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Original article

Biocontrol of toxinogenic *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *albedinis* by two rare Saharan actinomycetes strains and LC-ESI/MS-MS profiling of their antimicrobial products

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ABSTRACT

Fungi colonizing fruits in the field and post-harvest constitute a major threat to the global food sector. This study focuses on the biocontrol of *Aspergillus flavus* (aflatoxin-producing mold considered carcinogenic by IARC) and *Fusarium oxysporum* f. sp. albedinis (FOA) (phytopathogenic agent, causal of El Bayoud in the Algerian and Moroccan Sahara). These molds have a significant economic impact and pose a serious human health problem. The aim of this work is to study the antifungal activity of two rare actinomycetes strains; *Saccharothrix* sp. COL22 and *Actinomadura* sp. COL08 strains against toxinogenic *A. flavus* and *F. oxysporum* f. sp. albedinis. The strains are isolated from *Citrullus colocynthis* rhizosphere on different media: ISP2, GLM, TSA, Starch-casein-agar and WYE and with different treatments of the samples (physical, chemical treatment and enrichment). The antifungal tests against the pathogenic microorganisms were performed on ISP2, GLM and TSA medium by means of the agar cylinders method. The kinetics of antibiotic production were performed on ISP medium over 16 days. The characterization of the antimicrobial compounds by LC-ESI/MS-MS showed that the bacterial extracts contain Antibiotic SF 2738C, Tetrodecamycin and Aplysillamide B. The phenotypic and molecular studies showed that *Saccharothrix* sp. COL22 is closely related to the *Saccharothrix longispora* strain type and that *Actinomadura* sp. COL08 is closely related to the *Actinomadura hibisca* strain type. The two strains are rare and showed an interesting activity against toxinogenic *A. flavus* and *F. oxysporum* f. sp. albedinis.
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1. Introduction

Preventing the risk of contamination of food products by toxigenic molds is a major issue for all operators in the agri-food sector. Cases of poisoning have always hit the headlines; these molds can also have very serious economic consequences.

Aflatoxins, primarily produced by *Aspergillus flavus* are potentially carcinogenic and can affect many organs, in particular the liver and kidneys. Aflatoxins are mutagenic, genotoxic, and have the ability to cause birth defects. They also have an immunosuppressive effect, so they can reduce resistance to infectious agents.

Aflatoxins also pose a significant economic burden, causing the destruction of 25% or more of the world food crops every year (Frisvad et al., 2019; Camiletti et al., 2017).

Phœniciculture in Saharan agriculture constitutes the main resource for the inhabitants of the Saharan regions of Algeria. For more than 100 years, Bayoud, a vascular disease of the date palm, caused by *oxysporum* f. sp. albedinis (FOA) which is a soil fungus, is the most damaging disease of date palm in Algeria, without effective solution until now (Mezouari et al., 2019; Sedra and Zhar, 2010). This disease has devastated the palm groves of Morocco and Algeria (western and central Algerian Sahara, a region known as "Saoura"). F. *oxysporum* f. sp. albedinis particularly affects the best date-producing varieties such as Deglet Nour. However, its impact goes beyond the simple economic aspect linked to the loss

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of date production, because the date palm is of paramount importance in the oasis ecosystem (Sedra et al., 2011).

Biocontrol is one of the most feasible solutions to replace synthetic fungicides. The characteristics of an ideal biocontrol agent were defined by Wilson and Wisniewski (1989), and relate to biosafety, activity in various environments and pathogens, as well as ease of management and use (Arasu, 2021).

In view of the limited range of molecules used in fungal therapy and with the emergence of multidrug resistance, the pharmaceutical industry and researchers have initiated search for new antifungals that are more effective and less aggressive toward human health. The vast majority of natural antifungals are of microbial origin, and nearly half are synthesized by actinomycetes.

Actinomycetes are filamentous, septate, branched and Gram-positive bacteria with a Chargaff coefficient (GC%) of between 60 and 70%. Most of them are still immobile; their growth is slow with a generation time of 2–3 h. They are abundantly distributed in nature. Actinomycetes are important mainly because of their role in soil fertilization, synthesis of complex compounds such as enzymes, antimicrobial, antiviral, antitumor, antiparasitic, neuroprotective, antioxidant and immunosuppressive compounds (Djebbaha et al., 2022; Djebbah et al., 2021; Al-Dhabi et al., 2020).The species belonging to the genus *Streptomyces* constitute 50% of the total population of actinomycetes, the most prevalent genus in the environment. In addition, the so-called rare actinomycetal bacteria constitute a potentially important and as yet little explored source of new secondary metabolites with excellent antibacterial and antifungal activity. Their selective isolation from various unexplored ecosystems, led to the discovery of new interesting substances; among the genera we can cite: *Actinomadura*, *Saccharothrix* (Al-Dhabi et al., 2020).

In recent years, studies of actinomycetes in the rhizospheric soil of plants have shown that plant-associated actinomycetes are promising biocontrol agents in agriculture (stimulating effects of plant development and protective effects against phytopathogens). Most of these microorganisms have, however, been isolated from vegetable and fruit tree rhizospheric soils. Actinomycetes associated with medicinal plants could, on the one hand, constitute important sources of bioactive natural substances and, on the other hand, present biological activities and produce bioactive natural substances similar to those of the plant. Thus, *Citrullus colocynthis* is a plant recognized for its therapeutic virtues, especially its antimicrobial and antitumor activity. On the basis of this working hypothesis, this present work focuses on the isolation of actinomycetes strains from the *Citrullus colocynthis* rizosphere and studying their antifungal activity as well as identifying their antimicrobial molecules (Al-Dhabi et al., 2020).

2. Materials and methods

2.1. Sample collection and processing

Four soil samples from the rhizosphere of the colocynth (Citrullus colocynthis) were taken from a region 100 km south of Bechar (gateway to the Algerian Sahara: Longitude 30°49′46.2″N; Latitude, 2°44′33.4″W) according to the technique of Pochon and Tardieux 1962 (Chater et al., 2010).The isolation of the actinomycetes from their ecosystems constitutes a problem. That is why pretreatment samples (chemical and physical) is inevitable and essential for a better isolation of actinomycetes having a much lower growth rate compared to non-filamentous bacteria and fungi. To this end, each sample is divided into 4 lots. The first batch is heated at 110 °C for 10 min (physical pretreatment). The second batch consisting of 1 g of soil with 0.1 g of CaCO3 (chemical pretreatment)to decrease the number of vegetative bacterial cells, while allowing spores of actinomycetes to grow) and incubated at 28 °C for 7 days in an atmosphere saturated with humidity. The third batch undergoes enrichment by rehydration and centrifugation to release the spores. To do so, 50 mL of sterile 10 mM phosphate buffer containing 10% of soil extract is placed in an Erlenmeyer flask supplemented with 0.5g of dry soil. The whole is incubated at 30 °C for 90 min. At the same time, 8 mL of this solution is transferred to a conical tube and then centrifuged at 1500g for 20 min. The last batch does not undergo any treatment (Zitouni et al., 2005; Jorgensen et al., 2015).

2.2. Isolation of actinomycetal strains

Isolation was performed by suspension-dilutions: decimal dilutions in 9 mL of physiological water (NaCl 9 g/L) have been realized. 1 mL of each dilution (10-1 at 10-3) is seeded on the surface; three plates are seeded by dilution. The culture media used are:

- ISP2 medium (International Streptomyces Project 2: Yeast extract 4 g, Malt extract 10 g, glucose 4 g, agar 15 g).It is used for the characterization of Streptomyces species. Nitrogen, amino acids and vitamins are provided by yeast extract and malt extract, and dextrose is the carbon source.
- TSA medium (Trypto-casein soybean) is a universal nutrient medium suitable for a wide range of uses. In this medium, the growth of actinomycetes could be optimal because of the tryptone and papaic digestion provided by the soybean meal due to the soybean carbohydrates and the casein proteins. Sodium chloride maintains the osmotic balance.
- GLM medium (yeast extract 3 g, malt extract 3 g, glucose 10 g, peptone 5 g, agar 15 g), peptone supplies nitrogen, carbon, and amino acid. Malt extract is a source of nutrients essential for the growth of Actinomycetes. Dextrose is a source of carbon energy and yeast extract provides vitamins.
- Starch-casein-agar medium (Starch 10 g, casein 1 g, K2HPO4 0.5 g, Agar 15 g) is used for the isolation of actinomycetes. Starch is used as carbohydrate source and casein supplies nitrogen. The existence of the starch, casein and the nitrate in the isolation medium leads to a selective isolation of the actinomycetes, whereas the non-filamentous bacteria and fungi grow slowly.
- WYE (water-yeast extract-agar, modified by Reddi and Rao: oxide yeast extract, 0.25 g; oxide agar, 18 g).

The media are supplemented with 50 µg / mL amphotericin B as an antifungal agent. The dishes are incubated at 28 °C for 3 weeks or more (Barbut and Neyme, 2006; Goodfellow, 2010; Elbendary et al., 2018).The colonies having the morphological characteristics of actinomycetes are purified on ISP2, and then frozen at −20 °C (The culture medium used for the cryoconservation is nutrient broth supplemented with 15% glycerol as a cryoprotectant which reduces freezing damage by increasing viscosity. Instead of intercellular and extracellular water crystallizing, the syrupy solution becomes an amorphous ice by vitrifying). The cultural and micromorphological characteristics are noted after 14 days of incubation at 28 °C on ISP2 medium. These characteristics are also observed under an optical microscope at magnifications 100x and 400x (Elbendary et al., 2018).

2.3. Characteristics of the target microorganisms

A. flavus NRLL62477, aflatoxin B1 producer strain, was provided by the Laboratory of Biology of Microbial Systems (LBSM) of the higher school of Kouba Algiers. The toxicity test was verified on a YES medium (Yeast extract-sucrose: glucose 40 g, Yeast extract

20 g) and detection of fluorescence was at 365 nm by means of the thin-layer chromatography method (TLC) (Arifuzzaman et al., 2010; Dehnad et al., 2010).

F. *oxysporum* f. sp. albedinis was were isolated from different areas in southwest Algeria and molecularly identified at the LABIRIS Research Institute, Belgium.

2.4. Antifungal activity - preliminary screening

Several culture media were tested to find the best medium that promotes the production of antifungal biomolecules from the two isolates, namely ISP2, GLM and TSA all at 0.8% agar for better dissemination of biomolecules (Kumar and Jadeja, 2016; Degola et al., 2012). To study the antifungal activity, the following Agar cylinders method was performed: It is often used to demonstratean antagonistic interactionbetween microorganisms, and the technique is similar to the disk-diffusion method. It involves making an agar culture of the strain of interest (actinomycetes) on the three media by tight streaks on the plate surface, and incubated at 28 °C for 8–10 days. During their growth, the microbial cells excrete metabolites which diffuse in the medium. After the incubation period, a cylinder is cut aseptically and deposited on the surface of the agar(potato dextrose agar "PDA": Potato infusion 200 gm, Dextrose 20 gm, Agar 8 g) of another plate previously inoculated by the target microorganism at a rate of 10^5 cfu/mL (colony forming units/mL). The dishes are left at 4 °C for 2 to 4 h for good diffusion of any antifungal metabolites, and then incubated at 28 °C. The substances diffuse from the cylinders to the medium. The negative control consists of depositing a cylinder of medium agar which contains no strain, on a medium inoculated with pathogens. Then, the antimicrobial activity is demonstrated by the appearance of an inhibition zone around the agar cylinder. Inhibition diameters were measured after 72 h using a decimal rule (Kumar and Jadeja, 2016; Tao et al., 2018; AbdullahAl-Dhabi, 2020).

2.5. Kinetics of antifungal activity

The kinetics of the antifungal activity of the isolates was conducted in a liquid ISP2 medium. A preculture was prepared on this same medium as follows: a bone filled with spores of the isolates, aged 10 days and growing on a ISP2 medium was inoculated in a 250 mL Erlenmeyer containing 100 mL of liquid ISP2 as medium. Cultures were incubated at 28 °C with shaking at 250 rpm for 16 days (Djinni et al., 2019; Wang et al., 2020). The development of the antifungal activity was determined daily during these 16 days using the well diffusion method (100 µl of culture filtrate per well of 5 mm in diameter) (Kumar and Jadeja, 2016).

2.6. Extraction of antimicrobial metabolites - secondary screening

The culture conditions are the same as those which were described during the study of the kinetics of production of antibiotics. The extraction of antibiotics from the culture filtrate is carried out using 4 organic solvents immiscible with water and of increasing polarity (dichloromethane, ethyl acetate, n-hexane, and n-butanol). The culture is centrifuged and then filtered. 100 mL are mixed with an equal volume of each solvent. The organic and aqueous phases are collected separately then concentrated and tested using the disc diffusion method (Al-Dhabi et al., 2020). The organic extracts obtained from the culture filtrates and as well as the aqueous phases are tested against *A. flavus* and *F. oxysporum*. Disks of 6 mm in diameter are impregnated with 120 µl of extracts to be tested. They are dried at 37 °C. for 45 min, then sterilized under UV at 254 nm for 30 min. The discs are then deposited on the surface of the semi-solid ISP2 medium (8 g / L of agar), previously seeded with the target fungi and placed at 4 °C for 2 to 4 h to allow a good diffusion of the metabolites before the growth of the fungi, then they are incubated at 25 °C for 5 days. The results are read by measuring the diameter of the inhibition zone around the disc to know the best extraction solvent (Kumar and Jadeja, 2016).

2.7. Identification of the antimicrobial molecules by liquid chromatography (LC) coupled to tandem mass spectrometry (MS-MS)

Organic extracts showing the best antimicrobial activity were de-replicated by LC/MS-MS. The HPLC apparatus used is Shimadzu LabSolutions. The column used is the C18 (reverse phase). Mobile phase: acetonitrile and Deionized water with 1% formic acid. The pump is in Binary gradient mode, brand LC-20ADXR at a flow rate of 0.2000 mL/min. The injected volume is 1 µl. the sample is automatically injected into the column by an Autosampler Model SIL-20ACXR (Wang et al., 2020).

The HPLC is coupled to an Electrospray/tandem mass spectrometry "LC-ESI/Ms-Ms" with collision induced dissociation (CID) which is based on the fragmentation of mass selected primary ions in a reaction cell through collisions with an inert gas. Electrospray has the advantage of implementing a soft ionization method which makes it possible to obtain information on the complexes in solution and to study their stability in the gas phase. The primary ions are produced by Corona discharges of 4.50 kV. Mass analyzer scanned m/z range is from 150 to 2000 amu. Tandem mass spectrometry allows partial fragmentation of a previously selected ion (in the first quadruple) by collision on neutral gas molecules located in the collision chamber (second quadruple). This mode thus makes it possible to obtain information as to the structure and the stability in the gaseous phase of the selected ion. The ions resulting from the fragmentation are analyzed by the third quadrupole. The mass spectrum thus obtained shows the parent ion and the fragment ions whose intensities vary according to the collision energy (voltage applied between the first and the second quadrupole). MS data were acquired in positive ion mode (Djinni et al., 2019; Wang et al., 2020).

Identification was made by comparing our data (mass of each component and their retention time) with database of the Chapman & Hall Dictionary of Natural Products (Jakubieckrzesniak et al., 2018).

2.8. Molecular characteristics and phylogenetic analyzes

2.8.1. DNA extraction

The DNA of each actinomycetal strain was extracted using two methods:

a. The Boiling method: Based on heat shock causing cell lysis and release of cell constituents. The suspensions of young cultures (7 days of culture with agitation on ISP2 broth, at 28 °C) are centrifuged at 12,000 rpm for 15 min. The pellet was resuspended in molecular biology grade water and centrifuged at 12,000 rpm for 10 min. After removing the supernatant, the pellet was resuspended in 40 µl of molecular biology grade water and boiled at 100 °C in a water bath for 10 min, cooled immediately after on ice and centrifuged at 12,000 rpm. / min for 10 s before being stored at −20 °C. (Jakubieckrzesniak et al., 2018).
b. The use of phenol chloroform, according to the following protocol: from a young culture, the bacterial colonies are suspended in 550 µl of TE (Tris + EDTA: Tris HCl 10 mL pH 7.4, EDTA 1 mL pH 8). Then, 17 µl of lysozyme (35 mg / mL) are added and the mixture is incubated at 37° for 30 min. Protein hydrolysis is provided by 6 µl of proteinase K (10 mg / mL). After that, 30 µl of 10% SDS (Sodium dodecyl

sulfate) are added and the mixture is rigorously mixed and then incubated at 37 °C for 30 min. Subsequently, 100 µl of 5 M NaCl and 80 µl of CTAB (Cetyl Trim ethyl Ammonium Bromide: CTAB 100 g/L, NaCl 41 g/L) are added for the solubilization of the lipid membranes and the denaturation of the proteins. After incubation for 10 min at 65 °C, the proteins are separated from the aqueous phase by precipitation with 800 µl of CIA (Chloroform Isoamyl Alcohol, 24:1, v/v), followed by centrifugation at 12,000 rpm for 20 min. The aqueous phase containing the DNA is transferred to another Eppendorf tube and 800 µl of PCIA (Phenol Chloroform Isoamyl Alcohol, 25:24:1, v/v/v) are added. After centrifugation at 12000 rpm for 20 min, the aqueous phase is poured into a new tube.

DNA precipitation is ensured by adding 0.6 vol of isopropanol, and incubated at −20 °C for 2 h. After centrifugation for 5 min at 13,000 rpm, the pellet containing the DNA is recovered before being washed with 200 µl of 70° alcohol that will in turn be removed by centrifugation for 5 min at 13,000 rpm. The DNA obtained is dried, recovered in 100 µl of TER (TE + 1 µlRnase 20 mg/mL) and stored at −20 °C. The concentration of genomic DNA is assessed by electrophoresis on 0.8% of agarose gel and by spectrophotometry (NanoDrop) (Cao et al., 2020; Liu et al., 2019).

2.8.2. PCR amplification of the ITS 16S-23S transcribed intergenic spaces

Amplification of the intergenic regions was carried out using the universal primers: S-D-Bact-1494-a-S-20 and S-D-Bact-0035-a-A-15 to obtain the following sequences respectively:

5′GTCGTAACAAGGTAGCCGTA 3′ and 5′GCCAAGGCATCCACC 3′.

The PCR-ITS reaction mixture contains, in a final volume of 25 µl, the following concentration and volume reagents: 1X PCR buffer (2.5 µl), 2 mM MgCl2 (2 µl), 0.2 mMdNTP (0.2 µl), 0.1 µM primer F (0.1 µl), 0.1 µM primer R (0.1 µl), 0.5 U / R Taq polymerase (0.1 µl), and 1 µl of genomic DNA. Each reaction begins with a cycle of initial DNA denaturation at 95 °C for 4 min. It is followed by 30 cycles each of which iscomposed of (i) a denaturation step at 94 °C for 45 sec; (ii) a hybridizationstepof the pair of primers on the denatured template DNA at 55 °C for 30 sec, and finally; (iii) an elongation step at 72 °C for 45 sec. The amplification reaction is completed by a final extension cycle at 72 °C for 7 min (Cao et al., 2020).

2.8.3. Amplification of the 16S rDNA fragment

Amplification was carried out by a polymerase chain reaction (PCR). Two renowned universal primers were used in this study, and the concentration of each one is 25µMmol / µL molecular biology grade water.

27f (5′AGAGTTTGATCCTGGCTCAG 3′).

1492r (5′AAGGAGGTGATCCAGCCGCA 3′).

One microliter of the extracted DNA supernatant is diluted to 1/10, 1/20 and 1/50 and added to 24 µl of the reaction mixture containing, 25 mM MgCl2, 25 µM of each deoxynucleotide triphosphate, 5 Units of Hi-Taq DNA polymerase.

Amplification is performed in a thermal cycler (TC-Pro, Boeco, GERMANY); it begins with an initial denaturation cycle at 95 °C for 4 min then 30 cycles comprising 45 s of denaturation at 94 °C, 30 s of hybridization at 55 °C, 45 s of elongation at 72 °C, and 7 min followed by elongation at 72 °C (Cao et al., 2020; Buckingham, 2013).

2.8.4. Electrophoresis

After amplification, the samples are analyzed by horizontal electrophoresis for 45 min at 100 V in a 1.5% agarose gel in 0.5% TBE buffer in the presence of a size marker of 1 KB. After migration, the gel is treated with BET for 30 min then examined under ultraviolet (UV) light in a GelDoc to identify the amplified bands of size 1500 bp using the software IMAGE Lab 5.2.1. (Cao et al., 2020; Mahjoubi et al., 2013).

2.8.5. Sequencing

The sequencing is carried out according to the automated Sanger technique using the same pair of primers as for PCR. The determined sequence of the gene encoding 16S RNA is compared with the homologous sequences of reference microbial species listed in genomic banks, using "BLAST NCBI" (Cao et al., 2020; Rajivgandhi et al., 2019).The phylogenetic analysis is performed using MEGA software, version 10.1.8. (Saket and Ravishankar Rai, 2019). The sequence of the gene encoding the 16S RNA of the isolates are aligned using the Muscle algorithm with the homologous sequences closest to the strains of actinomycetes found in the genomic bank. The calculation of the evolution distance matrices is carried out by the Jukes and Cantor method (Khadayat et al., 2020).

The construction of the phylogenetic tree is done by means of the "neighbor-joining" algorithm (Boudjella et al., 2007). The statistical validation of the phylogenetic links established is carried out through the Bootstrap test, the values of which are based on the result of 1000 analyzes (Kumar et al., 2004).

2.8.6. Statistical analysis

The statistical analysis concerning the diameters of the antifungal activity is done by the Student test. The statistical analysis of the kinetics of the evolution of antifungal activities is done by the test of the ANOVA variance followed by a multiple comparison of Tukey-Kramer. The software package used for statistical analysis is Excel 2013. All results are the means of three independent replicates.

3. Results

3.1. Isolation of actinomycetal strains

ISP2 medium showed better growth and a higher number of actinomycetes compared to other media. The isolates COL22 and COL08 were isolated in ISP2. Purification of each colony is done on the same isolation medium and stored at 4 °C and −20 °C.

3.2. Antimicrobial activity

The antifungal activity of the isolates against the target fungi is given in Table 1 and Fig. 1. The COL22 isolate showed interesting activity on all the media used with a slight preference for ISP2 medium, against A. *flavus* and F. *oxysporum* f. sp. albedinis. Isolate COL08 showed antifungal activity only against A. *flavus*.

The statistical analysis of the results reported in Table 1 by the Student test reveals that:

The antifungal activity of the COL22 and COL08 isolates against A. *flavus*: t_Student = 2,43 > t_critical value. We notice the presence of a significant difference between the antifungal activity of COL22

Table 1
Mean and standard deviation of antifungal activity of COL22 and COL08 on ISP2 medium with agar cylinders method.

	COL22 Isolate	COL08 Isolate
A. *flavus*	22.66 ± 0.57 mm (a)	15 ± 0.5 mm (b)
F. *oxysporum* f. sp. albedinis	23.83 ± 0.28 mm (c)	00 mm (d)

* The statistical analysis used is the Student test.

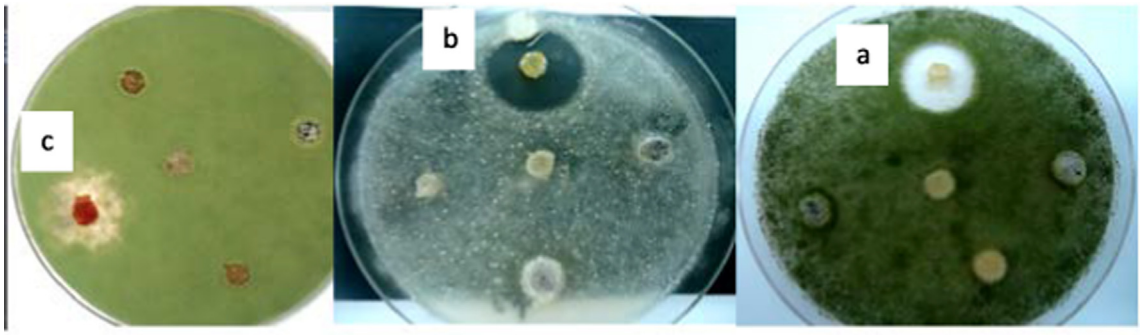


Fig. 1. Antifungal interactions vis-à-vis *A. flavus* and *F. oxysporum* f. sp. Albedinis using Agar cylinders on ISP2 medium. a: COL22 against *A. flavus*. b: COL22 against *F. oxysporum* f. sp. albedinis. c: COL08 against *A. flavus*. The cylinder in the center of each petri dish represents the negative control. The other three cylinders represent other isolated actinomycetes which showed no antifungal activity.

and COL08 against A. *flavus* was observed. t = 2,4364;P = 0,0209; dt = 30.

The antifungal activity of the COL22 and COL08 isolates against F. *oxysporum*:t_Student = 8 > t_critical value. We noticethe presence of a significant difference between the antifungal activity of COL22 and COL08 against F. *oxysporum*. t = 8,005; P = 6,18023 E-09; dt = 30.

3.3. Kinetics of antifungal activities

The kinetics of the antifungal activities, carried out on the ISP2 medium, are represented in Fig. 2. The activities begin on the third day. The activities peak on day 9–10 for isolate COL22 against both target molds, and on day 8 for isolate COL08 against A. *flavus*.

The statistical analysis of the results reported in Fig. 2 by ANOVA reveals p value = 0.055 greater than 0.05. Therefore, one can notice that there is not a significant difference between the three groups (COL22 against F. *oxysporum*, COL22 against A. *flavus* and COL08 against A. *flavus*). Fig. 3 shows multiple comparisons by the post-hoc Tukey-Kramer analysis between the three groups.

3.4. Extraction of antimicrobial metabolits

The COL22 strain showed antifungal activities in the organic phases of the dichloromethane, ethyl acetate and n-butanol extracts, but no activity was detected in the n-hexane extract.

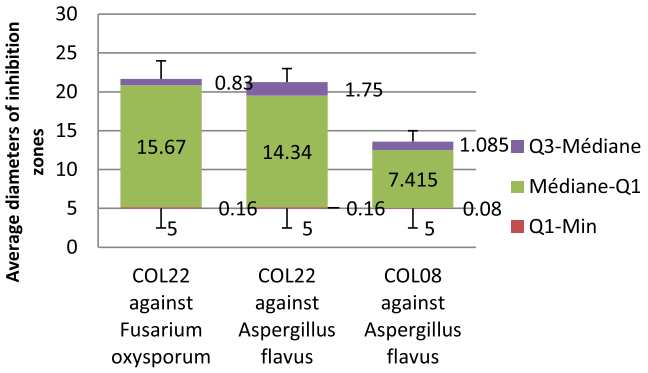


Fig. 3. Multiple comparison using the Tukey-Kramer method between the three groups (COL22 against *F. oxysporum*, COL22 against *A. flavus* and COL08 against *A. flavus*).

Dichloromethane will be maintained as the best extraction solvent, because it is the solvent that has shown better antimicrobial activity. The COL08 strain showed antifungal activities only in the n-butanol extract. Dichloromethane is known to be the solvent which extracts the fewest impurities thus making the final HPLC purification relatively easier, especially compared to n-butanol which extracts a lot of impurities which make the purifications more difficult (Jukes and Cantor, 1969).

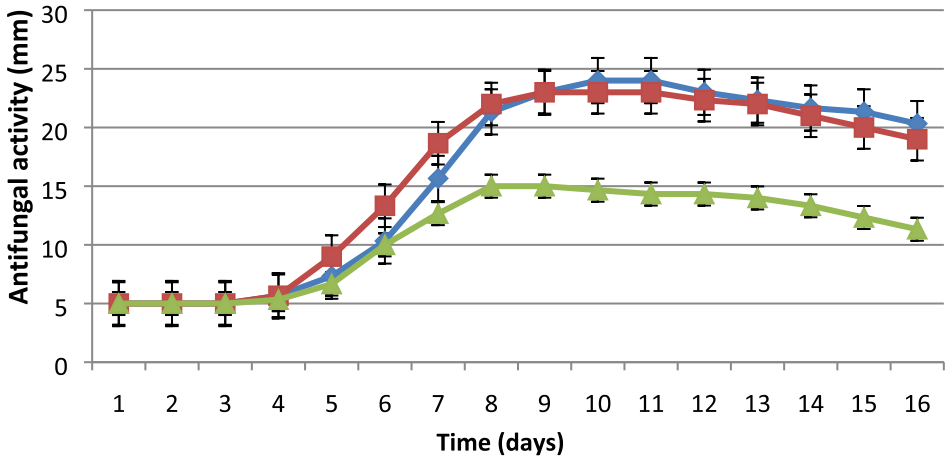


Fig. 2. Kinetics of the evolution of antifungal activities on ISP2 medium. COL22 against *A.flavus* (square), COL22 against *F. oxysporum* f. sp. albedinis (diamonds), COL08 against A. *flavus* (triangle). The activity is monitored by the method of agar cylinders, the diameter of thewell (5 mm) is included in the measurements.

3.5. Identification of the antimicrobial molecules by liquid chromatography (LC) coupled to tandem mass spectrometry (MS-MS)

3.5.1. Antimicrobial compounds produced by Saccharothrix longispora

The mass spectrum of *Saccharothrix longispora* extract, obtained in positive mode, presents 2 pseudo-molecular ions [M+H] + with an *m*/*z* ratio equal to 263.3 amu (atomic mass unit) and 337.4 amu (see Fig. 4 and Table 2) suggesting a molecular weight of 262.3 and 336.4 respectively. The mass spectrum of these ions also shows ionized fragments of the molecule with different mass losses (see Fig. 4). The identification is summarized in Table 2.

3.5.2. Antimicrobial compounds produced by Actinomadura hibisca

The mass spectrum of the *Actinomadura hibisca* extract, obtained in positive mode, presents a pseudo-molecular ion [M +H] + with an *m*/*z* ratio equal to 299.4 amu (atomic mass unit) (see Fig. 5 and Table 2) suggesting a molecular weight of 298.4. The mass spectrum of these ions also shows ionized fragments of the molecule with different mass losses (see Fig. 5). The identification is summarized in Table 2.

3.6. Identification of COL22 and COL08 isolates

The COL22 isolate grows very well on all media used. As shown in Fig. 6 (a), the colonies are yellow-orange in color on ISP2 medium and can form a crumpled bacterial mat. Micromorphologically at magnifications (400x) (as shown in Fig. 6 (b)), the COL22 isolate is characterized by the presence of abundant aerial filaments, long chains of non-motile rod spores resulting from anarchic fragmentation of aerial filaments.

The COL08 isolate grows very quickly on all media used. As shown in Fig. 6 (c), the colonies are reddish. Diffusible red pigments are secreted on the media and are particularly abundant on ISP2. Micromorphologically at magnifications (400x) and as shown in Fig. 6 (d), isolate COL08 is characterized by the presence of an unfragmented substrate filaments surmounted by a very sparsely produced aerial filaments.

The classic phenol–chloroform is one of the oldest DNA extraction protocols. As clarified earlier, this protocol benefits from the use of sodium dodecyl sulfate (SDS) for lysis of the cell wall, and proteinase K and RNase for digestion of protein and RNA, respectively. This method mostly gives high yields of good-quality DNA. As an alternative to the phenol–chloroform extraction procedure, the boiling method has the advantage of not using toxic chemicals like phenol and chloroform, but extraction gives poor quality DNA.

The partial sequence of the gene encoding16S RNA of the COL22 and COL08 isolates are determined and deposited at GenBank under the accession number of MW007750 and MW226995 respectively. Sequence analysis made it possible to relate the isolate COL22 and COL08 to the species *Saccharothrix* longispora and *Actinomadura* hibisca with a similar rate of 99.01% and 99.51% respectively. The phylogenetic position of these isolates relative to the closest species of the same genus is shown in Figs. 7 and 8.

4. Discussion

Fungal infections have increased in recent years due to the development of resistance to antifungals. There is therefore a need for research of new antifungal molecules with minimal side effects. The potential antifungal activity of *Streptomyces* strains from different ecosystems is well known. Nevertheless, limited studies on other genera of actinomycetes are available and global surveys have recognized that Saharan ecosystems are rich in microbial diversity. The genera *Saccharothrix* and *Actinomadura* are known for the production of several antibiotics of great structural diversity and an interesting spectrum of action.

These antibiotics belong to the families and groups of aminoglycosides and benzoquinones, glycopeptides, carboxylic nucleosides, heptadecaglycosides, dithiolopyrrolones, anthracyclines, macrolides, angucyclines, and benzene aromatics (Jukes and Cantor, 1969; Saitou and Nei, 1987; Felsenstein, 1985; Boudjella et al., 2007; Zitouni et al., 2004; Lamari et al., 2002; Labeda and Kroppenstedt, 2000; Zhuang et al., 2020).

The activity of molecules produced by *Saccharothrix* strains is directed mostly against Gram-positive bacteria, but not often against fungi and even more rarely against Gram-negative bacteria (Al-Dhabi et al., 2020; Liu et al., 2019; Zhuang et al., 2020).

The low percentage of antifungal activity was also noted by other authors (Abagana et al., 2016; Takahashi et al., 1986). Antifungal activity is found to be more interesting intheISP2 medium. Similar findings were reported by (Al-Dhabi et al., 2020; Rajivgandhi et al., 2019; Jukes and Cantor, 1969) who obtained good results with the media used, yet with a slight preference for theISP2 medium in general. The importance of culture media, certain components of which play an important role as precursors of antibiotics, should therefore not be overlooked. Indeed, the anti-

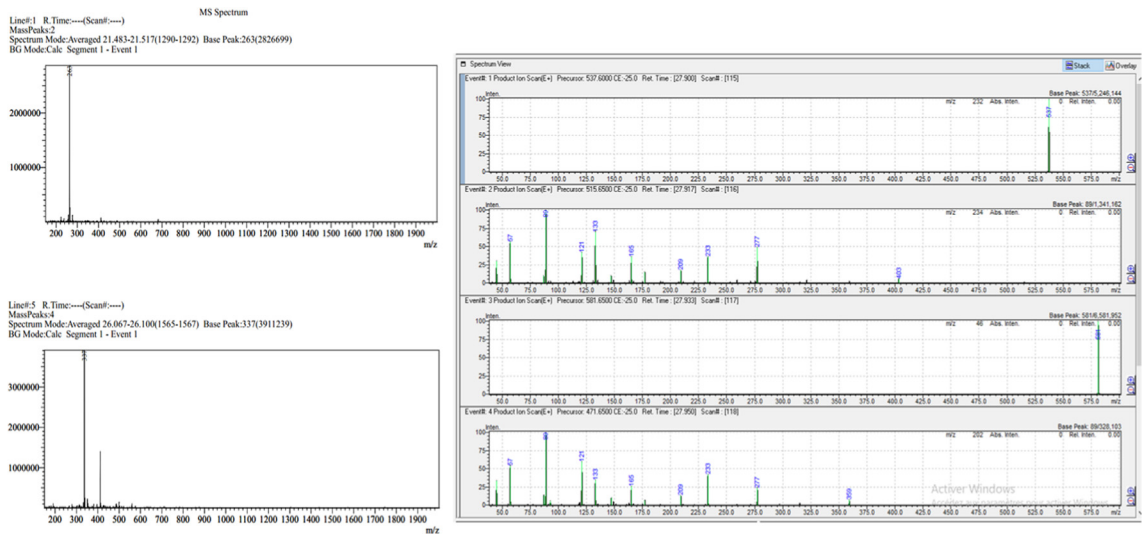
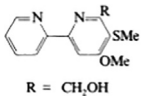
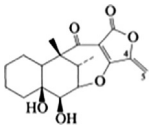
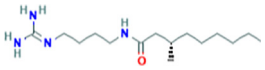


Fig. 4. LC–ESI/MS-MS positive ion mode spectra of Antibiotic SF 2738C and Tetrodecamycin produced by Saccharothrix longispora.

Table 2
Summary of the most interesting antimicrobial molecules obtained by LC–ESI/MS-MS positive ion mode of organic extracts of *Saccharothrix longispora* and *Actinomadura hibisca*. Compounds identified in the Chapman & Hall database (Jakubieckrzesniak et al., 2018).

Producing strains	Retention time (min)	Formula	Ion	Mass	Identification	Molecular formula	Biological activies
Saccharothrix longispora	21.513		$[M+H]^+$	262.3	Antibiotic SF 2738C4-Methoxy-5-(methylthio)-[2,2′ -bipyridine]-6-methanol. 6-Hydroxymethyl-4-methoxy-5-(methylthio) -2,2′ -bipyridine	$C_{13}H_{14}N_2O_2S$	Antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi, and exhibited cytotoxic activity
	26.095		$[M+H]^+$	336.4	Tetrodecamycin	$C_{18}H_{22}O_6$	Antiviral, antimicrobial and antitumor.
Actinomadura hibisca	24.728		$[M+H]^+$	298.4	Aplysillamide B	$C_{16}H_{34}N_4O$	Antimicrobial activity against some fungi and bacteria. Not cytotoxic.

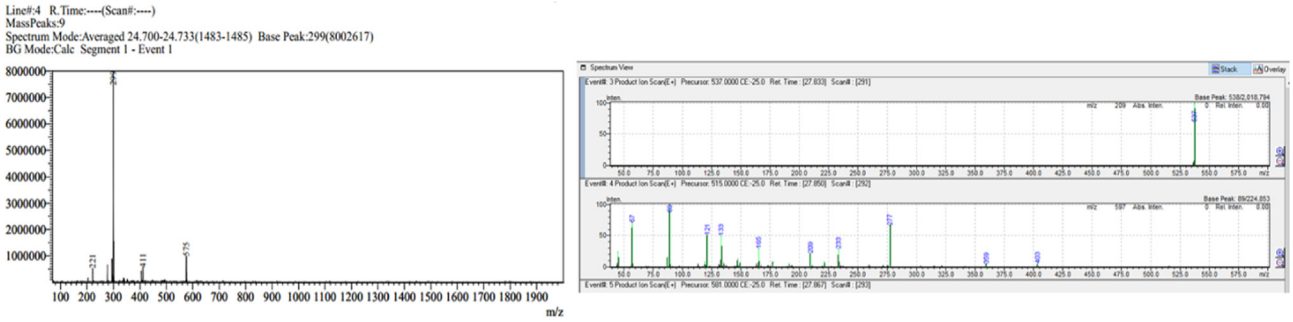


Fig. 5. LC–ESI/MS-MS positive ion mode spectra of Aplysillamide B produced by *Actinomadura hibisca*.

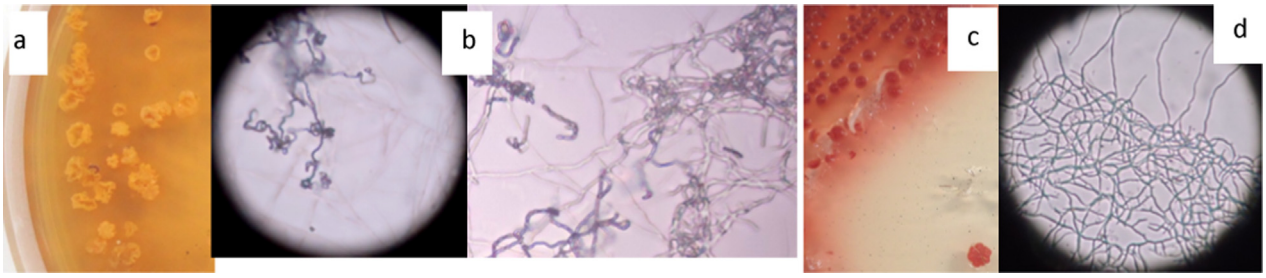


Fig. 6. a, b: Microscopic(magnifications (400x)) and macroscopic observation of COL22 isolate on TSA medium. c, d: Microscopic (magnifications (400x)) and macroscopic observation of COL08 isolate on TSA medium.

fungal activity is better on ISP2 (abundantin malt extract) than on TSA and GLM medium. It is therefore obvious that the components of the malt extract played an important role in the increase of this activity on theISP2 medium. It could also be that the glucose present in large quantities especially in the GLM medium (10 g / L against 4 g / L for ISP2) has played a damaging role. According to several authors (Rajivgandhi et al., 2019; Jukes and Cantor, 1969; Saitou and Nei, 1987; Felsenstein, 1985), for most of the microorganisms producing antibiotics, glucose, a rapidly assimilable carbon source, exerts at a certain concentration a catabolic repression on the production of antibiotics such as actinomycin, bacitracin, cephalosporin C, chloramphenicol, kanamycin, penicillin, etc. For a concentration of 10 g / L of glucose, (Felsenstein, 1985) showed a repressive effect of this sugar on the production of nucleoside antibiotics, but not on the macrolides synthesized by *Saccharothrix* sp. SA103 isolated from Saharan soil. Given the complexity of the media tested, it is difficult to attribute a rational explanation to the differences observed in production when moving from one medium to another. For these reasons, it is imperative to test the antibiotic activity of strains isolated on several media before deciding which is the most suitable.

The study of antifungal activity in general is of utmost importance in the hope of finding molecules among non-polyenics. Indeed, the original antifungal agents currently available and commonly used (clinically known as amphotericin B and nystatin) are polyene in nature. These molecules have very dangerous side effects; amphotericin B, for example, causes severe kidney infection. Oriented screening using a strain resistant to polyenes as the target germ is very useful, as it makes it possible to direct the investigations towards non-polyene molecules that have remained very poorly known to this day.

In our study, three antimicrobial molecules were identified in the organic extracts. In the literature, little work is done on these molecules. Antibiotic SF 2738C was first extracted by S. Gomi

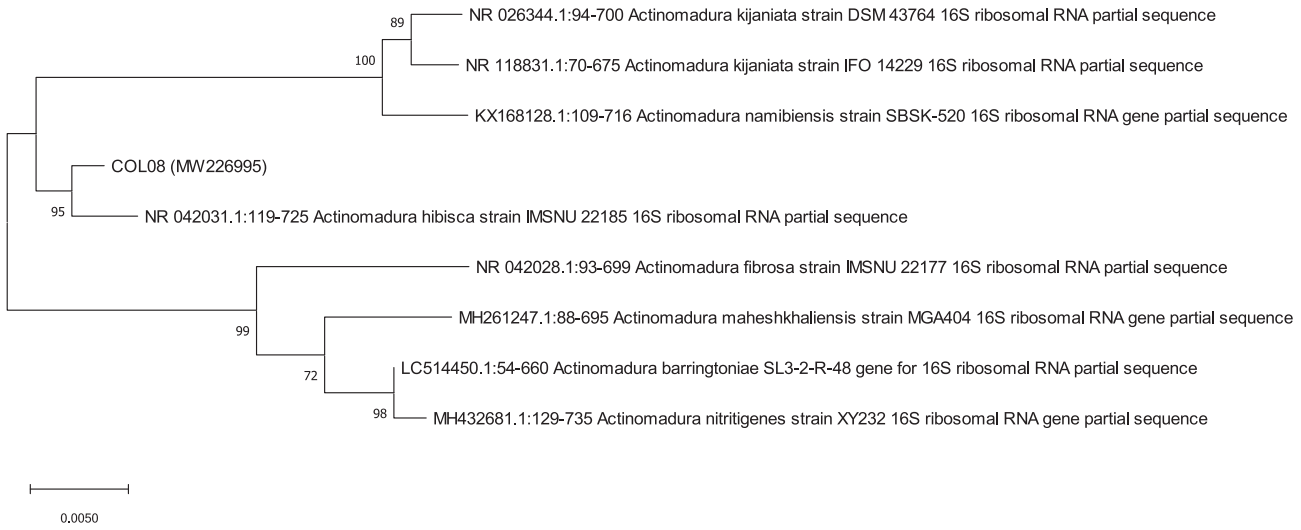


Fig. 7. Phylogenetic tree based on theanalysis of the sequences of the gene encoding 16S rRNA, showing the relationship between the COL08 isolate (MW226995) and the closest species of the genera *Actinomadura*. The Bootstrap values (greater than 70%) provided over 1000 analyzes are described at the level of the tree nodes.

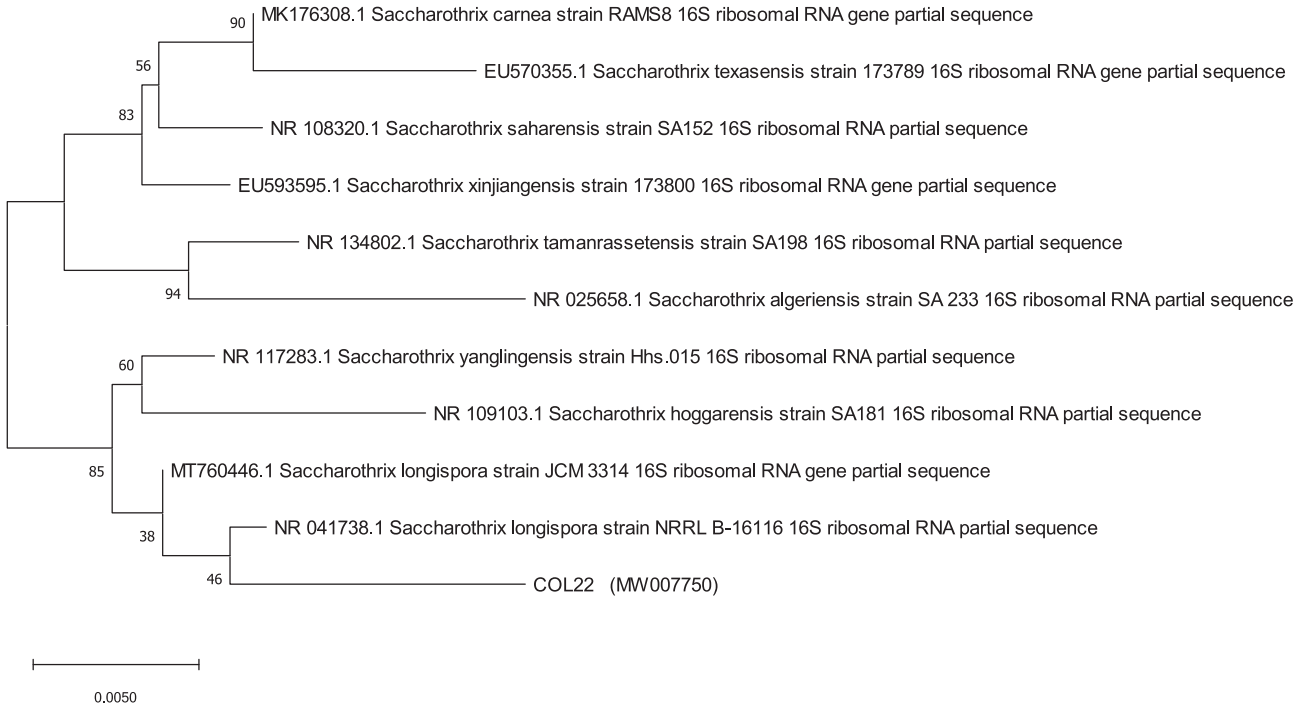


Fig. 8. Phylogenetic tree based on sequence analysis of the gene encoding 16S rRNA, showing the relationship between the COL22 isolate (MW007750) and the closest species of the genus Saccharothrix. Bootstrap values (greater than 40%) based on 1000 scans are shown at tree nodes.

et al from the culture broth of *Streptomyces* sp. The antibiotic showed an excellent antimimicrobial and anti-tumours abilities. It exhibited cytotoxic activity against P388 murine leukemia cells with IC50 values of 0.08, 0.25 and 7.5 Âµg/ml, respectively. Tetrodecamycins are secondary metabolites, known for their antibacterial activity against S.aureus and Photobacteriumdamselae ssp. Piscicida (A bacterium that causes pseudotuberculosis in some fish). In the literature, this molecule was produced by Streptomyces nashvillensis MJ885-mF8 isolated from a soil sample taken from Suginami-ku, Tokyo, Japan, but never previously from *Sacharrothrix*. The tetrodecamycins are known for their antibacterial, antifungal, antiviral and antitumor activities. At present, its molecular target and mechanism of action are very little studied and there is still a lot to do with this molecule. Knowing the mechanism of action of this molecule will lead to the knowledge of new molecular targets for the development of antibiotics. Aplysillamide B, antimicrobial guanidine alkaloids which, according to the bibliography ever produced by the actinomycetes in general (Takeuchi et al., 1992; Hacene et al., 1994; Hilali et al., 2002; Gomi et al., 1994; Honma et al., 1995).

The genus *Saccharothrix* comprises 12 species while the genus *Actinomadura* has up to37 (Jukes and Cantor, 1969). Broad spectrum (antibacterial and antifungal) *Saccharothrix* and *Actinomadura* isolates are often described in the literature, and in Algeria several strains of *Sacharrothrix* and *Actinomadura* have been isolated from soils, water and bark of trees (Saitou and Nei, 1987; Felsenstein,

1985; Boudjella et al., 2007; Zitouni et al., 2004; Lamari et al., 2002) but, to the best of our knowledge, never from the rhizosphere of *C. colocynthis*. It should also be noted that for *S. longispora* and *A.hibisca* no information concerning their antagonism vis-à-vis *A. flavus* and *F. oxysporum*f. sp. albedinis is mentioned in the available literature.

5. Conclusion

Several research strategies have been developed in order to bring new antibiotics to the market. There have been attempts to find out strains belonging to rare or infrequent genera of actinomycetes (other than the genus *Streptomyces*) and originating from extreme and particular ecosystems. It is in this context that the objective of our research workfalls. It aims to identify rare actinomycetes of Saharan origin and to study the antibiotics they produce. The isolates under study in the present work are rare actinomycetes: *Sacharrothrix longispora* and *Actinomadura hibisca.* They show great promise in terms of antifungal biomolecules (Gverzdys et al., 2016). At present, it is important to search for new strains that produce antimicrobial metabolites, because pre-existing antibiotics have failed due to the development of pathogen resistance to antibiotics. The present study is also a contribution to remedy this problem. Isolates which have shown activity against the fungi tested may be considered very good candidates for the search for potential antimicrobial compounds. The characterization of our antimicrobial compounds by LC-ESI/MS-MS showed that the bacterial extracts contain Antibiotic SF 2738C, Tetrodecamycin and Aplysillamide B. These antimicrobial molecules are rarely discussed in the bibliography. In conclusion, this is a pioneering study of antimicrobial activities of actinomycetes isolated from *Citrullus colocynthis* rizosphere against toxinogenic fungi to the best of our knowledge.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abagana, A.Y., Sun, P., Liu, C., Cao, T., Zheng, W., Zhao, S., Xiang, W., Wang, X., 2016. *Actinomadura* gamaensis sp. nov., a novel actinomycete isolated from soil in Gama, Chad. Antonie van Leeuwenhoek. J. Microbiol. 109 (6), 833–839.

Al-Dhabi, Naif Abdullah, Esmail, Galal Ali, Ghilan, Abdul-Kareem Mohammed, Arasu, Mariadhas Valan, Duraipandiyan, Veeramuthu, 2020. J. King Saud Univ. – Sci. 32 (1), 1226–1232. https://doi.org/10.1016/j.jksus.2019.11.018.

Al-Dhabi, Naif Abdullah, Ali, Galal, Abdul-Kareem, Esmail, Ghilan, Mohammed, Arasu, Mariadhas Valan, Duraipandiyan, Veeramuthu, Ponmurugan, Karuppiah, 2020. Characterization and fermentation optimization of novel thermo stable alkaline protease from Streptomyces sp. Al-Dhabi-82 from the Saudi Arabian environment for eco-friendly and industrial applications. J. King Saud Univ. – Sci. 32 (1), 1258–1264. https://doi.org/10.1016/j.jksus.2019.11.011.

Al-Dhabi, Naif Abdullah, Esmail, Galal Ali, Ghilan, Abdul-Kareem Mohammed, Arasu, Mariadhas Valan, Duraipandiyan, Veeramuthu, 2020. Metabolite profiling of Streptomyces sp. Al-Dhabi-100 isolated from the marine environment in Saudi Arabia with anti-bacterial, anti-tubercular and anti-oxidant potentials. J. King Saud Univ. – Sci. 32 (2), 1628–1633. https://doi.org/10.1016/j.jksus.2019.12.021.

Al-Dhabi, Naif Abdullah, Esmail, Galal Ali, Ghilan, Abdul-Kareem Mohammed, Arasu, Mariadhas Valan, 2020. Saudi J. Biol. Sci. 27(1), 474-479. 10.1016/j.sjbs.2019.11.011.

Arasu, Mariadhas Valan, 2021. Editorial on Antimicrobial Resistance and its Prevalence. J. Infect. Public Health 14 (12), 1739. https://doi.org/10.1016/j.jiph.2021.11.018.

Arifuzzaman, A., Khatun, M.R., RahmanIsolation, H., 2010. Screening of actinomycetes from Sundarbans soil for antibacterial activity. Afri. J. Biotech. 9 (29), 4615–4619.

Barbut, F., Neyme, D., 2006. Les difficultés d'interprétation des contrôles microbiologiques environnementaux. Revue Francophone des Laboratoires. 2006 (382), 27–32.

Boudjella, H., Bouti, K., Zitouni, A., Mathieu, F., Lebrihi, A., Sabaou, N., 2007. Isolation and partial characterization of pigment-like antibiotics produced by a new strain of Streptosporangium isolated from an Algerian soil. J. Appl. Microbiol. 103 (1), 228–236.

Boudjella, H., Bouti, K., Zitouni, A., Mathieu, F., Lebrihi, A., Sabaou, N., 2007. Isolation and partial characterization of pigment-like antibiotics produced by a new strain of Streptosporangium isolated from an Algerian soil. J. Appl. Microbiol. 103 (1), 228–236.

Buckingham, J., 2013. Dictionary of natural products on CDROM, third ed., Chapman & Hall, London.

Camiletti, B.X., Torrico, A.K., Fernando Maurino, M., 2017. Fungal screening and aflatoxin production by *Aspergillus* section Flavi isolated from pre-harvest maize ears grown in two Argentinean regions. Crop Protect. 92, 41–48.

Cao, Peng, Li, Chenxu, Wang, Han, Zhiyin, Yu, Xi, Xu, Wang, Xiangjing, Zhao, Junwei, Xiang, Wensheng, 2020. Community Structures and Antifungal Activity of Root-Associated Endophytic Actinobacteria in Healthy and Diseased Cucumber Plants and Streptomyces sp. HAAG3-15 as a Promising Biocontrol Agent. Microorganisms 8 (2), 236.

Chater, K.F., Biró, S., Lee, K.J., Palmer, T., Schrempf, H., 2010. The complex extracellular biology of Streptomyces. FEMS Microbiol. Rev. 34 (2), 171–198.

Degola, F., Dall'Asta, C., Restivo, F.M., 2012. Development of a simple and high-throughput method for detecting aflatoxins production in culture media. Lett. Appl. Microbiol. 55 (1), 82–89.

DehnadA, P.L., Bakhshi, R., Soofiani, S.A., Mokhtarzadeh, A., 2010. Investigation antibacterial activity of Streptomycetes isolates from soil samples, West of Iran. Afri. J. Microbiol. Res. 4 (14), 1542–1549.

Djebbah, Fatima Zohra, Belyagoubi, Larbi, Abdelouahid, Djamel Eddine, Kherbouche, Farid, Al-Dhabi, Naif Abdullah, Arasu, Mariadhas Valan, Ravindran, Balasubramani, 2021. Isolation and characterization of novel Streptomyces strain from Algeria and its in-vitro antimicrobial properties against microbial pathogens. J. Infect. Public Health 14 (11), 1671–1678. https://doi.org/10.1016/j.jiph.2021.09.019.

Djebbaha, Fatima Zohra, Al-Dhabi, Naif Abdullah, Belyagoubi, Mariadhas Valan Arasu Larbi, Kherbouche, Farid, Abdelouahid, Djamel Eddine, Ravindrane, Balasubramani, 2022. Isolation and characterisation of Streptomyces sp. Strain GLD25 with antimicrobial and antioxidant effects from Gueldaman cave (GLD1), Akbou-Algeria. J. King Saud Univ. – Sci. 34 (1), 101719. https://doi.org/10.1016/j.jksus.2021.101719.

Djinni, I., Defant, A., Kecha, M., Mancini, I., 2019. Actinobacteria Derived from Algerian Ecosystems as a Prominent Source of Antimicrobial Molecules. Antibiotics 8 (4), 172.

Elbendary, Afaf Ahmed, Hessain, Ashgan Mohamed, El-Hariri, Mahmoud Darderi, Seida, Ahmed Adel, Moussa, Ihab Mohamed, Mubarak, Ayman Salem, Kabli, Saleh, Hemeg, Hassan, Jakee, Jakeen Kamal El, 2018. Isolation of antimicrobial roducingActinobacteria from soil samples. Saudi J. Biol. Sci. 25 (1), 44–46.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approachusing the bootstrap. Evolution 39 (4), 783–791.

Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.-B., Nováková, A., Chen, A.J., Arzanlou, M., Larsen, T.O., Sklenář, F., Mahakarnchanakul, W., Samson, R.A., Houbraken, J., 2019. Taxonomy of *Aspergillus* section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Stud. Mycol. 93 (1), 1–63.

Gomi, Shuichi, Amano, Shoichi, Sato, Eriko, Miyadoh, Shinji, Kodama, Yoshio, 1994. Novel antibiotics SF2738A, B and C, and their analogs produced by *Streptomyces* sp. J. Antibio. (Tokyo) 47 (12), 1385–1394.

Goodfellow, M., 2010. Selective Isolation of Actinobacteria. In: Baltz, R., Demain, A., Davies, J., Bull, A., Junker, B., Katz, L., Lynd, L., Masurekar, P., Reeves, C., Zhao, H. (Eds.), Manual of Industrial Microbiology and Biotechnology, third ed., pp. 13-27.

Gverzdys, T., Kramer, G., Nodwell, J.R., 2016. Tetrodecamycin: An unusual and interesting tetronate antibiotic. Bioorg. Med. Chem. 24 (24), 6269–6275.

Hacene, H., Sabaou, N., Bounaga, N., Lefevre, G., 1994. Screenig for nonpolyenic antifungal antibiotics produced by rare Actinomycetales. Microbios. 79–81.

Hilali, L., Khattabi, A., Nssarlah, N., Malki, A., Finance, C., 2002. Isolement des nouvelles souches d'actinomycétales productrices de substances antifongiques à partir du milieu naturel Marocain. Rev. Biol. Biotech., 49–53

Honma, K., Tsuda, M., Mikami, Y., Kobayashi, J., 1995. Aplysillamides A and B, new antimicrobial guanidine alkaloids from the Okinawan marine sponge Psammaplysilla purea. Tetrahedron 51 (13), 3745–3748.

Jakubieckrzesniak, K., RajniszMateusiak, A., Guspiel, A., Ziemska, J., Solecka, J., 2018. Secondary Metabolites of Actinomycetes and their Antibacterial, Antifungal and Antiviral Properties. Pol. J. Microbiol. 67 (3), 259–272.

James Jorgensen, Karen Carroll, Guido Funke, Michael Pfaller, Marie Louise Landry, Sandra Richter, David Warnock, 2015. Chapter 29. Nocardia, Rhodococcus, Gordonia, *Actinomadura*, Streptomyces, and Other Aerobic Actinomycetes. Manual of Clinical Microbiology, 11th Ed.

Jukes, T.H., Cantor, C.R., 1969. Evolution of protein molecules. In: Munro, H.N. (Ed.), Mammalian Protein Metabolism. Academic Press, New York, pp. 21–132.

Khadayat, K., Sherpa, D.D., Malla, K.P., Shrestha, S., Rana, N., Marasini, B.P., Khanal, S., Rayamajhee, B., Bhattarai, B.R., Parajuli, N., 2020. Molecular Identification and Antimicrobial Potential of Streptomyces Species from Nepalese Soil. Int. J. Microbiol. 2020, 1–8.

Kumar, R.R., Jadeja, V.J., 2016. Isolation of Actinomycetes: a complete approach. Int. J. Curr. Microbiol. Appl. Sci. 5 (5), 606–618.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software formolecular evolutionary genetic analysis and sequence alignment. Brief Bioinform. 5, 150–163.

Labeda, D.P., Kroppenstedt, R.M., 2000. Phylogenetic analysis of Saccharothrix and related taxa: proposal for Actinosynnemataceae fam. nov. Int. J. Syst. Evolut. Microbiol. 50 (1).

Lamari, Lynda, Zitouni, Abdelghani, Boudjella, Hadjira, Badji, Boubekeur, Sabaou, Nasserdine, Lebrihi, Ahmed, Lefebvre, Gérard, Seguin, Elisabeth, Tillequin, François, 2002. New Dithiolopyrrolone Antibiotics from *Saccharothrix* sp. SA 233. I. Taxonomy, Fermentation, Isolation and Biological Activities. J. Antibiot. 55 (8), 696–701.

Liu, Chongxi, Zhuang, Xiaoxin, Zhiyin, Yu, Wang, Zhiyan, Wang, Yongjiang, Guo, Xiaowei, Xiang, Wensheng, Huang, Shengxiong, 2019. Community Structures and Antifungal Activity of Root-Associated Endophytic Actinobacteria of Healthy and Diseased Soybean. Microorganisms 243.

Mahjoubi, M., Jaouani, A., Guesmi, A., Ben Amor, S., Jouini, A., Cherif, H., Najjari, A., Boudabous, A., Koubaa, N., Cherif, A., 2013. Hydrocarbonoclastic bacteria isolated from petroleum contaminated sites in Tunisia: isolation, identification and characterization of the biotechnological potential. New Biotechnol. 30 (6), 723–733.

Mezouari, A., Makhloufi, A., Bendjima, K., Benlarbi, L., Boulanouar, A., Makhloufi, K., Gonzalez, M.D.J., 2019. Antifungal activity of Acacia tortilis subsp. raddiana tar on *Fusariumoxysporum*f. sp. albedinis, the cause of Bayoud disease of the date palm in Southwest Algeria. Indian. J. Agric. Res. 53 (09), 713–717. https://doi.org/10.18805/IJARe.A-417.

Rajivgandhi, G., Ramachandran, G., Maruthupandy, M., Vaseeharan, B., Manoharan, N., 2019. Molecular identification and structural characterization of marine endophytic actinomycetes Nocardiopsis sp. GRG 2 (KT 235641) and its antibacterial efficacy against isolated ESBL producing bacteria. Microbial Pathogenesis 126, 138–148.

Saitou, N., Nei, M., 1987. The neighbour-joining method: a new methodfor reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Saket, Siddharth, Ravishankar Rai, V., 2019. Isolation and characterization of bioactive compounds with antibacterial, antioxidant and enzyme inhibitory activities from marine-derived rare actinobacteria, Nocardiopsis sp. SCA21. Microbial. Pathogenesis 137, 103775. https://doi.org/10.1016/j.micpath.2019.103775.

Sedra, M.H., Lazrek, B.H., 2011. *Fusariumoxysporum* f. sp. albedinis Toxin Characterization and Use for Selection of Resistant Date Palm to Bayoud Disease. Date Palm Biotechnol. Springer, 253–270.

Sedra, M.H., Zhar, N., 2010. Genetic variability analysis of populations of *Fusariumoxysporum* f. sp. albedinis, causal agent of bayoud disease of date palm and other *Fusariumoxysporum* using molecular techniques. Acta Hortic. (882), 491–504 https://doi.org/10.17660/ActaHortic.2010.882.56.

Takahashi, A., Hotta, K., Saito, N., Okami, Y., Umezawa, H., 1986. Production of novel antibiotic, dopsisamine by new subspecies of nocardiopsismutabilis with multiple antibiotic resistance. J. Antibiot. 39, 175–183.

Takeuchi et al., 1992. Galacardines A and B, new glycopeptide antibiotics. J. Antibiots. 45, 297–305.

Tao, F., Yao, H., Hruska, Z., Burger, L.W., Rajasekaran, K., Bhatnagar, D., 2018. Recent development of optical methods in rapid and non-destructive detection of aflatoxin and fungal contamination in agricultural products. TrAC Trends Analy. Chem. 100, 65–81.

Wang, Z., Zhiyin, Y.u., Zhao, J., Zhuang, X., Cao, P., Guo, X., Liu, C., Xiang, W., 2020. Community Composition, Antifungal Activity and Chemical Analyses of Ant-Derived Actinobacteria. Front Microbiol. 11, 201.

Zhuang, X., Peng, C., Wang, Z., Zhao, J., Shen, Y., Liu, C., Xiang, W., 2020. *Actinomadura* physcomitrii sp. nov., a novel actinomycete isolated from moss [Physcomitrium sphaericum (Ludw) Fuernr]. Antonie van Leeuwenhoek. J. Microbiol. 113 (5), 677–685.

Zitouni, A., Lamari, L., Boudjella, H., Badji, B., Sabaou, N., Gaouar, A., Mathieu, F., Lebrihi, A., Labeda, D.P., 2004. Saccharothrixalgeriensis sp. nov., isolated from Saharan soil. Int. J. Syst. Evolut. Microbiol. 54 (4).

Zitouni, A., Boudjella, H., Lamari, L., Badji, B., Mathieu, F., Lebrihi, A., Sabaou, N., 2005. Nocardiopsis and *Saccharothrix* genera in Saharan soils in Algeria: Isolation, biological activities and partial characterization of antibiotics. Res. Microbiol. 156 (10), 984–993.

Further Reading

Kim, Min Kyeong, Kang, Hye Jeong, Roh, Su Gwon, Park, Ji Sun, Kim, Seung Bum, 2019. Streptomyces fodineus sp. nov., an actinobacterium with antifungal activity isolated from mine area soil. Int. J. Syst. Evolut. Microbiol. 69 (5).

National Library of medecine. National Center for Biotechnology Information. Pubchem. Aplysillamide B.

Zhang, J., Liang, X., Zhang, S., Song, Z., Wang, C., Xu, Y., Zhang, J., Maipomycin, A., 2021. a Novel Natural Compound With Promising Anti-biofilm Activity Against Gram-Negative Pathogenic Bacteria. Front Microbiol. 12 (11), 598024. https://doi.org/10.3389/fmicb.2020.598024.