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Full Length Research Paper

Characterization of Streptomyces strain SLO-105 isolated from Lake Oubeira sediments in North-East of Algeria

Houda Morakchi[1,2]*, Adel Ayari[1], Mira Taok[2], Djamila Kirane[1] and Nelly Cochet[2]

[1]Applied Microbial Laboratory, Department of Microbiology and biochemistry, Badji Mokhtar University, P. O. Box 12. 23000 Annaba, Algeria.
[2]University of Technology of Compiegne. BP 20 529. 60 205. Compiegne, France.

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A microbial strain, SLO-105, isolated from Lake Oubeira sediment was screened for its antimicrobial activity against pathogenic bacteria and fungi. The strain showed broad-spectrum antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* MRSA, *Bacillus subtilus*, *Micrococcus leutus*, *Streptococcus fecalis* and fungi *Aspergillus niger* and *Rodotorulla mucilaginosa.* However, no activity of the strain was observed against Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* as well as on fungi *Candida albicans.* Analysis of 16S rDNA sequence and the morphological and physiological characteristics of the strain suggested that the isolate belonged to *Streptomyces* genus.

Key words: *Streptomyces*, rDNA analysis, micro-morphology, physiology, antimicrobial.

INTRODUCTION

New microbial metabolites, particularly antibiotics, are permanently needed due to the increase of resistant pathogens, evolution of novel diseases and toxicity of currently used compounds (Hakvag et al., 2008; Jensen et al., 2005). Approaches to the discovery of new antibiotics are generally based on screening of naturally occurring microorganisms like Actinomycetes, (Donadio et al., 2002) in different environmental samples.

Actinomycetes represent a large group of Gram-positive filamentous bacteria that currently comprises over forty genera and hundreds of species among which is the important *Streptomyces* genus (Watve et al., 2001). Members of this group have been isolated from different soils, plant materials, waters and marine sediments (zaitlin et al., 2003; Jensen et al., 1991). They are able to produce a wide range of molecules with broad spectrum of activities, that is, antibacterial, antifungal, antitumural, antiparasitic and antiviral (Atta and Ahmad, 2009; Naeimpoor and Mavituna, 2000).

The present work described the cultural and physiological characteristics as well as the antibacterial activity of an Actinomycete strain, SLO-105, isolated from Lake Oubeira sediment.

MATERIALS AND METHODS

Strain isolation and storage

The strain SLO-105 was isolated from sediment sample collected from the Oubeira Lake. One gram of sample was diluted (10^{-1} to 10^{-6}) with sterile water and spread on glycerol-asparagines agar (L-asparagine: 0.1%, glycerol: 1%, K_2HPO_4: 0.1%, 1 ml of trace salts solution: $FeSO_4.7H_2O$: 0.1%, $MnCl_2.4H_2O$: 0.1%, $ZnSO_4.7H_2O$: 0.1%, pH 7.4) containing 50 µg/ml of cycloheximide in order to minimize fungal contamination. Plates were incubated for 2 weeks at 30°C (Matsukawa et al., 2007). The strain SLO-105 was confirmed by colony morphology as actinomycetes and then purified by streak plate technique (Thakur et al., 2007). For long storage for further use, spores stocks of the isolate were prepared as described by Hopwood et al. (1985). The spore suspension was analyzed by a spectrophotometer at 450 nm UVI light XS SECOMAM (Nova Analytics company) and the absorbance was adjusted to 0.05 (about 10^7 CFU/ml) by adding distilled water. The same volume of glycerol was added to the spore suspension and aliquots of 1 ml

*Corresponding author. E-mail: hmorakchi@yahoo.fr.

were stored in cryotubes at -20°C and – 80°C (Elmahdi et al., 2003; Hopwood et al., 1985).

Morphological characteristics

The micro morphology of the strain was carried out using a scanning electron microscope Philips XL 30 ESEM-FEG (Philips Optique Electronique, Limeil Brévannes, FRANCE). The sample used for observation has been cultured for 14 days on Yeast malt glucose agar (Bacto-yeast extract: 4.0 g/l, Bacto-malt extract: 10 g/l, glucose: 4.0 g/l, distilled water: 1000 ml, agar: 20 g/l. pH 7.3) (Boudjellel et al., 2006).

Cultural, physiological and biochemical characteristics

The morphological and cultural characteristics of strains SLO-105 were made according to the guidelines of the International Streptomyces Project (Shirling and Gottlieb, 1966). The cultural aspect of the pure strain was studied on different ISP medium after 14 days incubation at 28°C. Colors were determined according to ISCC-NBS color charts (Kenneth, 1958).

Carbohydrates utilization was determined by growth on carbon utilization medium (ISP_9) supplemented with 1% carbon sources (Pridham and Gottlieb, 1948) and incubate at 30°C. Melanin production was observed on peptone yeast iron agar and tyrosine agar (Pridham et al., 1957).

Hydrolysis of gelatin was evaluated according to Waksman (1961) method. Degradation of adenine, guanine and L-Tyrosine were determinate using Goodfellow (1971) medium. Hydrolysis of casein and starch were evaluated by using the media of Gordon et al. (1974). Tween 80 was observed on Sierra and Greuell medium (1957), lecithinase and chitinase activity were evaluated respectively on egg-yolk medium (Nitsch and Kutzner, 1969) and Powdered Chitin Agar (Hsu and Lockwood, 1975).

Antibiotic bioassay

The antimicrobial activity of strain SLO-105 was evaluated on solid media by double layers method (Mellouli et al., 2003; Hu et al., 2000). After incubation of the strain for 8 days at 30°C, plates were covered by 4 ml of top agar previously seeded with 50 µl of fresh suspension of the test organisms. Plates were incubated 24 h at 37°C for bacteria and 3 - 5 at 30°C for fungi.

The test organisms were *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 9027*, *Bacillus subtilus ATCC 6633*, *Micrococus luteus* ATCC 4698, *Streptococcus fecalis ATTC 49332,* the pathogenic methicillin resistant *Staphylococcus aureus* MRSA ATCC 43300, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231 and *Rodotorulla mucilaginosa* ATCC 9449.

DNA isolation

A loop of culture of strain cultured on solid medium (ISP_2) was suspended in 0.5 ml of Tris-EDTA buffer (10 mM Tris, 1 mm EDTA, pH 8). 50 µl of lysozyme solution (100mg/ml) was added to the buffer. After overnight incubation at 37°C, 70 µl of a solution in 10% SDS and 6 µl of proteinase K (10 mg/mL) were added to the buffer and the solution was incubated at 65°C for 10 min. 500 µl of chloroform-isoamyl alcohol (24:1) were added to the solution and vortexed for 10 s, then centrifuge at 3000 g for 10 min. DNA was precipitated with 600 µl of isopropanol at -20°C for 30 min and then centrifuged at 15,000 rpm for 10 min. The pellet was washed 2 times with ethanol, dried at room temperature and then resuspended in 10 µl of 1X TE buffer and stored at -20°C (Valenzuela-Tovar et al., 2005)

Amplification and sequencing of the 16S rRNA gene

The amplification of the 16S gene was conducted using the kit Accuprime Taq and two primers 63F (CAGGCCTAACACATG CAACTC) and 1387r (GGGCGGGTGTACAAGGC). The PCR mixture consisted of 5 µl of 10X PCR buffer (final concentration: 1X) 0.6 µl primer 16S-63F, 0.74 µl primer 16S-1387r, 5 units / µl of Taq Polymerase and 1 µL genomic DNA to a final volume of 50 µl.

PCR was carried out in a thermocycler (Eppendorf mastercycler gradient) using a program that consisted of an initial denaturation at 94°C for 2 min, then 30 cycles of elongation (92°C for 30 s, 50°C for 30 s and 72°C for 1 min). The last step was performed at 72°C for 5 min. Two blanks containing all components of the reaction mixture except the DNA sample are routinely used as controls (Hu et al., 2002). The PCR products were analyzed by agarose gel electrophoresis.

The sequencing was performed (Genoscreen, France) using the BigDye Terminator v3.1 Cycle Sequencing Kit and the 3730xl sequencer (Applied Biosytems, Fermont, Calif.). The 16S DNA sequences obtained was compared with sequences deposited in GenBank genomic bank using the BLAST program (Version 2.0) available on the site http://www.ncbi.nlm.nih.gov/Blast. Sequence data for related species were retrieved and aligned using the Clustal X program. The phylogenetic tree was constructed via the neighbor-joining algorithm (Saitou and Nei, 1987) using the software MEGA 4.0.2 (Tamura et al., 2007). The sequence data was deposited in the Genbank Database, under the accession number GQ371155.

RESULTS AND DISCUSSION

The strain SLO-105 was isolated from lake Oubeira sediment situated in The National Park of El Kala (North-East of Algeria) which covers an area of 78 400 ha and contains a set of wetlands of high ecological value (Van Dijk and Ledant, 1983). The Lake Oubeira is a fully protected area and include a large wetland territory (76,438 ha). It has been stated of International Importance under the Ramsar Convention.

The scanning electron micrograph of the strain SLO-105 (Figure 1) revealed that the aerial mycelia were monopodially branched with compact sporophore. The spores had a smooth surface, no fragmentation of substrate mycelia was observed and no sclerotia, sporangia, or flagellated spores were formed.

The cultural characteristics of the strain listed in Table 1 showed moderate to good growth on most media. Typically, the colony was elevated, spreading and covered with vivid orange yellow aerial mycelium. The reverse side showed a deep orange yellow color and the strain produce a vivid yellow pigment on most media except on the ISP_5.

The morphological and cultural characteristics of the isolate were compared with known Actinomycetes species described in Bergey's manual of systematic bacteriology and they suggested that SLO-105 strain belong to Streptomyces genus. This was confirmed by 16S rDNA gene sequence analyzed. The alignment of this sequence with the 16S rDNA retrieved from the

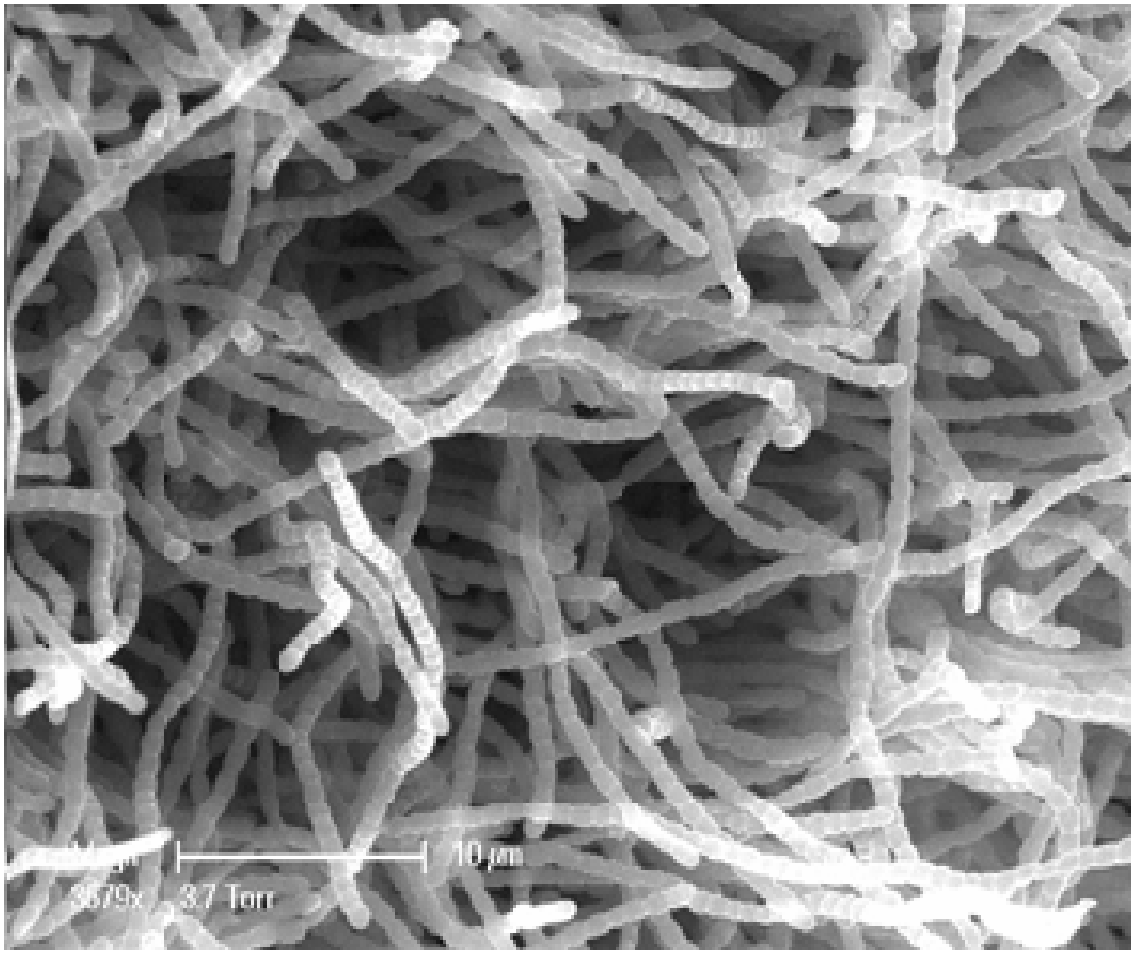

Figure 1. Scanning electron micrographs of the spore chains of strain SLO-105 grown on Yeast malt glucose agar at 30°C for 14 days.

Table 1. Cultural characteristics of strain SLO-105.

Medium	Growth	Aerial mycelium	Substrate mycelium	Soluble pigment
Tryptone yeast extract (ISP1)	Poor	Vivid Orange Yellow	deep orange yellow	Vivid Yellow
Yeast-malt-glucose (ISP2)	Good	Vivid Orange Yellow	deep orange yellow	Vivid Yellow
Oatmeal (ISP3)	Moderate	Light Gray	deep orange yellow	Vivid Yellow
Inorganic salts starch (ISP4)	Moderate	Vivid Orange Yellow	deep orange yellow	Vivid Yellow
Glycerol asparagines (ISP5)	Moderate	Light Orange Yellow	light orange yellow	None

genbank database revealed a high degree of similarity (98-99%) with Streptomyces 16S rDNA gene. Based on phylogenetic analysis (Figure 2) the strain was highly related to *Streptomyces galbus* strain DSM 40089 with 99% of similitude.

The physiological and biochemical characteristics of the isolate shown in Table 2 indicated that SLO-105 strain have the ability to utilize all tested carbon sources. The isolate produce melanin pigment when cultivated on either ISP_6 or ISP_7 and produced hydrogen sulfide. It was also able to degrade adenine, tween 80 and liquefy gelatin but not able to degrade casein, starch, guanine and L-tyrosine. The strain shown a lecithinase activity on egg-yolk agar but no chitinase activity was observed.

The active metabolites produced by SLO-105 strain exhibited various degrees of activities against tested organisms (Table 3). Antimicrobial activities expressed as the inhibition zone diameter showed that highest activities were observed on Gram positives bacteria *S. fecalis* (76 mm), *M. luteus* (72 mm), *S. aureus* MRSA (60 mm) and was also active against *A. niger* (45 mm) and *R. mucilaginosa* (55 mm). No activity was observed on fungi

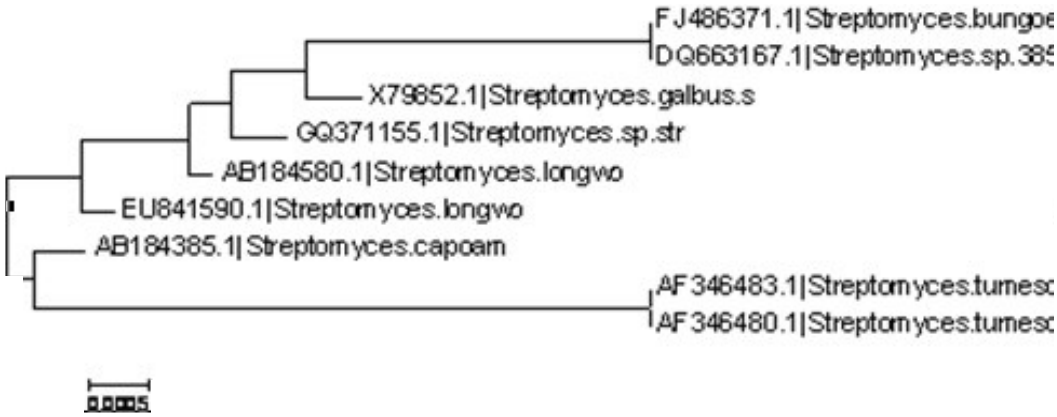

Figure 2. The phylogenetic position of SLO-105 strain among neighboring species.

Table 2. Physiological and biochemical characteristics of strain SLO-105.

Characteristics	Strain SLO-105
Carbon utilization	
Glucose	+
Arabinose	+
Fructose	+
Sucrose	+
Xylose	+
Lactose	+
Mannitol	+
Production of melanin pigment	
peptone-yeast extract-iron agar (ISP6)	+
Tyrosin agar (ISP7)	+
Degradation activity	
Adenine	+
Guanine	-
L-Tyrosine	-
Tween 80	+
Starch	-
Others	
Hydrogen sulfide production	+
Hydrolysis of casein	-
Liquefaction of gelatin	+
Lecithinase production	+
Chitinase production	-

Table 3. Antibacterial activity of SLO-105 strain on solid media.

Organism test	Inhibition zone diameter (mm)
Bacteria	
B. subtilis	68
E. coli	-
P. aeruginosa	-
S. aureus MRSA	60
M. luteus	72
S. fecalis	76
Fungi	
C. albicans	-
A. niger	45
R. mucilaginosa	55

Candida albicans and Gram negative tested bacteria.

In this work, we have shown the ability of the strain SLO-105 to produce antimicrobial compounds against microorganisms, especially the multiple antibiotic resistant Gram positive bacteria MRSA ATCC 43300 and the fungi *A. niger* and *R. mucilaginosa.* Further investigations on the extraction, purification and characterization of the active compound are currently in progress.

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