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Full Length Research Paper

Aerococcus sp. with an antimycobacterial effect

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Tuberculosis is an infectious disease that causes fatality every year because of drugs resistance of the *Mycobacterium* complex; this is why the development of new antibiotics becomes an urgent need. To reach that target, a main approach was led; consisting on screening of active substances producing microorganism. In this study, we reported data on a strain that was isolated from different areas of Fez (Morocco), which present an antagonistic effect against *Mycobacterium smegematis* and possessed a large spectrum against bacteria of Gram positive and negative. The antimycobacterial compounds producer, ZI1, was identified as *Aerococcus* sp. on the basis of PCR amplification of 16S ribosomal RNA gene followed by sequencing (99 and 100% of homology, respectively by RS16 and FD1), and by using biochemical tests, the unknown bacterium ZI1 was so close to either the species *Aerococcus viridans* or *Aerococcus urinaeequi,* but it was distinguishable from the other five *Aerococcus* species. The antimycobacterial compounds were synthesised in the exponential growth phase of *Aerococcus* sp. and were fully affected following heat treatment and protolytic enzymes which indicated the proteinaceous nature of the active agents.

Key words: Tuberculosis, antimycobacterial compounds, *Aerococcus* sp.

INTRODUCTION

Tuberculosis is a perilous and contagious bacterial infection caused by the complex *Mycobacterium* (*M.*) including *M. tuberculosis*, *M. bovis, M. africanum*, *M. microti*, *M. canettii* and *M. pinnipedii* (Ernst et al., 2007).

This disease is characterized by an inflammatory response that leads to containment, but not eradication of bacteria within granulomas in the lung, and the success of the causative agent as a pathogen is its ability to evade host immunity by multiplying into macrophages and establishing a chronic infection (Bhatt and Salgame, 2007; Rengarajan et al., 2008).

Moreover, this infection remains between the curable diseases that provoke a big number of casualties (Dye, 2006); the statistics speak about more than 1.7 million dead people in 2009 (World Health Organization, 2010).

Due to the decline of socioeconomic standards, the human immunodeficiency virus pandemic aggravated by the increasing numbers of multidrug-resistant strains. Furthermore, the only vaccine currently licensed for use against tuberculosis, the live attenuated *Mycobacterium bovis* strain Bacille Calmette-Guérin (BCG), is effective only in providing protection against severe childhood forms of the disease such as tuberculous meningitis and miliary tuberculosis, and its efficacy for providing protection against the more common adult pulmonary form of the disease has been highly variable and has ranged from 0 to 80% in different clinical trials (Whelana et al., 2008). All this has made the whole world in a hurry to find new anti-tubercular agents with novel modes of actions.

Natural products or their semi-synthetic derivatives, well defined as providing novel examples of anti-infective drug leads, currently play important roles in the chemo-therapy of tuberculosis and form one avenue in the search for new antitubercular agents. For example, Aminoglycoside antibiotics (streptomycin) isolated from *Streptomyces griseus*, and cyclic peptides (capreomycin) isolated from *Streptomyces capreolus* NRRL 2773.

These natural products exhibited wide-ranging *in vitro* potency towards *M. tuberculosis* (Copp, 2003).

This is why we present here an antimycobacterial

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Abbreviations: ***A,*** *Aerococcus*; **LB**, Luria-Bertoni; ***M,*** *Mycobacterium.*

compounds producing bacterium isolated from different ecological zones of Fez (Morocco) which is *Aerococcus* sp.

The antibacterial activity of the genus *Aerococcus* have been reported in few studies such as in Zaria's research (Zaria, 1993), which found that *A. viridans* is able to inhibit the growth of four *Staphylococcus hyicus*, three *Staphylococcus aureus* strains and some other Gram-positive bacteria, including *Streptococcus* group A and D *Corynebacteria* (Zaria, 1993). However, its antimycobacterial effect has never been elucidated by any other studies.

The objectives of this study include (a) screening antimycobacterial compounds-producing bacteria active against *M. smegmatis* (b) identifying strains on the basis of gram stain, biochemical characteristics and PCR followed by DNA sequencing of 16S ribosomal RNA gene (c) evaluating antimicrobial activity of the isolated bacterium against a broad spectrum of gram positive and negative bacteria and (d) partially characterizing of the secreted substances.

MATERIALS AND METHODS

Bacterial strains and media

ZI1 is an isolate obtained from different ecological zones of Fez Morocco which was selected for its ability to inhibit the growth of *M. smegmatis*.

M. smegmatis MC² 155 is used as an indicator bacterium of antagonistic activity; it's a non pathogenic atypical strain with a generation time of approximately 3 h (Grosset et al., 1989). Other bacteria chosen as indicators for the inhibitory spectrum assay were: *Mycobacterium aurum* A^+: non pathogenic bacterium with a generation time of approximately 6 h. This strain was used as a model to evaluate the effect of active substances on the growth of *M. tuberculosis* (Chung et al., 1995).

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). *Staphyloccus haemolyticus* (Hassi et al., 2007), *Bacillus subtilis* ILP 142B (Hamadi and Latrache, 2008), *Escherichia coli* Dh5α (Microbial biotechnology laboratory of Techniques and Sciences Faculty, Fès), *Erwinia chrysanthemi* 3937 (Hassouni et al., 1999) were friendly provided by Dr. Hassouni (LCB-CNRS-Marseille).

These strains were propagated in Luria-Bertani (LB) at 37°C or at 30°C for *Erwinia chrysanthemi*.

The isolate was stored at -70°C in LB broth supplemented with 25% glycerol. Throughout the experiments, the strains were subcultured every week on agar media and held at 4°C. Different media in broth or on agar plates were used including Luria-Bertani medium containing peptone (10 g), yeast extract (5 g), NaCl (10 g) per litre of distilled water, Soc medium containing tryptone (20 g), yeast extract (5 g) NaCl (0.58 g), KCl (0.18 g) MgCl2 (0.95 g), MgSO4 (2.46 g), glucose (3.6 g) per litre of distilled water, respectively and YPG medium containing 20 g of peptone, 10 g of yeast extract, 20 g glucose, 60 µg/ ml of ampicilline and 30 µg/ ml of kanamycine per litre of distilled water (Sambrook et al., 1989).

Selection procedure for antimycobacterial compounds producing bacteria from water and soil samples

Twelve samples of water and soil were collected from ecological areas Fez Morocco. These samples were treated independently according to the method followed by Hassi et al. (2007). Colonies which present halos of inhibition against *M. smegmatis* were picked up and transferred to sterile Soc or LB agar plates; these were incubated at 37°C and conserved at 4°C for later assays.

Antimycobacterial activity assay

Antimycobacterial activity was done to confirm previous results and it was performed by two different methods, that is, (1) agar-well diffusion assay as led by Muriana and Klaenhammer (1991). The presence of inhibition zone around each well was evaluated by measuring its diameter. (2) A modified spot-on-lawn assay where a colony of the isolated strain was spotted onto the surface of Soc agar plates which had been already spread with a broth culture of indicator *M. smegmatis*. These plates were incubated at 37°C for 24 h and the antimycobacterial activity was detected by the observation of inhibition area surrounding on test strains (Tagg et al., 1976). These assays were done three times and they were also carried out to evaluate the inhibitory activity of *E. coli* Dh5α used as a control.

Identification of antimycobacterial compounds-producing strains

Antimycobacterial compounds producing strain was examined for cellular morphology, gram stained characteristics using a microscope. The biochemical identification was also performed according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

After that isolate was identified by molecular methods which consist to amplify the 16S rRNA gene of the strain by PCR and to determine 16S rDNA sequence by direct sequencing.

The PCR amplification was performed with the primers RS 16 and FD1 targeted against regions of 16S rDNA. The amplification protocol was carried out in thermal cycler (Techgene^R) under the following conditions: denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1min 30 s. The final extension was at 72°C for 5 min. PCR reaction composed of 20 µl of 10 × reaction Taq buffer with 25 mM $MgCl_2$, 1 mM deoxynucleoside triphosphate, 1 U/µl of Taq polymerase, 10 µM of each primer, distilled water and 2 µl of genomic DNA which was used as a template for amplification (Saiki et al., 1985; Weisberg et al., 1991).

PCR products were resolved by electrophoresis in 1% agarose gel and visualized by ethidium bromide (10 mg/ml) staining. PCR amplicons were purified and sequenced using the Big Dye Terminator with primers while automated sequencing of both strands of the PCR products was done on a BIOSYSTEME 3130 automated gene sequencer (Sanger et al., 1977).

Identification analysis was realized by an alignment of sequence consensus of the 16S rDNA genes collected in an international database (Genebank) present at the NCBI website located at http://www.ncbi.nlm.nih.gov/BLAST. These results were then expressed in percentage of homology between the submitted sequence and the sequences resulting from the database.

Inhibitory spectrum of isolated strain

Spot-on-lawn assay was used to evaluate the inhibitory spectrum of the isolated culture strain. Gram-positive and Gram-negative bacteria were assayed comprising *Mycobacterium aurum, Staphyloccus haemolyticus*, *Bacillus subtilis*, *Escherichia coli* Dh5 α and *Erwinia chrysanthemi*.

This inhibition was scored on an abstract scale as follows: -, No

inhibition; +, presence of zone of inhibition. The assay was repeated three times.

Compounds extraction

The active substance was extracted by ethyl acetate as will be described below in the determination of the kinetics paragraph and also by ammonium sulfate according to the method of Naclerio and collaborators (1993).

The obtained solutions will be referred to as "crude extract" and their antimycobacterial well-diffusion assays were carried out on LB agar plates at 37°C then subsequently examined by measuring the diameter of inhibition zone. Extraction of *E. coli* Dh5 α culture by both ethyl acetate and ammonium sulfate was used as control. The assays were done three times and the averages were calculated.

Determination of the kinetics

Synthesis of antimycobacterial compounds was monitored during the growth cycle by growing the producer strain culture in Soc broth under shaking condition during 48 h at 37°C. After every 2 h, optical density at 595 nm was recorded. The cells were removed by centrifugation at 6000 rpm for 10 min and bioactive supernatants were then extracted with an equal volume ethyl acetate (Ten Brink et al., 1994; Abo-Amer, 2007). The crude extract was tested for antimycobacterial activity using well-diffusion assay on LB agar plates at 37°C. The assay was performed two times and the average of the inhibition zone diameter was calculated.

Physico-chemical characterization

Thermostability

To check the thermal stability, strain acetyl ethyl and ammonium sulfate extracts were exposed to 80°C (30 min), 100°C (15 min) and 4°C (1 month), and the activity was checked by well-diffusion assay which was done three times and the average of the inhibition zone diameter was calculated (Iqbal et al., 2001).

Effect of enzymes

Pepsin and proteinase K were tested for their proteolysis activity on the antimycobacterial compounds contained in the strain acetyl ethyl and ammonium sulfate extracts.

The assays were performed at a final concentration of 1mg/ml, respectively at pH 3 and 7 (Ahmad and Rasool, 2003; Noonpakdee et al., 2003). The samples with and without enzymes were held at 37°C for 3 h and the remaining activity was determined by well-diffusion assay using *M. smegmatis* as indicator strain. The assay was done three times and the average was calculated. Extracts not treated by proteases or by heat were used as controls.

RESULTS AND DISCUSSION

Selection procedure for antimycobacterial compounds producing bacteria from water and soil samples and antimycobacterial activity assay

The screening of bacteria isolated from water and soil areas of Fez morocco exhibited one isolate, ZI1, having inhibitory properties. After that this antimycobacterial activity was confirmed by both spot-on-lawn assay and agar-well diffusion assay revealing a diameter of inhibition about 8 ± 0.1 mm.

Identification of antimycobacterial compounds-producing strains

As reported in the literature, PCR amplification of the 16S rRNA gene with the FD1 and RS16 primers allowed the amplification of a DNA fragment of approximately 1.5 kb (Weisberg et al., 1991). These primers were shown to be able to amplify the 16S rRNA gene from eubacteria (Weisberg et al., 1991).

After DNA sequencing, the sequences obtained with RS16 and FD1 primers were about 565 bp bi-directionally. It was reported that the initial 500 bp sequence provides adequate differentiation for bacterial identification (Clarridge, 2004).

BlastN search showed that the nucleotide sequence of 16S rRNA gene of the isolated strain ZI1 had a homology of 99 and 100%, respectively by RS16 and FD1 primes to that of *Aerococcus* sp. P3-2 (GenBank accession no EU376006.1) and mostly with *Aerococcus viridans* Rabin (GenBank accession no AY707782.1), *Aerococcus viridans* Newfoundland (GenBank accession no AY707781.1), *Aerococcus viridans* Maine (GenBank accession no AY707780.1), *Aerococcus viridans* ATCC 700406 (GenBank accession no AY707779.1), *Aerococcus viridans* ATCC 29838 (GenBank accession no AY707778.1), *Aerococcus viridans* ATCC 10400 (GenBank accession no AY707777.1), *Aerococcus viridans* 1032 (GenBank accession no AY707776.1) and *Aerococcus viridans* 1030 (GenBank accession no AY707775.1).

According to the criterions defined by Drancourt et al. (2000), we concluded that ZI1 belonged to *Aerococcus* sp. strain.

In order to clarify the phenotypic resemblance between the unknown isolate ZI1 and *Aerococci*, biochemical characteristics were examined according to Bergey's manual of determinative bacteriology, thus, morphologically, colonies were non-pigmented, circular and small (about 1 mm in diameter) when grown on LB agar. It was Gram-positive cocci, arranged paired, or in tetrads, no motile. It was able to hydrolyze esculin and citrate wasn't utilized. Acid was produced from glucose, maltose, sucrose, mannitol, lactose and amygdalin but not from arabinose, rhamnose, inositol, melibiose and sorbitol. It grew in the presence of 6.5% NaCl with a maximum development at 30°C and growth occurred on solid media at 22°C but failed at 50°C. Negative reactions were observed for catalase, oxidase, arginine dihydrolase, gelatin liquefaction, starch hydrolysis, ornithine decarboxylase, lysine decarboxylase, urea splitting, Voges Proskauer test, H2S and indole production.

On the basis of its cellular morphological and biochemical characteristics, the isolate ZI1 resembled members of the genus *Aerococcus*, in particular *A.*

viridans, since each of *A. sanguicola, A . urinaehominis* and *A. suis* differed from this species by failing to produce acid from lactose and mannitol (the majority of *A. viridans* strains ferment these substrates) (Lawson et al., 2001a, b; Facklam et al., 2003; Vela et al., 2007), and *A. suis* was more distinguishable from *A. viridans* by producing arginine dihydrolase (Vela et al., 2007). Besides that all the following tests were negative for *A. christensenii*: hydrolyze esculin, acid production from maltose, sucrose, lactose, mannitol (Collins et al., 1999) and in the case of *A. urinae,* it can readily be distinguished from ZI1 by its failure to hydrolyze esculin and to ferment maltose and lactose, but acid was formed from sorbitol (Aguirre and Collins, 1992; Zhang et al., 2000; Christensen et al., 1997). Therefore, the unidentified isolate ZI1 was found to be phenotypically distinct from *A. sanguicola, A. suis, A. urinaehominis, A. christensenii* and *A. urinae* but so close to *A. viridans.*

Moreover, the isolate ZI1 satisfied the criteria for *A. viridans* given by Bergey's manual of determinative bacteriology (Holt et al., 1994) and these results corroborate also with the researches of other studies (Williams et al., 1953; Clausen, 1964; Razeq et al., 1999; Whittenbury, 1965; Lawson et al., 2001a, b; Tsujimura et al., 2001; Greenwood et al., 2005; Facklam et al., 2003; Zhang et al., 2000; Wiik et al., 1986).

Nevertheless, contrary to Vela et al. (2007) study which successfully distinguish *A. suis* from other *Aerococci* by using biochemical tests, herein these couldn't make an evident discrimination between *A. viridans* and *A. urinaeequi* (Felis et al., 2005), although, this latter differed by fermenting melibiose (Whittenbury, 1965), but it was not enough and this made the differentiation between the two species more difficult and 16S rRNA gene identity wasn't sufficient to guarantee the identity between the two species because of a similarity value of 99.9% between their 16S rRNA gene sequences, as was described by Felis et al. (2005). Though, ZI1 could be either *A. viridans* or *A. urinaeequi,* so further methods are required to differentiate between these two species as DNA–DNA hybridization data (Felis et al., 2005) and electrophoretic analysis of whole-cell proteins (Lawson et al., 2001a, b; Collins et al., 1999).

Finally, regardless to its morphology, cultural appearance, physiologic and biochemical characteristics mentioned above, together with the phylogenetic analysis, the strain ZI1 was assigned to the genus *Aerococcus*. Based on these data, we assigned our strain as *Aerococcus* sp. strain ZI1.

Until now, no described antimycobacterial actives substances producer bacterium has been related to *Aerococcus* sp.

Spectrum activity

Spot-on-lawn assay was performed to assess the antagonistic activity of *Aerococcus* sp. ZI1 against indicator strains including *M. aurum, S. haemolyticus*, *B. subtilis*, *E. coli* Dh5α and *Erwinia chrysanthemi*. The strain showed a larger antimicrobial spectrum for being active against all bacteria tested.

There are few studies demonstrating antibiosis effect of *Aerococcus* species. Nevertheless, our results corroborate with some studies showing the antagonistic effect of *A. viridans* 167 on *Staphylococcus aureus* (Gorbunova et al., 1989; Zhurylo and Drozd, 1999) and also with the authors revealing that *A. viridans* was able to inhibit the growth of *Staphylococcus hyicus*, *S. aureus* and some other gram-positive bacteria, including *Streptococcus* group A and D *Corynebacteria* (Allaker et al., 1989; Zaria, 1993), while in Kremenchutskiĭ and Samoĭlenko research, it has been shown that *A. viridans* inhibited *E. coli* and *B. subtilis* by producing hydrogen peroxide (Kremenchutskiĭ and Samoĭlenko, 1987). Our findings agree also with the results obtained from Ballester et al. (1980) study showing that the growth of a large number of Gram-negative and Gram-positive bacteria was inhibited by viridicin produced by *A. viridans* (Ballester et al., 1980). However, other studies have shown *Aerococcus sp* had effect against either *Erwinia chrysanthemi* or *Mycobacterium* sp.

Furthermore, the broad spectrum of activity of the strain suggests that its target is highly conserved; this finding prompted us to focus on the compounds mode of action.

Compounds extraction

The extraction was done both by ethyl acetate and ammonium sulfate. Two substances which are widely used to extract antimicrobial compounds from Gram positive and negative bacteria (Duncan et al., 1989; Streitenberger et al., 2001; Naclerio et al., 1993; Muriana and Klaenhammer, 1991; Márcia et al., 2006; Parada et al., 2007; El-Shafie et al., 2008; Li et al., 2008; Santos et al., 2011). The crude extract of the antimycobacterial substances prepared from *Aerococcus* sp. ZI1 was tested against *M. smegmatis*. Thus, the antimycobacterial assays showed inhibition zone in which the diameters were 19 +/-2 and 18 +/- 2 mm, respectively after extraction by ethyl acetate and ammonium sulfate, while the crude extract of *E. coli* Dh5α used as control didn't exhibit any inhibitory activity against the indicator strain, which suggested that *Aerococcus* sp. ZI1 acted by a substance secreted in the medium, and these results indicated also that the produced compounds were soluble in ethyl acetate solvent.

Determination of the kinetics

Aerococcus sp. ZI1 was toxically incubated in Soc media at 37°C in a rotary shaker and the compounds production was evaluated every 2 h by the well-diffusion assay.

Measurements of the optical density of the cultures

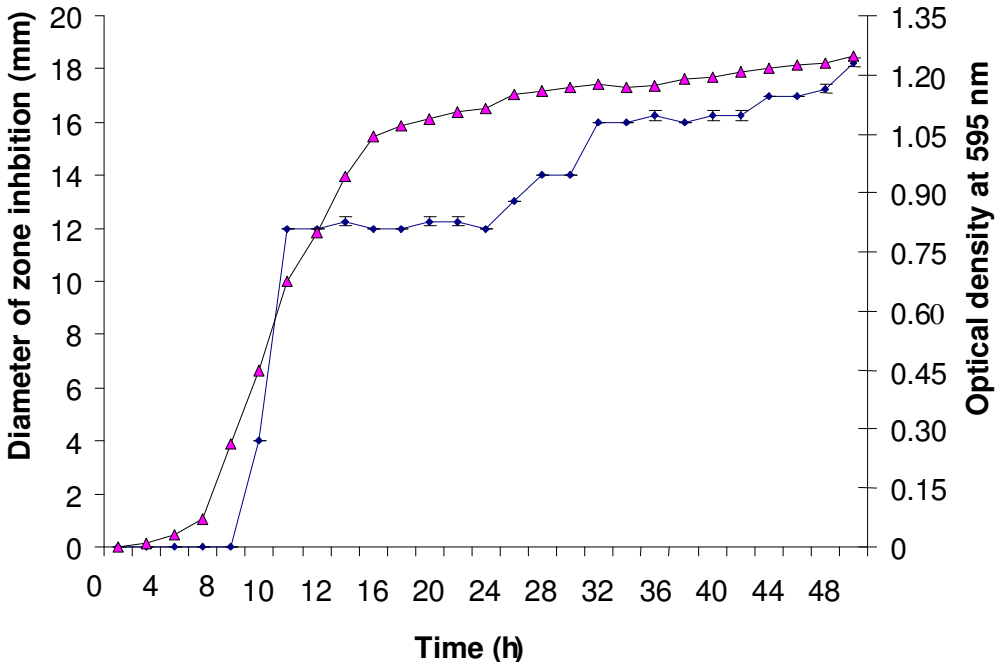

Figure 1. Growth kinetic and antibacterial agent production by *Aerococcus* sp. (▲) growth kinetic, (♦) antibacterial production. *Aerococcus* sp. cells were cultured in Soc broth and were incubated for 48 h at 37°C. The OD and antimycobacterial activity were measured. The experiments were repeated two times and results represent the mean ± standard error to the mean.

during 48 h of incubation showed that there was a lag time of about 9 h before the synthesized compounds appeared in the culture medium, and a slight inhibition of *M. smegmatis* growth occurred in the beginning of the logarithmic growth phase. An increasing of the halos of inhibition diameter was observed during the incubation period and the maximum of the antibacterial activity was reflected during stationary growth phase after 48 h (Figure 1).

This result suggested that the growth inhibition was due to the accumulation of antimicrobial substance in the culture supernatant which was produced as a primary metabolite.

Previously, this same finding had been showed in some concerned studies, bacteriocins was reported to be produced in early growth phase by Gram positive bacteria such as *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus mundtii* which retained maximum activity throughout the stationary phase (Hartnett et al., 2002). This also joins the results of Rajoka et al. (2003) study, showing that the production of beta-galactosidase from *Kluyveromyces marxianus* was apparently growth-associated.

Effect of heat and proteolytic enzymes

The sensitivity of the antimycobacterial substances produced by *Aerococcus* sp. ZI1 to the heat treatment and the proteolytic enzymes proteinase K and pepsin, was evaluated by measuring residual activity against *M. smegmatis* in the well-diffusion assay.

Aerococcus sp. ZI1 compounds were fully inactivated by all the proteases tested and their activity was also completely lost after heat treatments at 80 and 100°C which indicated proteinaceous nature of the active agent, while the storage of the active substance at 4°C for a month did not influence the activity for the strain and the extracts not treated by proteases or by heat treatment showed inhibition zones against *M. smegmatis* toward the wells (Table 1).

These findings agree with Ballester et al. (1980) study which aimed the purification of the viridicin produced by *A. viridans*. Indeed, a crude preparation of this macromolecule was sensitive to the action of protease.

The genus *Aercoccus* belongs to lactose acid bacteria (Liu, 2003; Stiles and Holzapfel, 1997; Simpson and Taguchi, 1995; Parada et al., 2007; Leroi, 2010) which namely produce bacteriocins, antimicrobial peptides known to be sensitive to most proteases (Ballester et al., 1977, 1980; Wu et al., 2004; Papagianni and Anastasiadou, 2009) and their antibacterial activity could be affected by high heat treatment (Galvez et al., 2007; Noonpakdee et al., 2003; Jack et al., 1996), hence, the antimycobacterial compounds of *Aercoccus sp* ZI1 may be a bacteriocin or it can be just another protein such as lactate oxidase of *A. viridians* possessing antagonistic activity relative to 27 test-cultures of bacteria

Table 1. Effect of different treatments on extract of *Aerococcus* sp. Relative activity was measured by well-diffusion agar test against *Mycobacterium smegmatis.*

Treatment	Relative activity
Enzymatic treatments	
Proteinase K	-[a]
Pepsin	-
Control (extracted by ethyl acetate)	+[b]
Control (extracted by ethyl ammonium sulfate)	+
Heath treatments	
80°C	-
100°C	-
Control (extracted by ethyl acetate)	+
Control (extracted by ethyl ammonium sulfate)	+

-[a]: No inhibition, +[b]: inhibition.

(Kremenchutskiĭ and Arenkov, 1989). Therefore, further study is required to examine deeply characteristics of antimycobacterial molecules.

Moreover, to contribute efficiently in finding a new drug to cure tuberculosis, other investigations should be executed. Firstly, the antimycobacterial effect of the substances produced by the antagonistic *Aercoccus* sp. ZI1 must also be proven against *M. tuberculosis,* and not only against *M. smegmatis* which was used in this study because it is a rapid grower and a non pathogenic atypical *Mycobacterium*. Besides that preclinical study aiming the revelation of the antimycobacterial activity of these substances *in vivo*, paired with the research of their pharmacokinetic, pharmacodynamic properties, tolerability and toxicology in different species have to be conducted. Each of these steps is important before judging whether or not it is possible to use *Aercoccus* sp. ZI1 substances as anti-tubercular agent.

Conclusion

Tuberculosis is the leading killer among all infectious diseases worldwide and presents, therefore, a major threat to the health of millions of inhabitants of developing as well as developed countries. Not only *M. tuberculosis*, but also other species of *Mycobacterium* are emerging as health concerns, and new antimycobacterial drugs are desperately needed to counteract growing resistance towards currently available drugs (Kuete, et al., 2007; McGaw et al., 2008).

This present investigation highlighted the isolation of *Aerococcus* sp. ZI1 which revealed its ability to inhibit the growth of *M. smegmatis* used as a model to test the effect on *M. tuberculosis*, and this is the first study which elucidates its antimycobacterial effect. The isolated strain exhibited also a broad spectrum of activity against both Gram-positive and Gram-negative tested bacteria which suggest that the compounds may warrant further investigation with regard to their mode of action.

The findings of this study suggest that *Aerococcus* sp. ZI1 produces proteinaceous inhibitory substances which should be purified and identified, then determine their inhibitory minimal concentration in further work.

Finally, more studies are required to demonstrate their effectiveness *in vitro* against *M. tuberculosis,* the real causative agent of tuberculosis, followed by other investigations having as a aim looking for the efficacy, safety, toxicology, and the pharmacodynamic properties of these substances *in vivo* and that will contribute to establish their possible beneficial effects which may suggest their probable use in the therapeutic purpose against tuberculosis.

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