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Isolation of a new *Staphylococcus epidermidis* strain with anti-mycobacterial activity and determination of the nature of its active substance

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Tuberculosis is the second cause of death from infectious disease. The resurgence of tuberculosis and the increase in mycobacterial infections, as well as the multidrug-resistance of these bacteria to available antibiotics, has encouraged research on new antimycobacterial agents. In this context, water and soil samples from different Moroccan biotopes have led to the isolation of a bacterial strain, capable of inhibiting mycobacterial growth (*Mycobacterium smegmatis* MC^2 155 and *Mycobacterium aurum A*$^+$). This effect was found to be due to a secreted substance. 16S RNAr gene sequencing identified the isolate as *Staphylococcus epidermidis*. The biologically active substance from this strain was extracted using ethyl acetate. The precipitate was not sensitive to proteinase K and therefore not a protein. Analysis of the ethyl acetate extract by thin layer chromatography allowed the identification of the anti-mycobacterial fraction. A phytochemical study identified polyphenol in the bioactive fraction responsible for the observed antimycobacterial agents.

Key words: Tuberculosis, mycobacteria, antimycobacterial agents, *Staphylococcus epidermidis*, polyphenol.

INTRODUCTION

Tuberculosis caused by *Mycobacterium tuberculosis*, as well as other members of the *M. tuberculosis* complex, remains undeniably a menace to Public Health (Musser, 1995; Berthet et al., 1999; Rossetti et al., 2002). Nevertheless, the discovery of effective antituberculosis agents and the advent of chemotherapy (Boyars, 1990), the implementation of large scale *Mycobacterium bovis* BCG (*Bacille de Calmette et Guérin*) vaccination programs (Ann Ginsberg, 2002; Bonnaud, 1996) and the improvement in living conditions resulted in a considerable decline of the disease in the industrialized nations (the infection rate dropped from 200/100.000 inhabitants in 1900 to less than 10/100.000 inhabitants in 1980) (Rastogi et al., 2001; Jost et al., 2001).

However, in the early 1990's, a global rise in incidence was observed due to increased population mobility, human migrations of economic and political origin and deficiencies in control programs where many patients failed to received adequate treatment (Newton et al., 2000). This context has favored the problematic appearance of multidrug resistant strains (Jost et al., 2001). Moreover, immune suppressed patients, namely with human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS) disease, are

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particularly vulnerable (Mohle-Boetani et al., 2002; Chin and Hopewell, 1996). Developing nations continue to be the most affected by tuberculosis with mortality levels of 40% as compared to 7% in industrialized countries (Emile, 1996).

In spite of scientific advancements, this disease continues to plague the poorer regions of the world (Dharmarajan et al., 2007). According to the World Health Organization (WHO) Global Tuberculosis Control Report in 2010, more than 2 billion people, close to one third of the world's population, were infected by the tuberculosis bacillus. One in every ten infected individuals will develop the disease during the course of his lifetime. The risk is by far greater for people living with HIV.

Tuberculosis (TB) remains a major global health problem. While mortality levels are about 2% each year, nearly 3 million TB cases are undiagnosed or unreported in 2012. In 2012, among 8.6 million people with tuberculosis, 1.3 million died due to this disease. In addition, the number of drug-resistant cases (MDR-TB) is increasing. Nearly 3.7% of new TB cases in the world have drug-resistant strains. About 60% of new cases of MDR-TB in 2011 - estimated at about 500,000 - are observed in the 'BRIC' countries only (Brazil, Russia, India, China and South Africa) (WHO, 2013). The WHO estimates that between 2000 and 2010, close to one billion people will be newly infected and 200 million of these will go on to develop the disease, of which 35 million will die from tuberculosis if nothing is done to improve infection control (OMS, 2005).

In Morocco, 500 to 1000 people die each year from tuberculosis. According to the latest reports from the Ministry of Health, the kingdom registered 25.500 new cases in 2007, corresponding to 82 cases per 100.000 inhabitants. Seventy percent of the patients were between 15 and 45 years of age (Ben cheikh et al., 1996), and the more densely populated urban areas were the most vulnerable.

The rise in tuberculosis incidence is mainly due to the emergence of multidrug resistant strains and the advent of AIDS. Drug resistance has been attributed to insufficient dosage and length of treatment. It is of two types, multidrug-resistance and extensively drug-resistance. In 2007, the number of cases due to multidrug-resistant strains worldwide was estimated to be 500.000 (MDR-TB); 85% from 27 countries (15 of which are situated in the European region). At the end of 2008, 55 countries and territories had reported at least one case of extensively drug-resistant tuberculosis (XDR-TB) (OMS, 2009), indicating the urgent need for new and effective antimycobacterial agents (Newton et al., 2000).

The production of antimicrobial substances is observed in an important number of bacteria, especially Gram positive bacteria (Sosunov et al., 2007). This phenomenon represents a defense mechanisms used by these bacteria in producing antibiotics, organic acids and lytic enzymes such as lysozymes. Moreover, various types of proteins, exotoxins and bacteriocins are produced (Mota et al., 2004).

In this context, the purpose of the present investigation was to search for new substances with antimycobacterial effect from the Moroccan biotopes, which has not been extensively explored in this regard.

MATERIALS AND METHODS

Bacterial strains

The tests were carried out on non-pathogenic mycobacteria. The bacterial strains used were the following:

1. *Mycobacterium aurum* A^+: A scotochromogenic rapid growing species, with a generation time of 6 h, having a similar spectrum of drug susceptibility as *M. tuberculosis* (Chung et al., 1995).
2. *Mycobacterium smegmatis* MC^2 *155*: A thermophilic, non pigmented species (cultures may turn yellow in time), that is a rapid growing strain with a generation time of 3 h.

The mycobacteria were kindly provided by Dr. Suzana David (Centro de Tuberculose e Micobactérias, Instituto Nacional de Saúde Dr.Ricardo Jorge Delegação do Porto, Portugal).

Isolation of a bacterial strain with an antimycobacterial effect

Several samples (water, soil and water) were taken from different Moroccan ecological niches. The samples were kept under agitation for two hours. The supernatant was recovered after sedimentation of the heavier particles. Serial dilutions (of 10^{-1} to 10^{-5}) were carried out in sterile Luria Bertoni (LB) broth (Hassi et al., 2007) (peptone: 10 g/l; yeast extract: 5 g/l; sodium chloride: 10 g/l). An aliquot of 70 µl of each dilution was spread on LB-agar previously inoculated with 70 µl of *M. aurum* A^+ or *M. smegmatis* cultures having 0.3 optical density at 600 nm (OD_{600nm}). After incubation at 37°C for 48 h, colonies surrounded by an inhibition zone were purified. The isolate thus obtained was designated S_3.

To confirm that the antimycobacterial effect was due to secreted substances, the culture of the isolate was filtered and the filtrate was tested on mycobacterial cultures using the well or diffusion method according to the following protocol: a 50 ml culture of the strain in LB broth, 48 h at 37°C with agitation; was centrifuged at 6000 rpm for 5 min to remove bacterial cells in suspension. The supernatant was recovered and filtered using 0.45 µm porosity filters. The filtrate obtained was tested by depositing 100 µl in wells cut out from solid media in plates previously inoculated with 100 µl of mycobacterial culture (*M. aurum* or *M. smegmatis*) at OD_{600nm} = 0.3. The plates were incubated at 37°C for 48 h. After incubation, the presence or absence of an inhibition zone was reported. The inhibition tests were repeated three times.

The control used corresponded to a culture filtrate from *Esherichia coli DH5α* prepared under the same conditions. 100 µl of this filtrate was deposited in the wells prepared in plates previously inoculated with mycobacterial culture (*M. aurum* A^+ or *M. smegmatis* MC^2 *155*) at OD_{600nm} = 0.3. The plates were incubated at 37°C for 48 h. After incubation, the presence or absence of an inhibition zone was reported.

Activity spectrum of the isolate (S_3)

In order to get an idea on the activity spectrum, the antimicrobial

effect of the isolate under study was evaluated against the following bacteria: *M. aurum A*$^{+}$; *M. smegmatis MC*^{2} *155*; *E. coli DH5α*; *Bacillus subtilis CIP 5262*; *Staphylococcus haemolyticus* (Hassi et al., 2007).

A few colonies from young S_3 cultures were deposited at the center of a plate previously inoculated with 100 µl of *E. coli DH5α, B. subtilis CIP 5262* and *S. haemolyticus* cultures at an OD_{600nm} of 0.5, and *M. aurum A*$^{+}$ and *M. smegmatis MC*^{2} *155* cultures at an OD_{600nm} of 0.3. The plates are incubated at 37°C for 48 h. After incubation, the presence or absence of an inhibition zone was reported. The test was repeated three times.

Identification of the studied isolate (S_3)

The identification of the isolate S_3 was based on 16S rRNA gene sequencing. As the most frequently used molecular approach for bacterial phylogeny (Woese et al., 1990), this method has permitted the elaboration of large databases (Maidack et al., 1996; Van de Peer et al., 1999). It has been successfully used in the identification of several environmental microorganisms as well as others that were impossible to grow in culture (Relman et al., 1992; Strous et al., 1999).

A young culture (24 h old) of the isolate S_3 was prepared in LB broth. A 1 ml aliquot of the culture was transferred to a 1.5 ml microtube and centrifuged at 6000 rpm for 5 min. The pellets was resuspended in 100 µl of sterile distilled water, frozen at -20°C for 30 min, then heated at 95°C for 3 min. This thermolysis procedure was repeated twice in order to burst a large number of cells. After centrifugation at 7000 rpm for 10 min, 2 µl of the supernatant (containing the DNA) were used in the amplification reaction (Rodrigues et al., 2007).

For, amplification, universal primers, fD1 (5' AGAGTTTGATCCTGGCT CAG 3') and Rs16 (5' TACGGCTACCTTGTTACGACTT 3'), used in the identification of bacteria by sequencing of 16S rDNA were selected (Weisberg et al., 1991). The reaction mix was prepared in a final reaction volume of 20 µl and contained: 4 µl of Taq buffer (5 x), 1.2 µl of $MgCl_2$ (25 mM), 4 µl of dNTPs (1 mM), 2 µl of fD1 (10 µM), 2 µl of Rs16 (10 µM), 0.2 µl of Taq polymerase (5 U/µl), 4.6 µl of pure H_2O and 2 µl of the DNA. The amplification conditions consisted of an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 1 min 30 s; final extension was performed at 72°C for 10 min. The amplified fragment of approximately 1.5 Kb was visualized by 1% agarose gel electrophoresis.

For sequencing, PCR products were purified using a PCR Product Purification Kit (JETquick, Genomed) as described by the manufacturer. Amplification was carried out using 2.7 µl of the DNA product. The sequencing reaction mixture contained 2 µl BigDye V 1.1 and 0.25 µl of the primer (forward or reverse), PCR grade water was added for a final reaction volume of 10 µl. Amplification conditions were the following: initial denaturation at 96°C (3 min), 35 cycles of denaturation at 96°C (20 s), annealing at 60°C (5 seconds) and extension at 60°C (4 min). All sequences were determined in an automated DNA capillary sequencer AbiPrism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, USA) using BigDye^{TM} Terminator Cycle Sequencing Ready reaction Kit (PE Biosystems, Applied Biosystems, Foster City, USA). Two sequencing reactions were performed using the two primers used for PCR amplification.

The 16S rRNA gene sequences obtained were compared with those of the same gene from different bacterial reference strains. The degree of similarity was determined according to sequence homology. The sequences were analyzed using the data base of the National Center for Biotechnology Information (NCBI) and the BLAST N program.

Other than sequencing of the 16S rRNA gene, the molecular identification of the isolate was complemented using conventional preliminary tests including microscopic observation, Gram stain and growth at 50°C (Meyer et al., 2004).

Determination of the nature of the active substance from the study isolate (S_3)

Preparation of the proteins extract from isolate (S_3) using ammonium sulfate

Ammonium sulfate is frequently used to precipitate proteins in aqueous solutions. It is one of the most frequently used methods for concentrating active substances of proteic nature secreted by microorganisms in culture medium. The precipitation protocol used was described by Shimei and collaborators for the purification and characterization of antimicrobial peptides (Subpeptin JM_4-A and JM_4-B), produced by *B. subtilis* JM_4 (Shimei et al., 2005).

A 100 ml volume of LB broth was inoculated with the selected strain and incubated at 37°C under rotary agitation at 150 rpm for 48 h. After incubation, it was centrifuged at 6000 rpm, at 4°C, for 5 min. The supernatant was recovered and 52.3 grams of ammonium sulfate were progressively added under agitation. The mixture was left overnight under agitation at 4°C. The precipitate was centrifuged at 10000 rpm, at 10°C, for 20 min. The pellet was re-suspended in 200 µl of buffer (KH_2 PO_4 50 mM, pH = 6). The suspension obtained was dialyzed against the same buffer at 4°C for 12 h. The dialisate was filtered using a 0.45 µm porosity filter. The effect of the dialisate was then tested against *M. aurum* and *M. smegmatis* using the well method. The tests were repeated three times.

The control used in this experiment was the protein extract from a *Bacillus* sp. strain that was previously isolated by our group and was shown to secrete a protein capable of inhibiting mycobaterial growth (El Guendouzi et al., 2011). This extract was prepared in the same manner as that described for the S_3 isolate. The inhibition test was repeated three times.

Ethyl acetate extraction of the antimycobacterial substance

The technique used allowed the separation of substances according to their partition coefficient in two non-miscible solvents such as ethyl acetate and water (Botosoa, 2010). A 100 ml volume of sterile LB broth was inoculated with strain S_3. The culture was incubated at 37°C under rotary agitation at 150 rpm for 48 h, and then centrifuged at 7000 rpm, at 4°C, for 5 min. After centrifugation, the supernatant was recovered and an equal volume of ethyl acetate was added. This mixture was kept under agitation at ambient temperature (24°C) for one hour. After decantation, the organic phase was evaporated, and the residue dissolved in 1 ml of sterile distilled water. The biological effect of the extract thus obtained was tested against *M. aurum* and *M. smegmatis* using the well method.

The controls used in this experiment were LB broth without inoculums and LB broth inoculated with *E. coli*. These controls were treated using the same ethyl acetate protocol used to prepare the extract from the strain tested. The biological effect of the extracts from the two controls was tested against *M. aurum A+* and *M. smegmatis MC*^{2} *155* using the well method. The inhibition test was repeated three times.

Sensitivity of the precipitated metabolite to proteinase K degradation

A 100 µl volume of the ammonium sulfate or ethyl acetate precipitates

Table 1. Antimycobacterial effect of the filtrate from the studied isolate S_3.

Filtrate	Diameter of the inhibition zone (mm) against	
	MC^2	A^+
Filtrate from S_3	25.04 ± 0.91	28.05 ± 0.52
Filtrate from *Escherichia coli*	0	0

MC^2, *Mycobacterium smegmatis* MC^2 *155*; A^+, *Mycobacterium aurum A+*.

obtained from strain S_3, were added to 40 µl of a proteinase K solution (1mg/ml). The mixture was homogenized and incubated at 37°C for 3 h. The effect of the proteinase K precipitate was tested against *M. aurum* and *M. smegmatis* using the well method. The control used was a proteinase K solution of the same concentration. The test was repeated three times.

Thin layer chromatography fractionation of the precipitate from the study isolate (S_3)

Thin layer chromatography (TLC) is a rapid analytical technique used to separate and identify metabolites. It is based on the phenomenon of adsorption and can be applied to pure molecules, extracts (complex mixtures of metabolites) and biological samples (Levine, 1990; Botosoa, 2010). Silica gel, on plates 4 cm wide and 12 cm high, was used as the adsorbent (stationary phase). The eluent (mobile phase) consisted of the organic solvent methanol-hexane (6:4, v/v), selected from previously tested mixtures for optimal separation of the components. For TLC analysis, 20 µl of ethyl acetate extract was spotted in bands onto the silica gel plate. After development, the plates were air dried. The retention factors (Rf) of the separated components were then determined.

Identification of the fractions responsible for antimycobacterial activity

In order to identify which molecules in the ethyl acetate extract of the isolate S_3 were responsible for the antimycobacterial activity, an adapted version of a bioautographic method from earlier reports was used (Afolayan and Meyer, 1997; Caccamese et al., 1989; Györgyi et al., 2010).

The developed TLC plate was transferred aseptically into a Petri plate. A *M. smegmatis* inoculum was added to 50 ml of LB agar at a temperature of approximately 45°C. This suspension was poured over the chromatogram, under aseptic conditions, in order to create a thin layer of agar medium over the silica gel. The plates were then incubated at 37°C for 24 h. Results consisted of the detection of growth inhibition zones of *M. smegmatis* on the chromatogram. This method allowed the detection of one active and three inactive fractions. In order to confirm the results from this experiment, the silica gel around the inhibition zone was scraped into a microtube and eluted using 1 ml of ethyl acetate. The eluate was centrifuged at 3000 rpm for 5 min. The supernatant was recovered and concentrated under vacuum. The extract obtained was dissolved in 100 µl of sterile distilled water and tested for its growth inhibitory effect against *M. smegmatis*. This test was performed as follows: Aliquots of 100 µl from mycobacterial culture, containing approximately 10^6 CFU/ml, were spread on LB-agar where well, 6 mm in diameter, had previously been cut. The prepared organic extract was deposited in the well. The plates were incubated at 37°C. The diameters of the inhibition zones were measured after 48 h of incubation at 37°C. Likewise, the three bands showing no biological activity on the chromatogram were also analyzed using this method. The tests were repeated three times.

Detection of polyphenol in the bioactive fraction

The reaction for polyphenol detection (Singleton et al., 1999) was performed on the crude extract from the strain under study as well as on the purified bioactive fraction from the chromatogram according to the following protocol. 500 µl of the Folin-Ciocalteu reagent (10^{-1} dilution) and 400 µl of a Na_2CO_3 solution (75 mg/ ml), were added to l00 µl of the extract, and incubated at 40°C for 5 min. The appearance of a green blue color indicated the presence of polyphenols. For the control, the same protocol was used except that the extract was replaced by ethyl acetate. The test was repeated three times.

RESULTS

Isolation of a bacterial strain with an antimycobacterial effect

A bacterial strain S_3 was isolated and shown to be capable of inhibiting the growth of mycobacterial reference strains. The filtrate from the isolate created an inhibition zone whereas the filtrate from *E. coli* used as control showed no antimycobacterial activity (Table 1). The inhibitory effect observed was relatively higher against *M. aurum as* compared to *M. smegmatis*.

Activity spectrum of the studied isolate (S_3)

The results obtained on the activity spectrum of the isolate S_3 (Table 2) revealed an antibacterial effect against Gram positive (*B. subtilis, S. haemolyticus*) and Gram negative (*E. coli*) bacteria as well as mycobacteria (*M. aurum*, *M. smegmatis*).

Identification of the studied isolate

In order to classify the microorganism into a genus and species, a certain number of identification criteria were respected. The molecular definition of the genus stipulates that the identity of 16S rRNA gene sequences should be superior or equal to 97%. Similarity superior or equal to 99% corresponds to an identical species (Drancourt

Table 2. Activity spectrum of the studied isolate S_3.

	Inhibitory effect in the presence of				
	Mycobacteria		**Gram negative**	**Gram positive**	
Stain	*M. aurum*	*M. smegmatis*	*E. coli*	*B. subtilis*	*S. haemolyticus*
S_3	+	+	+	+	+

+, presence of an inhibition zone.

Table 3. Identification of the strain under study.

Size of the sequenced fragment generated by primer Rs16	Degree of similarity with the sequence obtained using primer Rs16 (%)	Size of the sequenced fragment generated by primer fD1	Degree of similarity with the sequence obtained using primer fD1 (%)	Bacterial species showing a high degree of similarity with the isolate under study
555 bp	100	466 bp	100	*S. epidermidis* (EU428955.1)[a]

a, Acession number; bp, base pairs.

Table 4. Degradation of the *S. epidermidis* extracts by proteinase K.

	Diameter of the inhibition zone (mm)			
	Extract without proteinase K treatment		**Proteinase K treated extract[a]**	
	Effect against *MC²*	**Effect against *A⁺***	**Effect against *MC²***	**Effect against *A⁺***
Ethyl acetate extract form the study isolate	26.12 ± 1.02	29.1 ± 0.4	26.02 ± 0.91	28.1 ± 0.9
Ammonium sulfate extract from the study isolate	0	0	-	-
Ammonium sulfate extract from *Bacillus* sp. (control)[b]	28 ± 1.5	33 ± 2.1	0	0
Ethyl acetate extract from *E. coli* (control)	0	0	-	-
Ethyl acetate extract from LB medium (control)	0	0	-	-

a, proteinase K solution used as control showed no antimycobacterial activity (data not shown); *MC²*, *Mycobacterium smegmatis*; *A⁺*, *Mycobacterium aurum*; -, test not performed because the corresponding extract showed no biological effect. b, El Guendouzi et al. (2011).

et al., 2000). Results are shown in Table 3.

According to the defined molecular identification criteria, the results obtained showed that the isolate corresponds to a *S. epidermidis* strain. This was also confirmed morphologically and by the Gram stain (Gram positive) as well as its inability to grow at 50°C (Meyer et al., 2004).

Determination of the chemical nature of the active substance from *S. epidermidis*

Degradation by proteinase K

After precipitation of the *S. epidermidis* extracts using ammonium sulfate and ethyl acetate, proteinase K degradation of these percipitates was studied. Results are shown in Table 4.

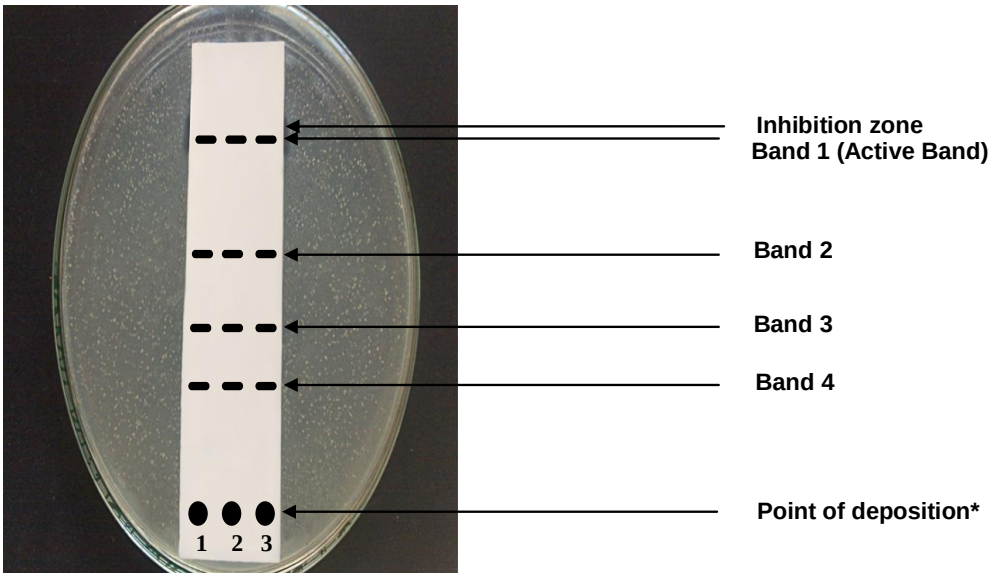
The active substance from the *S. epidermidis* ammonium sulfate precipitate did not show antimycobacterial activity (Table 4). Since this chemical precipitates proteins (Shimei et al., 2005), it was concluded that the active substance was not a protein.

The active substance extracted using ethyl acetate was able to inhibit mycobacterial growth (Table 4), as shown by the formation of an inhibition zone around the wells. Moreover, this precipitate was not sensitive to proteinase K (Table 4). These results indicate that the isolate's antimycobacterial effect was due to a non protein active substance.

Table 5. Antimycobacterial effect of the active TLC fraction from *S. epidermidis* against *M. smegmatis* growth.

TLC fraction	Retention factor (Rf)	Diameter of the inhibition zone (mm)
Band 1	0.91	24.2 ± 1.3
Band 2	0.7	0
Band 3	0.56	0
Band 4	0.45	0

These results show that the TLC fraction band 1 inhibited mycobacterial growth.



Scheme 1. Bioautography of the *S. epidermidis* extract. *At the points of deposition (1, 2 and 3), the spots of the same extract were deposited. The spots were faintly colored in yellow, thus they are invisible in the chromatogram.

Fractionation of the ethyl acetate extract and identification of the biologically active fraction

TLC fractionation of the ethyl acetate extract produced four distinct spots that are very faintly colored and therefore they are not visible in the photo taken (Scheme 1 and Table 5). TLC evidenced the antimycobacterial effect of the ethyl acetate extract, which resulted in the formation of an inhibition zone around the component containing the antimycobacterial substance. The Rf value for this component was 0.91 (Scheme 1). In order to confirm this result, all spots were purified and tested against *M. smegmatis.* Results are shown in Table 5.

Evidence for polyphenol in the bioactive TLC fraction

The crude precipitate, as well as the purified bioactive TLC fraction (active band), from the study strain were tested for the presence of polyphenol. The results obtained are shown in Table 6.

These results indicate that the activity observed was due to the presence of a polyphenol in the bioactive fraction from *S. epidermidis*.

DISCUSSION

The samples analyzed led to the isolation of a bacterial strain (S_3). This isolate showed a growth inhibitory effect against *M. smegmatis* and *M. aurum*. Its antimycobacterial activity was observed through the presence of zones of mycobacterial growth inhibition as a result of diffusion, through the LB agar medium, of bioactive substances. The filtrate from the isolate also showed a growth inhibitory effect. The absence of mycobacterial growth around the wells containing the filtrate, confirmed

Table 6. Evidence for polyphenol in the ethyl acetate of the bioactive TLC fraction from *S. epidermidis*.

Staphylococcus epidermidis	Polyphenol
Ethyl acetate extract	+
Bioactive TLC fraction (band 1, Rf = 0.91)	+
Control[a]	-

+, Presence; -, absence; a, for the control, the same protocol was used except that the extract was replaced by ethyl acetate.

that the observed antimycobacterial effect was due to the diffusion of secreted substances in the agar medium. The inhibitory effect of the active substance from the study strain was relatively more pronounced against *M. aurum* than *M. smegmatis*. This observation could be due to the longer generation time of *M. aurum* as compared to *M. smegmatis*, which would allow for more extensive diffusion of the inhibitor.

The filtrate inhibited the growth of *M. smegmatis* and *M. aurum* that present a susceptibility profile to antituberculous agents similar to that of *M. tuberculosis* (Mitscher and Baker, 1998; Chaturvedi et al., 2007). Their growth inhibition is highly predictive of the activity against *M. tuberculosis* (Chung et al., 1995). This suggests that the filtrate could be equally active against Tuberculousis bacilli. Indeed, these two non-pathogenic mycobacteria have been considered as surrogates for the evaluation of active substances against *M. tuberculosis* growth (Newton et al., 2000, 2002).

The results obtained on the activity spectrum of the isolate S_3 revealed an antibacterial effect against Gram positive (*B. subtilis, S. haemolyticus*) and Gram negative (*E. coli*) bacteria as well as mycobacteria (*M. aurum* and *M. smegmatis*).

According to the defined molecular identification criteria, the results obtained showed that the isolate corresponds to a *Staphylococcus epidermidis* strain. This was also confirmed morphologically and by the Gram stain (Gram positive) as well as its inability to grow at 50°C (Meyer et al., 2004). This study shows for the first time that this species secretes an active substance against mycobacterial growth.

The active substance from the *S. epidermidis* ammonium sulfate precipitate did not show antimycobacterial activity. Since this chemical precipitates proteins (Shimei et al., 2005), it was concluded that the active substance was not a protein.

The active substance extracted using ethyl acetate was able to inhibit mycobacterial growth, as shown by the formation of an inhibition zone around the wells. Moreover, this precipitate was not sensitive to proteinase K. These results indicated that the isolate's antimycobacterial effect was due to a non protein active substance.

The antimycobacterial activity of *S. epidermidis* was also demonstrated by bioautography. By this method the TLC separation of the different constituents from the ethyl acetate extract of this bacteria was first obtained, followed by the evaluation and localization on the TLC plate of the active substance (Band 1, Rf = 0.91). TLC was also used for the purpose of subsequent purification and identification of the active substance. The nature of the biologically active substance responsible for the antimycobacterial effect of the component identified by TLC, Rf value of 0.91, was determined using phytochemical test. These indicated the presence of polyphenol in the crude extract and in the TLC component, which made us to conclude that the active substance was a phenolic compound.

Although polyphenols are mainly synthesized by plants (Kablan et al., 2008; Karumi et al., 2004), there are some microorganisms that are also capable of producing phenolic compounds (Max et al., 2012a, 2012b). These include, for example, *Debaryomyces hansenii* and *Streptomyces setonii* that metabolizes ferulic acid in various phenolic compounds (Max et al., 2012a, b). Other studies have shown that some human colonic bacteria produce phenolic and indolic compounds (Smith and Macfarlane, 1996).

The polyphenols are well known for their excellent biological activities including the inhibition of dental cavities (Sakanaka et al., 1989), inhibition of allergies (Yeo et al., 1995), reduction of blood pressure (An, 1998), prevention of gout (An et al., 1996) and the inhibition of oxydation. Moreover, several studies show that these molecules have an antimycobacterial effect. Indeed, it was reported that several polyphenolic extracts from medicinal plants, traditionally used in the treatment of respiratory illnesses, inhibit *M. tuberculosis* growth *in vitro* (Newton et al., 2002; Okunade et al., 2004; Seephonkai et al., 2002). Another study showed that polyphenols from green tea inhibit *M. tuberculosis* growth *in vivo* (Anand et al., 2006).

In conclusion, the analysis of samples from new ecological niches from Morocco has evidenced novel antimycobacterial substances. In the present investigation, the use of soil and water from different Moroccan biotopes allowed the isolation of a strain of *Staphylococcus* capable of secreting a polyphenolic substance that inhibits mycobacterial growth.

Since mycobateria are capable of infecting macrophages, the effect of the active substance from this strain will be studied *ex vivo*. This effect will also be studied against pathogenic bacteria namely those responsible for nosocomial infections. Finally, the *ex vivo* active substance will be purified and the chemical structure elucidated.

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