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Molecular identification, antibacterial activity, and production of hydrolytic enzymes by halotolerant bacterium *Streptomyces* sp. ESM2-25 GTF strain isolated from extreme environment in north-east of Algeria

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Abstract

The aim of this study is to establish the taxonomic position of the halotolerant bacterium ESM2-25 GTF strain isolated from the extreme environment of El-Mellah lagoon water, which is situated in the city of El-Kala in the northeast of Algeria and to study its phenotypic characteristics, antibacterial activity against several pathogenic bacteria, and hydrolytic enzymes production. The novel bacterium ESM2-25 GTF strain was isolated from water samples by the dilution agar plating method using the starch-casein medium, screened *in vitro* for its hydrolytic enzymes production and antibacterial activity. The phenotypic and molecular characteristics show that the strain ESM2-25 GTF belongs to the genus *Streptomyces*. However, the comparison of the morphological and physiological characteristics of the strain ESM2-25 GTF with those of the nearest species showed significant differences. This strain showed an antibacterial activity against *Bacillus subtilis* subsp. *spizizenii* CIP 106094, *Escherichia coli* ATCC 29522, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *S. aureus* MRSA ATCC 43300, while it is not active against *Pseudomonas aeruginosa* ATCC 27853. Furthermore, the strain ECM2-25 GTF was able to produce different hydrolytic enzymes such as lipase, protease, amylase, catalase, and gelatinase, which have applications in the field of food and industry. The interesting antibacterial activity of the strain ESM2-25 GTF against pathogenic bacteria and hydrolytic enzymes production indicate the importance of the exploitation of marine actinomycetes for biotechnological applications and the discovery of new antibacterial molecules and could encourage further research on the bioactive molecules secreted by this strain.

Introduction

Late blowing is the major cause of spoilage in hard Microbial resistance to antibiotics increased with the COVID-19 pandemic, as although physical distancing played a positive role in decreasing transmission, it was countered by the overuse of broad-spectrum antibiotics to treat patients infected with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (Rodríguez-Baño *et al.*

2020; Lai *et al*. 2021; Ukuhor 2021). There is also the problem of the toxicity of certain drugs for the environment and human health (Rowan *et al*. 2016; Hasani *et al*. 2020; Han *et al*. 2021). These factors represent a significant public health risk that requires active research into new, less toxic, naturally occurring antimicrobial molecules, especially as all efforts have currently been directed towards research into the COVID 19 pandemic (Marston *et al*. 2016; Rodríguez-Baño *et al*. 2020).

Actinomycetes are Gram positive filamentous bacteria forming a mycelium and with a high percentage of G + C (Lechevalier and Lechevalier 1967; Mazza *et al*. 2003). Based on *their ability* to produce antibiotics, the enzymes and other bioactive substances of actinomycetes are *considered* the most useful and beneficial bioactive molecules producing living organisms for humanity (Benedict 1953; Cai 2009; Mitousis *et al*. 2020). In fact, these microorganisms produce 45 % of the bioactive molecules (Berdy 2005).

Among the actinomycetes, the genus *Streptomyces* is the most important producer of bioactive molecules (Kieser *et al*. 2000; Hopwood 2007a; Kovácsová *et al*. 2017; Mahasneh *et al*. 2021) including antibacterial agents such as tetracyclines, antifungal agents such as amphotericin B, ancticancer drugs exemplified by adriamycin, the immunosuppressant tacrolimus, enzymes such as amylase, lipase, protease, cellulase, and chitinase (Miyadoh 1993; Hopwood 2007b; Suthindhiran and Kannabiran 2009; Kumar *et al*. 2020). *Streptomyces* species have been reported to produce nearly 80 % of the antibiotics known currently (Miyadoh 1993; Kieser *et al*. 2000).

As in the case of the antibiotics produced by *Streptomyces*, the extracellular enzymes produced from them could also be used instead of toxic products that are being used in various industries and affecting human health and the environment (Kumar *et al*. 2020).

Scientists are also faced with a decline in the discovery of new bioactive molecules, due to the depletion of resources caused by the overexploitation of the natural environment (Demain and Sanchez 2009).

The exploration of new and extreme environments that were previously unexplored would allow the discovery of new bioactive molecules. Among these unexplored and extreme environments, the El-Mellah lagoon located in the north-east of Algeria as a part of the El-Kala National Park, illustrates a good example of wetlands in the Mediterranean region. This brackish water lagoon is registered on the Ramsar list of wetlands of international importance to be protected (Boumezbeur and Bouteldji 2005).

The aim of the present work is to describe the phenotypic and molecular characteristics, as well as the antibacterial activity and hydrolytic enzymes production of a new marine bacterial isolate from the water of El-Mellah lagoon.

Experimental

Isolation of the actinomycete strain

Water samples were collected from El-Mellah Lagoon of El Kala (Fig. 1) (northeast of Algeria; latitude 8°20'E, longitude 36°54'N), kept at 4 °C and transferred immediately to the laboratory. Strain ECM2-25 GTF was isolated using the dilution method after 14 days of incubation at 28 °C from water samples on starch-casein agar medium (10 g starch; 0.3 g casein; 2 g K_2HPO_4; 2 g KNO_3; 0.02 g $CaCO_3$; 0.01 g $FeSO_4 \cdot 7H_2O$; 0.05 g $MgSO_4 \cdot 7\ H_2O$; 20 g agar; 1,000 mL distilled water; pH 7.2) (Williams and Cross 1971) on Petri plates without any antibacterial agents.

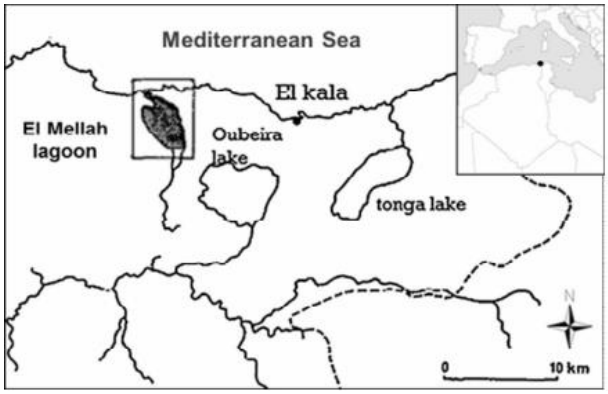

Fig. 1. Geographical localization of El-Mellah Lagoon (36°54'N, 8°20'E) (Bensaâd-Bendjedid *et al.* 2018).

Identification of the actinomycete strain

Morphological studies

The observation of the micro-morphology of the strain ESM2-25 GTF was carried out using a scanning electron microscope PHILLIPS XL30 ESEM-EFEI (Europe NanoPort, Eindhoven, Netherlands). The strain used for observation has been cultured for 14 days at 28 °C on ISP2 medium (4.0 g yeast extract; 10 g Bacto-malt extract; 4.0 g glucose; 1,000 mL distilled water; 20 g agar; pH 7.2) (Boudjella *et al*. 2006; Morakchi *et al*. 2009; Alliouch-Kerboua *et al*. 2015).

Cultural and physiological characteristics

Cultural *characteristics* of strain ESM2-25GTF were characterized following the guidelines of the International *Streptomyces* Project (ISP) (Shirling and Gottlieb 1966; Mohamed *et al*. 2013). These characteristics were observed on the medium ISP2 after 21 days of incubation at 28 °C. Colors were determined according to ISCCNBS color charts (Kenneth and Judd 1976). The growth in the presence of NaCl was evaluated according to Larpent and Larpent Gourgaud (1997). The production of melanoid pigments was observed on ISP7 agar medium (15 g glycerol; 0.5 g L-tyrosine; 1 g L-asparagine; 0.5 g K_2HPO_4; 0.5 g $MgSO_4 \cdot 7H_2O$; 0.5 g NaCl; 0.01 g $FeSO_4 \cdot 7H_2O$; 1 mL standard saline solution; 18 g agar; 1,000 mL distilled water; pH 7.2) as described by Shirling and Gottlieb (1966).

Genomic DNA extraction

DNA extraction was performed directly on actinomycete colonies. The strain was inoculated on the blood horse agar medium and incubated at 28 °C for 14 days. A bacterial suspension (200 µL) was prepared with sterile ultrapure water for the ESM2-25 GTF strain. After homogenization, the mixture was centrifuged, and the DNA was recovered (supernatant) (Bonnet *et al*. 2011; Alliouch-Kerboua *et al*. 2015).

Amplification and 16S rRNA gene analysis

For the sequencing of 16S rDNA first, the amplification of the 16S gene was conducted using the Kit Phusion Finnzymes HF Buffer (Finnzymes, Keilaranta, Finland) and two universal primers, 536F 5'-CAG-CAG-CCG-CGG-TAA-TAC-3'and rp2-5'ACG-GCT-ACC-TTG-TTA-CTT-3'. The reaction mixture contains for a final volume of 50 mL: PCR buffer 1 (10 mM TrisHCl, 20 µL KCl), 4 µL of 5×HF buffer, 0.4 µL of dNTP, 1 µL of each primer, 0.2 µL of *Taq* polymerase, 2 µL of DNA and 12.4 µL of QSP water to give a final volume of 20 µL. The amplification of the 16S RNA gene is performed in an "AB Applied Biosystem 2720 Thermal Cycler" (Life Technologies, Carlsbad, USA) with the following profile: an initial denaturation step at 98 °C for 30 s (98 °C for 30 s instead of 95 °C for 10 s, this increase corresponds to heat shock), followed by 35 cycles of amplification at 98 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s. At the end of the cycles, a final step is to hold the reaction mixture at 72 °C for 10 min, which is then cooled to 4 °C (La Scola and Raoult 1998). The resulting amplifiers are detected by agarose gel electrophoresis and visualized under ultraviolet (UV) after addition of ethidium bromide.

Phylogenetic analysis

The 16S rRNA sequence was determined by a 3130x/Genetic Analyser (Applied Biosystems, Hitachi, Japan), with the same primers used. The 16S rRNA sequences obtained were compared with sequences deposited in the GenBank genomic bank using the "BLAST NCBI" program available on the website: https://blast.ncbi.nlm.nih.gov/Blast.cgi. The phylogenetic tree was constructed via the maximum likelihood algorithm using the software MEGA version 5.2.2. (Tamura *et al*. 2011). The rRNA gene sequence of the strain ESM25-GTF was aligned using MUSCLE software with nucleotide sequences of representatives of the genus *Streptomyces* obtained from Genbank database (Edgar 2004). The evolutionary distance matrices were made as described by Kimura (1980). The tree topology of the maximum likelihood data was evaluated by bootstrap analysis

with 1000 re-samplings (Felsenstein 1981; Felsenstein 1985). Finally, the nucleotide sequence of the strain of the actinomycete was deposited in GenBank (EMBL) under the accession number OK384567.

Screening of extracellular enzymes production

For the detection of extracellular enzymes, the assays were performed on agar plates after incubation at 28 °C for 7 – 14 days. The starting culture used for enzyme screening was obtained by growing the *Streptomyces* sp. ESM2-25 GTF isolate on agar plates on ISP2 medium after incubation at 28 °C for 7 days. All experiments were conducted according to the standard protocols described below.

Protease activity

Proteolytic activity of the isolate *Streptomyces* sp. ESM2-25 GTF was determined in skim milk based medium: (10 g skim milk; 3.6 g agar; 110 mL distilled water; pH 7.5). Clear zones around the growth after 14 days indicated protease activity (Gordon *et al.* 1974).

Amylase activity

Amylase activity was tested on a starch based medium: (10 g soluble starch; 100 mL nutrient agar; pH 7.5). The starch hydrolysis was observed by flooding plates with Iodine-potassium iodide solution (Lugol's iodine solution) (1.0 g iodine; 5.0 g potassium iodide; 330 mL distilled water) and a clear zone around the colony showing hydrolysis of starch (Gordon et Smith 1953).

Gelatinase hydrolysis

Gelatin hydrolysis was screened using gelatin-based agar: (10g gelatin; 1g yeast extract; 20g agar; 1,000 mL distilled water; pH 7.2). After 7 days, plates were flooded with the following solution: 15 g $HgCl_2$; 20 mL concentrated HCl; 100 mL distilled water. Gelatinase hydrolysis was observed when clear zones appeared around colonies (Gordon and Smith 1953).

Lipolytic activity

Lipolytic activity was detected on Tween 80 based medium: (10 mL Tween 80; 1g $NaNO_3$; 5 g yeast extract; 50 mL salt solution; 0.1 g $CaCl_2 \cdot H_2O$; 18g agar; 1,000 mL distilled water; pH 7.2). After 14 days a turbid or good-visible halo around the colonies indicated lipolysis (Sierra 1957).

Hemolytic activity

Hemolytic activity was tested using Columbia agar: (17 g polypeptone; 3 g pancreatic peptone; 3 g yeast extract; 1 g cornstarch; 5 g NaCl; 13.5 g agar; 1,000 mL distilled water; 100 mL horse blood; pH 7.3). After 7 days, a clear zone around the colonies showed hemolytic activity (Larpent and Larpent Gourgaud 1997).

Antibacterial activity

The antibacterial activity of the strain ESM2-25 GTF was studied by the cross-streak agar plate method. A longitudinal streak of the actinomycete strain was streaked on ISP2 medium. After incubation at 28 °C for 7 days, the tested strains (*Escherichia coli* ATCC 29522, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* subsp. *spizizenii* CIP 106094, *Staphylococcus aureus* ATCC 29213) were inoculated in streaks crossing the actinomycete, then the plates were re-incubated at 28 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone on both sides of the streak (Boudjella *et al*. 2006).

Results

Identification of the strain ESM2-25 GTF

Cultural and morphological characteristics of the strain

The scanning electron micrograph of the strain ESM2-25GTF (Fig. 2B) showed long chains of

spores formed on aerial hyphae. These chains were straight to flexuous (*Rectiflexibles*) and spiral (*Spirales*). The spores had the smooth surface and an ovoid shape. No sclerotia, sporangia or flagellated spores were observed, and no fragmentation of substrate mycelium was formed.

The cultural characteristics (Fig. 2A) showed a whitish gray aerial mycelium with good growth on ISP2 medium. The colony was elevated and spreading.

The morphological and cultural characteristics of strain ESM2-25 GTF were compared with those of known species of actinomycetes described in the in Bergey's manual of systematic bacteriology (Whitman *et al*. 2012), and they strongly suggested that strain ESM2-25 GTF belong to genus *Streptomyces.*

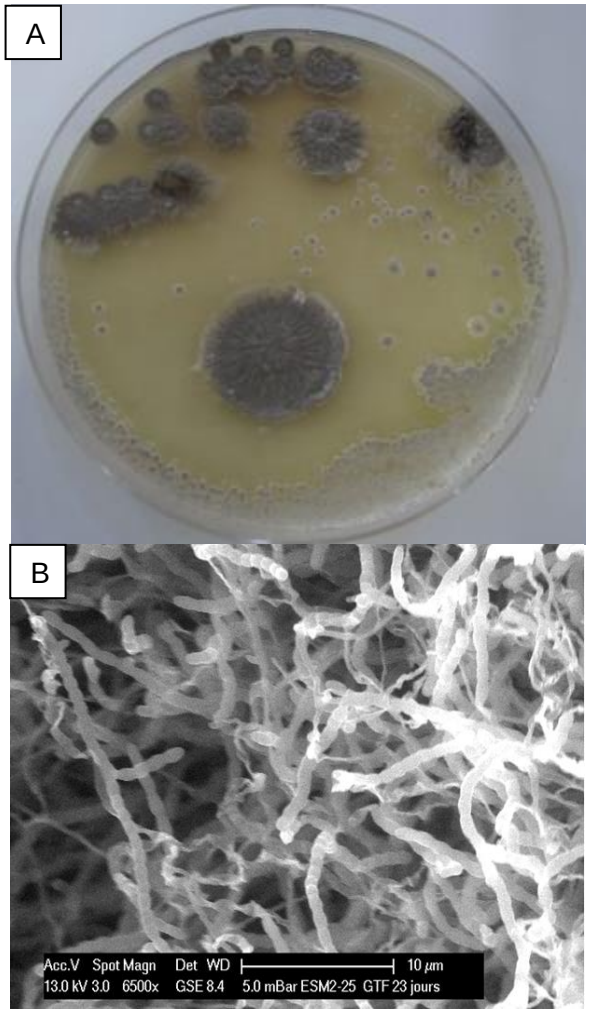

Fig. 2. Colonies of *Streptomyces* sp. ESM2-25 GTF grown on ISP2 solid medium at 28 °C after 14 days of incubation (**A**). Scanning electron micrographs of the chains of spores of *Streptomyces* sp. ESM2-25GTF in ISP2 solid medium at 28 °C after 14 days of incubation (**B**).

Physiological characteristics of the isolated strain

Physiological characteristics listed in Table 1 showed that isolate ESM2-25 GTF is not able to assimilate citrate. The isolated strain can grow with 0 %, 4 %, 7 %, 10 %, and 13 % of NaCl. The catalase and oxidase reaction were positive. In addition, this strain produces a light yellow melanoid pigment on the ISP7 medium.

Table 1. Physiological characteristics and extracellular enzymes production by the strain ESM2-25GTF (+: Growth or positive reaction of the test strain, -: no growth or negative reaction, ±: moderate growth).

Characteristics	**Strain ESM2-25GTF**
Carbon utilization	
Citrate	-
Extracellular enzymes	
Starch	+
Casein	+
Tween 80	+
Gelatin	+
Hemolysis	+s
Tolerance to NaCl:	
0 %	+
4 %	±
7 %	±
10 %	±
13 %	-
Catalase	+
Oxidase	+
Melanoid pigment production	
Tyrosine agar (ISP7)	+ Light yelow

Phylogenetic analysis

The alignment of the isolate ESM2-25 GTF 16S rRNA gene sequence (922 bp) with the 16S rRNA retrieved from the Genbank database has given 98 % similarity with *Streptomyces pluricolorescens* NRRL B-2121^T and 99 % similarity with *Streptomyces cinereorectus* NBRC 15395^T. The maximum likelihood tree shows the relationship between ESM2-25 GTF, *Streptomyces pluricolorescens* NRRL B-2121^T, *Streptomyces cinereorectus* NBRC 15395^T and other representatives *Streptomyces*. The position of ESM2-25 GTF in the maximum likelihood phylogenetic tree is shown in Fig. 3. The strain of actinomycete was deposited in GenBank under the accession number OK384567.

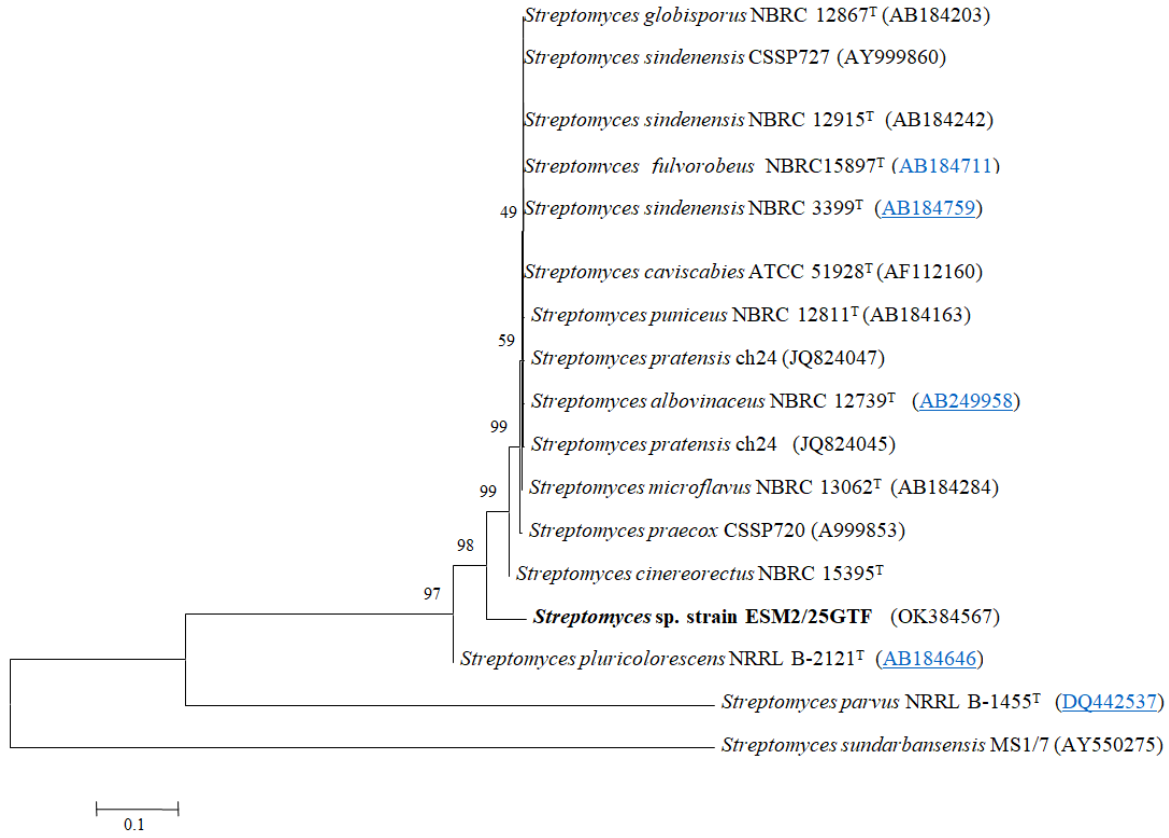

Fig. 3. Maximum likelihood tree based on 16S rRNA sequences showing relationship between *Streptomyces* sp. ESM2-25 GTF and related sequences obtained from "NCBI Blast". Bootstrap = 1,000. The scale bar indicates 0.1 substitution per site.

Extracellular enzymes production

Extracellular enzymes production listed in Table 1 showed that isolate ESM2-25 GTF degrades starch, casein, Tween 80 and Gelatin. This strain also shows a hemolytic activity.

Antibacterial activity

The activity of the strain ESM2-25GTF is shown in Table 2.

Table 2. Antibacterial activity of ESM2-25GTF strain on ISP2 solid medium. The diameters of the inhibition zones of target microorganisms are shown in millimetres (mm).

Target microorganisms	**Inhibition zone diameter [mm]**
Bacillus subtilis subsp. *spizizenii* CIP 106094	41
Escherichia coli ATCC 29522	35
Enterococcus faecalis ATCC 29212	34
Staphylococcus aureus ATCC 29213	18
Staphylococcus aureus MRSA ATCC 43300	3
Pseudomonas aeruginosa ATCC 27853	0

The strain exhibited a strong antibacterial activity against *Bacillus subtilis* subsp. *spizizenii* CIP 106094, *Escherichia coli* ATCC 29522 and *Enterococcus faecalis* ATCC 29212, moderate activity against *Staphylococcus aureus* ATCC 29213, and low activity against *S. aureus* MRSA ATCC 43300. It has no activity against *Pseudomonas aeruginosa* ATCC 27853.

Discussion

Today, microbial resistance constitutes major public health problems, expressed both at the hospital level by nosocomial infections, and at the community level (Boucher *et al*. 2009). The causes of this microbial resistance are multiple and various, such as the frequency of the mutations, the number and the type of mutations required for the expression of the resistance, the spectrum of activity, the concentration of the drug, and also the antimicrobial abuse that promotes the transfer of resistance through vertical and horizontal transfer mechanisms (Levy 2000). The COVID 19

pandemic and coronavirus infections have increased the problem of microbial resistance due to the prescription of broad-spectrum antibiotics for coinfections (Rawson *et al*. 2020). Indeed, several microbes have been found in coinfections with coronavirus such as: *Escherichia coli*, *Pseudomonas aeruginosa*, multi-resistant *Staphylococcus aureus* MRSA, *Enterococcus* spp. which correspond to the strains chosen for testing the antibacterial activity of the strain ESM2-25 GTF (Rawson *et al*. 2020).

Despite their ability to heal, some medications, including antibiotics, are still harmful to health, because of their toxicity (Demain and Sanchez 2009).

Currently, microbial enzymes are replacing chemical catalysts in manufacturing of chemicals, food, leather, and pharmaceutical industries. Moreover, due to the quick doubling time of microbes with special features compared to plants and animals, the microbial fermentation process may possibly meet the current market demand for the industrial enzyme (Kumar and Takagi 1999; Hames-Kocabas and Uzel 2007; Sathya and Ushadevi 2014).

There is also the problem of decreasing the discovery of new bioactive molecules, and the continuing and persistent demand for new biological sources of industrial enzymes that are competitive on the market compared to the non-biological products, particularly in terms of cost and convenience of use, especially antibiotics and enzymes due to resource depletion, caused by overexploitation of normal environments (Botella 2009; Demain and Sanchez 2009).

Scientists are faced with the challenges of discovering new antibiotics and new sources of enzymes from underused or untapped environments such as extreme environments (Davies-Bolorunduro *et al*. 2021).

Many new antibiotics such as dithiolopyrrolones, mzabimycins, Mutactimycin PR, and 7-deoxy-8-O-methyltetrangomycin have been reported from extremophilic actinomycetes isolated from Algeria (Lamari *et al*. 2002; Zitouni *et al*. 2004; Hadj Rabia-Boukhalfa *et al*. 2017; Corral *et al*. 2019; Tata *et al*. 2019).

The extreme environment chosen for our study is El-Mellah lagoon, which by its rich flora and fauna offers an ecosystem whose biodiversity makes it suitable for the development of new molecules of biotechnological interest. However, one of the most important characteristics of El-Mellah lagoon is its membership of the marine domain, because of the existence of a channel linking it to the Mediterranean Sea. It has been established that actinomycetes inhabit marine environments, including the species of *Streptomyces*, have the ability to produce different bioactive molecules from those produced in the terrestrial environment, including antibiotics and enzymes, which are of great interest to the scientific community (Ramesh *et al*. 2009; Manivasagan *et al*. 2013; Sathya and Ushadevi 2014).

El-Mellah lagoon is a brackish water lagoon with salinity ranging from 34.8 to 25.4 $mg.L^{-1}$ depending on the season (Chaoui *et al*. 2006). This makes it an extreme environment containing an extremophile microflora including actinomycetes offering the opportunity for the discovery of new bioactive molecules of therapeutic interest. Several recent studies have highlighted the existence of marine *Streptomyces* producing bioactive molecules (Gebreyohannes *et al*. 2013; Alliouch-Kerboua *et al*. 2015).

Many new halotolerant and halophile actinomycetes have been reported from different Algerian ecological niches. Among the halotolerant actinomycetes there is *Streptomyces* sp. SY BS5 isolated from an arid region in south of Algeria (Souagui *et al.* 2015), *Nocardiopsis* sp. HR-4 isolated from the salt-lake soil named Sebkha of Ain (Djinni *et al.* 2019) and *Melghirimyces algeriensis* sp. nov. isolated from soil of an Algerian salt lake (Addou *et al.* 2013). For halophilic actinomycetes we can mention *Actinopolyspora mzabensis* sp.nov. isolated from a Saharan soil sample collected from the Mzab region of Algerian Sahara (Meklat *et al*. 2013), *Actinoalloteichus hoggarensis* sp. nov. isolated from Tamanrasset an arid area in the south of Algeria (Hoggar) (Boudjelal *et al.* 2015) and *Actinopolyspora biskrensis* sp. nov. isolated from a Saharan soil from Biskra from northeast of Algeria (Saker *et al.* 2015).

The study of macro-morphological characteristics showed that this isolate belongs to the series of gray, this color is specific to bacteria of the genus

Streptomyces (Tresner and Backus 1963). The micro-morphological characterization showed that the isolate exhibits smooth spores, and spiral type and straight to flexuous that are typical of the genus *Streptomyces* (Dietz and Mathews 1971).

Physiologically, the isolated strain was metabolically active. This activity was manifested by various degradation activities. However, given the brackish character of El-Mellah lagoon and its impact on its microbial microflora, among the most important physiological characteristics in our study and which inevitably influences all the other physiological characteristics, it is "the tolerance to the different concentrations of NaCl". For this reason, our first interest was focused on this character. In fact, by testing different NaCl concentrations, it was found that our isolate tolerates NaCl concentrations up to 10 %, with optimum growth at 0 % NaCl concentration, making it halotolerant.

The isolate showed the ability to produce different extracellular hydrolytic enzymes such as protease, amylase, lipase, and gelatinase. Several studies have demonstrated production of these enzymes by marine halotolerant *Streptomyces* (Suthindhiran and Kannabiran 2009; Sathya and Ushadevi 2014; Tatar *et al*. 2014; Ashraf *et al*. 2021). The hemolytic activity of *Streptomyces* sp. ESM2-25GTF suggests that this isolate could be clinically more important and must be investigated further for the cytotoxic and anticancer properties. Suthindhiran and Kannabiran (2009) also demonstrated the hemolytic activity of a moderately halophilic *Streptomyces* sp.

The isolated strain is able to produce melanoid pigment like halotolerant *Streptomyces* reported in many other studies (Sonya *et al*. 2005; Mohamed *et al*. 2013).

Based on morphological and physiological characteristics, a first preliminary identification of the genus *Streptomyces* could be made (Shirling and Gottlieb 1966; Whitman *et al*. 2012). Nevertheless, it is only by realizing the phylogenetic characterization that this observation has been confirmed. The genus *Streptomyces* is the most abundant in nature, especially in soil, and its number is increasing (Lucas *et al*. 2013). They also inhabit other terrestrial and aquatic niches (Hopwood 2007a). The phylogenetic analysis was based on the 16S gene sequencing, which is characterized by its high stability (Woese 1987). Therefore, it is the most accurate and adapted molecular chronometer for this type of study (Stackebrandt *et al*. 2002). Indeed, the bacteria belonging to the genus *Streptomyces*, have the notoriety of being very unstable genetically. The characters most affected by this phenomenon are aerial mycelium formation, spore production, antibiotic production and/or resistance, and the formation of extracellular enzymes, such as tyrosinase involved in pigmentation (Leblond *et al*. 1990). These mutations are mainly due to chromosomal deletions and amplification of DNA (Leblond *et al*. 1990). For this reason, the 16S rRNA gene sequence of the isolate ESM2-25 GTF (922 bp) was amplified by PCR, sequenced, and submitted to GenBank. The maximum likelihood tree (Fig. 3) showed that *Streptomyces* sp. ESM2-25GTF (Genbank accession number OK384567) forms a separate line in the phylogenetic tree, it is closest to *Streptomyces cinereorectus* NBRC 15395^T with a bootstrap value of 99 %, and to *Streptomyces pluricolorescens* NRRL B-2121^T with a bootstrap value of 98 %. Contrary to our strain, *Streptomyces cinereorectus* species NBRC 15395^T and *Streptomyces pluricolorescens* species NRRL B-2121^T do not produce melanoid pigment. In addition, *Streptomyces pluricolorescens* species NRRL B-2121^T aerial mycelium was yellow or red color series and the substrate mycelium was grayed yellow to yellow brown. Moreover, *Streptomyces cinereorectus* species NBRC 15395^T had short spore chains with up to 10 spores per chain. By relying on these results, the strain *Streptomyces* sp. ESM2-25 GTF could be a new species however, the DNA-DNA hybridization analysis is necessary to confirm this result.

Conclusion

Based on the results, the ESM2-25 GTF strain showed an antibacterial activity against pathogenic bacteria: *Bacillus subtilis* subsp. *spizizenii* CIP 106094, *Escherichia coli* ATCC 29522 *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, but it is not active against *Pseudomonas aeruginosa* ATCC 27853. Our data

also shows that the ESM2-25 GTF strain was able to produce various hydrolytic enzymes such as protease, amylase, lipase, and gelatinase. These promising results indicate the importance of the exploitation of marine actinomycetes for the discovery of new bioactive molecules, using cultural methods such as antibacterial molecules which are a tool for controlling bacterial infections, and extracellular enzymes with potential applications in the fields of biotechnology and industry. Further investigations to extract, purify, and characterise the bioactive molecules are currently in progress.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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