium, the antimicrobial activity was determined by the plate diffusion method (Bauer et al., 1966). After incubation of the selected strains for 7 days at the appropriate growth temperature, an agar disk (10 mm in diameter) was recuperated and placed in LB plates covered by 3 ml of top agar containing 50 µL of a 5 h culture of *M. luteus* LB 14110 or *E. coli* ATCC 8739 test strains. Plates were first kept in a refrigerator (4°C) for at least 2 h to allow the diffusion of any antibiotics produced, then incubated overnight at 30°C for *M. luteus* and at 37°C for *E. coli*. For antifungal activity determination, plates containing Sabouraud agar medium were covered with 3 ml of top agar containing 100 µL of spore suspension already prepared from *V. dahliae* or *Fusarium* sp. or *F. oxysporum* sp. *albidenis* (Foa) and by 50 µL of *C. tropicalis* R2 CIP203 or *C. albicans* ATCC 2091 culture. After 2 h at 4°C, the plates were incubated at 30°C. The antimicrobial activity was observed after 24 h for bacteria and 48 h for fungi and yeast.

Moreover, in liquid medium, a paper disk was impregnated with 50 µL of the corresponding sample and then laid on the surface of an agar plate containing 3 ml of top agar seeded by 40 µL of a 5-h old culture of one of the bacteria used for antibacterial tests: *M. luteus* LB 14110, *E. coli* ATCC 8739 and by 50 µL of *C. tropicalis*

R2 CIP203 or *C. albicans* ATCC 2091 culture or 100 µL of spore suspension of *V. dahliae* or *Fusarium* sp. for antifungal activities. After 2 h at 4°C, plates containing *M. luteus*, *C. tropicalis* R2 CIP203, *V. dahliae* and *Fusarium* sp. were incubated at 30°C and those inoculated with *E. coli* at 37°C, all overnight except *V. dahliae* and *Fusarium* sp. for 48 h.

Organic crude extracts preparation and thin layer chromatography (TLC) analysis

Crude antimicrobial compound was recovered from the culture filtrate of each active isolate by solvent extraction with ethyl acetate. Ethyl acetate was added to the filtrate in the ratio 1:1 (v/v) and shaken vigorously for 20 min. The organic layers were collected and the organic solvent was evaporated to dryness in a vacuum evaporator at 40°C to obtain a gummy crude extract which was recuperated in 0.5 ml of ethyl acetate and assayed against indicator microorganisms. For TLC analysis of the crude extract of the isolate TN80, a small drop of a sample was spotted onto TLC plate (Silica gel 60 F_{254}) with a capillary and dried; the spotting process was repeated by superimposing more drops on the original spot for obtaining appropriate quantity (2 to 5 µg) of the sample on the plate. The TLC plate was developed with a CH_2Cl_2/5% MeOH solvent system, and was sprayed with anisaldehyde/sulphuric acid.

Screening for antifungal polyenic and nonpolyenic metabolites

In order to determine the effect of active compounds from selected *Streptomyces* isolates on the ergosterol present in the fungal cell membrane, ergosterol was used as the reversal agent to test for its ability to reverse the inhibition of *C. albicans* by antibiotic (Ouhdouch et al., 2001). Sabouraud agar plates with 50 mg/ml ergosterol were prepared along with a control without ergosterol. The plates were seeded with *C. albicans* and ergosterol inhibition was tested by disc diffusion method. Sterile filter paper discs were impregnated with 50 µL organic crude extract suspension, dried and placed onto plates previously seeded with test microorganisms. The plates were incubated at the appropriate growth temperature of the indicator microorganisms for 24 h and then observed for the inhibition zone. More also, the absorption spectrum of active extracts in methanol were recorded in the region (200 to 440 nm) by using a UV-vis spectrophotometer and compared with those of known polyenic antifungal.

RESULTS AND DISCUSSION

Isolation of *Streptomyces*

This study was undertaken with the aim of highlighting the presence of *Streptomyces* in Tunisian oases ecosystem and selecting the strains with antibacterial and antifungal activity. Using the selective medium and the cultivation conditions previously described, a total of 68 different *Streptomyces* isolates were obtained from a soil sample collected from the Tunisian oases ecosystem: rhizosphere soil of date palm ("Deglet Nour"). All *Streptomyces* were isolated at mesophilic temperatures (25 to 37°C). These results are in agreement with other authors (Williams et al., 1989; Awad et al., 2009) who found that most species of *Streptomyces* were isolated at mesophilic temperature. With the exception of some *Streptomyces* that can adapt to wide pH range (Thakur et al., 2007), *Streptomyces* are usually neutrophiles and cultivated in medium of pH 7.0 to 7.5 at temperatures of 25 to 37°C for 7 days.

Furthermore, these strains were isolated on the *Streptomyces* isolation agar medium. This medium seems to be the most specific and sensitive for *Streptomyces*, since it contains sodium propionate which acts as an antifungal agent to inhibit the fungal contamination and glucose, which most *Streptomyces* use as a carbon source. In addition, its transparency facilitates colony observation. Several authors rightly consider that the use of antibiotics is an essential precaution in the isolation of *Streptomyces* (Kitouni et al., 2005; Errakhi et al., 2009). On the other hand, ampicillin (5 µg/ml) was added to the medium to inhibit bacterial contamination. The addition of antifungal agents to the isolation medium suppresses the growth of fungal species on the plates. For this purpose either cycloheximid (50 µg/ml) or nystatin (50 µg/ml) were used. These results were therefore anticipated because several studies have shown the importance of the constituents of the screening medium under which the producing microorganisms were cultivated (Iwai and Omura, 1992).

Characterization of *Streptomyces* isolates

All isolates grew on a range of agar medium showing morphology typical of *Streptomyces* (Locci, 1989), since the colonies were shown growing, aerobic, chalky, folded and with aerial and substrate mycelium of different colours. In addition, all colonies possessed an earthy odour.

The cultural characteristics (pigment production) and morphological characteristics (colour series) of the 68 *Streptomyces* isolates are presented in Table 1. All of these isolates fitted the genus description as previously reported (Williams et al., 1943, 1983). The colour of the substrate mycelium and aerial spore mass also varied. *Streptomyces* isolates were categorized into nine colour series according to the colour of their mature sporulated aerial mycelium, with gray and white colour series being the most abundant (Table 1). Members of the gray series were found to represent 30.8% of the total number of isolates; however, the lowest occurrence was noted for the orange, violet and red series (2.9% for each). The highest occurrence of isolates of the gray series was in agreement with that earlier reported (Ndond and Semu, 2000; Barakate et al., 2002). Nevertheless, Thakur (2007) in his study on distribution of *Streptomyces* in forest areas reported that the white colour class dominated (46%).

Table 1 also shows that of the 68 *Streptomyces* isolates, 13 isolates (19.11%) produced melanin and 30 isolates (44.11%) produced soluble pigment. These percentages were less than that obtained by Ndonde

Table 1. Morphological and cultural characteristics of the sixty-eight *Streptomyces* isolates.

Parameter	Colour series									
	Gray	White	Yellow	Green	Cream	Pink	Orange	Violet	Red	Total
Number of isolate	21 (30.8)	16 (23.5)	10 (14.7)	3 (4.4)	7 (10.2)	5 (7.3)	2 (2.9)	2 (2.9)	2 (2.9)	68 (100)
Pigment production										
Melanin	5	2	1	0	3	1	0	0	1	13 (19.11)
Soluble	12	4	7	2	3	1	0	1	0	30 (44.11)

Numbers in parentheses represent the percentage out of the total isolates.

(2000) and comparable to that obtained by Thakur (2007). The production of melanoid pigments varied in all the series with the exception of the strains grouped in the orange series, where none produced melanoid pigments. The differences in colour of the aerial mycelium of the isolates as well as those of the pigments they produce may be an indication of the diversity of *Streptomyces* isolates in the site investigated.

Antimicrobial activity

In order to assess whether *Streptomyces* isolated from the rhizospheric soil of date palm ("Deglet Nour") from Tunisian oasis may be useful in screening for natural bioactive compounds, the antimicrobial activity of the isolates was determined (Table 2). Our result indicate that 67% of isolates were active against one or more of the test organisms. This percentage was higher than as describe by many authors studying the activity of soil actinomycetes (Saadoun et al., 1998; Barakate et al., 2002). However, others reported almost an equal percentage (Ndond and Semu, 2002). Data in Table 2 also showed that the percentage of active isolates varied within each colour series. The most active compounds producing isolates belonged to the yellow and red colour series (100% for each) and only 85.7% of cream isolates were active against one or more of the test organisms. Barakate et al. (2002) while studying the antimicrobial activity of isolates from Moroccan habitats soil found that the higher percentage of active isolates was found in white and red ones. Arai (1976) however, reported that most active species of *Streptomyces* were found in the gray and yellow series of no chromogenic type and no antibiotic-producing species were described in the white and green series of chromogenic type.

Moreover, almost 22 of *Streptomyces* isolates (32.35%) did not show any antibiotic activity towards the test organisms using Bennett's agar as medium of production, although, it is probable that they produced other useful compounds for which they were not screened in this study. In fact, it should be noted that production of secondary metabolites by microorganisms is often connected with and influence by primary metabolism. The composition and concentrations of the constituents of the media are closely linked with the metabolic capacities of the producing organism and greatly influence the biosynthesis of the bioactive molecules (Elleuch et al., 2010). Porter (1971) stated that probably all *Streptomyces* possessed some antimicrobial proprieties if proper conditions were taken into consideration during culturing of these organisms for purposes of assessing their antibiotic production.

In Table 2, antibacterial activity was observed in 40 isolates (58.82%), 18 isolates (26.47%) exhibited antifungal activity, while only twelve isolates of *Streptomyces* (17.64%) showed the both activity. In previous studies, it was shown that the isolation rate of *Streptomyces* with antimicrobial activity is higher than 40% (Lemriss et al., 2003) and in others less than 10% (Jiang and Xu, 1996). These results confirm that the *Streptomyces* are able to produce a wide variety of antifungal activity. The comparison of the antimicrobial activity between all the colour classes against the tested organisms showed that isolates in the gray and yellow series displayed the highest antibiosis against the bacteria organism tested; while no activity was noted in the red and orange series against this test organism. Isolates in the yellow series were found to be most active against fungi. These differences in percentage of antimicrobial activity may imply that the investigated *Streptomyces* isolates belonged to different species or to the same, but they produced different bioactive compounds. In fact, we notice that in the same colour class, most of the isolates showed different activity spectrum.

Furthermore, results of antimicrobial activity expressed in terms of the diameter of inhibition zone seem to confirm this hypothesis (Table 3). The inhibition zone of antimicrobial activity of *Streptomyces* isolates against the test microorganisms is different; some isolates exhibited a strong activity (>30 mm) especially against Gram-positive bacteria, but the most active isolates show a moderate antimicrobial activity (<20 mm). The highest percentage of active isolates was obtained against *M. luteus* (55.88%); while the lowest percentage was exhibited against *E. coli* (19.11%). The antifungal activity of *Streptomyces* strains against *Fusarium* sp. and *V. dahliae* was almost equal (26.47%). Many authors

Table 2. Antimicrobial activity of the sixty-eight *Streptomyces* isolates.

Parameter	Colour series									
	Gray	White	Yellow	Green	Cream	Pink	Orange	Violet	Red	Total
Number of isolate	21	16	10	3	7	5	2	2	2	68 (100)
Number of active isolate	12 (57.1)	9 (56.2)	10 (100)	1 (33.3)	6 (85.7)	3 (60)	1 (50)	1 (50)	2 (100)	46 (67.64)
Number of active isolates against bacteria	12	8	10	1	5	3	0	1	0	40 (58.82)
Number of active isolates against fungi	1	1	8	0	1	3	1	1	2	18 (26.47)
ATB + ATF[a]	1	1	6	0	0	3	0	1	0	12 (17.64)

Numbers in parentheses represent the percentage out of the total isolates. [a]ATB, Antibacterial activity; AFA, antifungal activity.

also that the *Streptomyces* isolates appear to be highly active against Gram-positive bacteria (Barakate et al., 2002; Thakur et al., 2007). However, the percentage of isolates active against fungi seems to be lower than that reported previously in *Streptomyces* screening.

The antifungal activity of isolates was determined by the agar diffusion method against *Fusarium* sp. and *V. dahliae*. This plant pathogen is a causal agent of verticillium wilts of tomato, potato and olive-tree. *F. oxysporum* sp. *albidenis* (Foa) (El Hadrami et al., 1997; Fernandez et al., 1998) an other plant pathogen, has caused destruction of a large number of palms in the oases of Algeria and Morocco, but not to those in Tunisia. In the course of study for antimicrobial activity against *F. oxysporum* sp. *albidenis*, five different isolates of *Streptomyces* were selected for their strong antifungal activity using those crude extracts. According to the Table 3, these isolates nominated, TN80, TN84, TN86, TN87 and TN93, were categorized into five colour series yellow, red, orange, pink and cream, respectively. As indicated in Table 4 however, three isolates (TN84, TN86 and TN93) did not showed any antibacterial activities but the two isolates (TN80 and TN87) presented an antibacterial activities against Gram positive (*M. Luteus* LB 14110).

More also, according to the data in Table 4, the TN80 strain showed the strongest antifungal activities against both fungi, *F. oxysporum* sp. *albidenis* and *Fusarium* sp. TLC silica gel analysis of the active crude extract of the culture of the TN80 showed three dark bands having respectively the following R_f values: B1 = 0.4; B2 = 0.53 and B3 = 0.63 (Figure 1). Hu et al. (2006) reported that in sulphuric acid reaction in TLC analysis, macrolides appear generally dark. Taking into account this finding and in consideration of our results, we can assume the hypothesis that the strain TN80 produces simultaneously three different macrolide molecules. Nevertheless, due to their complex structure and high molecular weight, macrolide antibiotics, are not able to penetrate the cell walls of most Gram-negative bacteria (Mellouli et al., 2003). Therefore, we can explain the inhibitory activity against the Gram-negative bacterium (*E. coli* ATCC 8739), of the strain TN80 (in solid and liquid media), by the fact that TN80 isolate secret at least one other active compound, beside the three macrolide molecules that inhibits the growth of Gram-negative bacteria.

Detection of polyenic and nonpolyenic antifungal activity

As shown in Table 2, among the 68 isolates, 18 strains (26.47%) showed antifungal activity against at least one of the tested fungi, while 13 (19.11%) out of these 18 isolates inhibited the *C. tropicalis* R2, an amphotericin B-nystatin resistant strain. The production of nonpolyenic antifungal substances by the eighteen isolates having antifungal activities was investigated using several criteria: antibacterial activity, ergosterol inhibition and UV-vis spectra of active extracts (Ouhdouch et al., 2001; Lemriss et al., 2003). Ergosterol present in fungal cell membrane has a very high affinity towards polyene antibiotics. Polyene drugs form complexes with ergosterol, which open channels in the fungal membrane that cause leakage of critical intracellular constituents and subsequent cell death. This behaviour is exploited in a detection method developed to identify the presence of polyene class of antibiotics (Bastide et al., 1986). Interpretation of resultants are as follows: reduced zone in size in presence of ergosterol-polyene type of antibiotic present and no reduced zone in presence of ergosterol-polyene type of antibiotic absent (Table 5). The use of spectroscopy to distinguish polyenic from nonpolyenic substances were also used by several authors (Thakur et al., 2007; Saadoun et al., 2009). The UV spectral data for the ethyl acetate extract of selected active fermented broth are shown in Table 5. According to this table,

Table 3. Inhibition zones of antimicrobial activity of *Streptomyces* isolates against test microorganisms.

Test microorganism	Inhibition zone			Number of active isolate
	<20	20 to 30	>30	
Escherichia coli	9	3	1	13 (19.11)
Micrococcus luteus	26	9	3	38 (55.88)
Fusarium sp.	10	7	1	18 (26.47)
Verticillium dahliae	13	4	1	18 (26.47)

Numbers in parentheses represent the percentage out of the total isolates.

Table 4. Inhibition zones of antifungal activity of *Streptomyces* isolates against *Fusarium oxysporum* sp. *albidenis* (Foa) and *Fusarium* sp.

Isolates (colours)	ATB[a]	Inhibition zones (mm) of antifungal activity against	
		F. oxysporum* sp. *albidenis	***Fusarium* sp.**
TN80 (yellow)	+	45	60
TN87 (pink)	+	25	30
TN86 (orange)	-	20	28
TN84 (red)	-	14	20
TN93 (cream	-	11	16

[a]Antibacterial activity against *M. Luteus*.

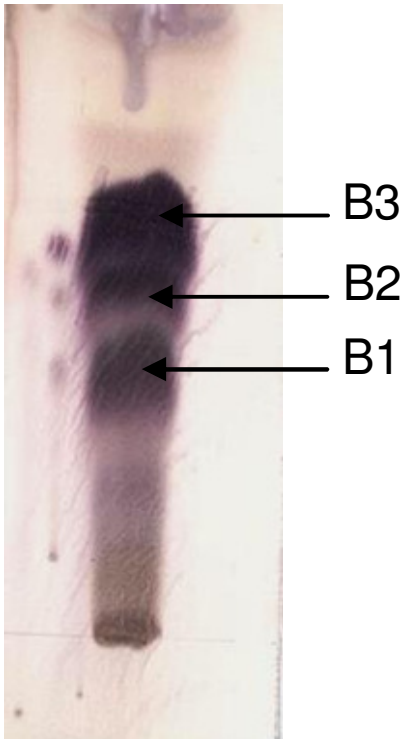

Figure 1. TLC analysis of the crude extract of TN80 isolate after treatment with anisaldehyde/H_2SO_4 solution.

thirteen isolates (TN80, TN84, TN86, TN87, TN93, TN210, TN211, TN212, TN223, TN224, TN226, TN236 and TN245) appeared promising because of activity against *E. coli* (cell membrane without sterols) and no

Table 5. Results of screening for nonpolyenic antifungal producing *Streptomyces.*

Isolates	Antibacterial Activity[a]	Activity against *C. tropicalis* R2	Ergosterol's effect against *C. albicans* ATCC 2091[b]		UV-vis spectral
			With	Without	
TN80	+	+	54	55	NP
TN84	+	+	32	32	NP
TN86	+	+	37	38	NP
TN87	+	+	42	44	NP
TN93	+	+	26	25	NP
TN97	-	-	0	20	P
TN201	-	-	0	22	P
TN210	+	+	13	12	NP
TN211	+	+	19	17	NP
TN212	+	+	22	22	NP
TN223	+	+	14	12	NP
TN224	+	+	16	18	NP
TN226	+	+	38	36	NP
TN236	+	+	12	17	NP
TN244	-	-	0	18	P
TN245	+	+	20	21	NP
TN246	-	-	0	13	P
TN247	-	-	0	20	P
Reference[c]	-	-	4	15	P

[a]Activity against *E. coli* ; [c] Amphotericin B ; NP: nonpolyenic, P: polyenic; [b] inhibition zone in mm in Sabouraud agar medium with or without 50 mg/ml of ergosterol.

marked inhibition of antifungal activity by exogen ergosterol (target of polyenic antifungal compounds). In addition, maximum absorbance peaks range between 200 and 440 nm and the characteristics of absorption peaks indicate a highly polyene nature. The broad-spectrum of activity shown in these isolates is possibly due to the production of different compounds. The metabolites produced by those thirteen isolates did not show a UV-vis spectrum characteristic of a polyenic structure. Of particular interest, these thirteen isolates (19.11%) apparently do not synthesize polyene-like substances. Similarly, Lemriss et al. (2003) when screening for nonpolyenic antifungal metabolites in clinical isolates of actinomycetes, found that only five *Streptomyces* (4.54%) exhibited nonpolyenic antifungal activity. In our study, it was shown that the isolation rate of *Streptomyces* with non polyenic antifungal activity was higher than 11% (Thakur et al., 2007) and more than 15% (Ouhdouch et al., 2001). More also, the Thirteen (19.11%) isolates (TN80, TN84, TN86, TN87, TN93, TN210, TN211, TN212, TN223, TN224, TN226, TN236 and TN245) have been selected for addition studies. The identification of these selected isolates, as well as the isolation, purification and the structural elucidation of corresponding active compounds are under investigation.

Conclusion

The southern region of Tunisia is characterized by the date palm oases, which constitute the principal financial resources of oases population. In this region, the major palm plantations are marked by prevalence of the elite variety "Deglet Nour". This variety is highly susceptible to the fungus *Fusarium oxysporum* sp. *albidenis* known as 'bayoud', which destroys a high number of palms in the oases of Algeria and Morocco, but unexpectedly not in those of Tunisia. Reasons for this surprising fact are still unknown, but may have an origin in antagonistic microorganisms as we assumed. The investigation of bacteria producing activity against fungi especially against 'bayoud' may therefore provide a significant contribution to understand the resistance phenomena of the Tunisia oasis palms and also an important ecological and economical contribution.

In this study, sixty-eight *Streptomyces* strains were isolated from Tunisian oases ecosystem. Among these isolates, forty strains (58.82%) showed antibacterial activity, eighteen strains (26.47%) showed antifungal activities; among them thirteen promising isolates (19.11%) seems to produce nonpolyenic antifungal activities. This high level of isolates producing antifungal

and nonpolyenic active compounds, compared to previous works (Ouhdouch et al., 2001; Lemriss et al., 2003; Thakur et al., 2007), may explain the protected areas of Tunisian oases ecosystem against the phyto-pathogen fungus such as *F. oxysporum* sp. *albidenis*. Equally, this Tunisian oases ecosystem represents a potential source for the discovery of new nonpolyenic antifungal compounds.

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