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Characterization of Actinomycetes Strains Isolated from Cheliff Estuary in the North-West of Algeria

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Abstract

This study focused on the investigation of Actinomycetes strains isolated from sediment sample of Cheliff River estuary in Mostaganem (North-west of Algeria). Identification of the 5 strains of Actinomycetes isolated from sediments by morphological, biochemical methods and by16S rRNA gene sequence analysis revealed that four strains belonged to the genus *Streptomyces* and one - to the genus *Nocardia.* No strains of Actinomycetes were isolated from water samples. The optimum growth of all strains was recorded between 28°C and 30°C and at pH 7-9. All strains had halotolerant and mesophile behavior except SSG which grew very well at 45°C and could be considered as thermotolerant bacteria. Antibacterial activity was tested against five pathogenic bacteria as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Salmonella typhi* ATCC 9289, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 2785. All isolated strains exhibited an inhibitory activity against at least one human pathogenic bacterium. *Streptomyces* sp. strain SSG showed antibacterial effect against four pathogenic tested bacteria followed by *Streptomyces sp.* SSB1, *Streptomyces sp.* SSM and *Nocardia sp.* NSR. This study revealed that Cheliff River estuary contains Actinomycete species (*Streptomyces* and *Nocardia*) with proved antibacterial activity and could be a new source for isolation of antibiotic producing bacteria against human pathogenic bacteria.

Keywords: Actinomycetes, Cheliff River, Estuary, Sediments, Phylogeny, Antimicrobial activity.

1. Introduction

Marine microorganisms such as *Actinobacteria* are important sources of biomolecules (Shepherd *et al.*, 2010; Blunt *et al.*, 2016; Giraou *et al.*, 2019). This important microbial group has been explored for biotechnology and provides excellent sources of medication especially against multidrug resistant pathogens (Abdelfattah, 2016; Newman, 2016; Ramachandran *et al.*, 2019). Previous studies were focused on new bioactive natural compounds from unexploited habitats (freshwater, mangrove, invertebrate, macroalgae) to find out potential diseases therapy (Ganesan *et al.*, 2016, Rotich *et al.*, 2017; Rangseekaew and Pathom-aree, 2019). Aquatic environment provides interesting prospects to find out new bioactive components (Gomez-Escribano *et al.*, 2016, Lee *et al.*, 2018). The *Actinobacteria* are widespread in various environments, and their presence in estuarine ecosystem might be promoted probably by the high nutrient content of this setting (Rosmine and Sramma, 2016). *Actinobacteria* are Gram-positive bacteria known to produce a panel of natural compounds exhibiting a wide range of biological activities (Prasad *et al.*, 2013; Abdelfattah, 2014; Sharma, 2016; Gamaleldin *et al.*, 2020). A single actinomycete strain can produce from 10 to 20 different secondary metabolites molecules (Sosio *et al.*, 2000; Bentley *et al.*, 2002) such as antibiotic (Tetracyclines) (Hopwood, 2007); antifungal (Amphotericin), anticancer (Adriamycin) and immunosuppressant molecules (Tacrolymus).

Our work was oriented to the estuary of Cheliff River, which is one of the longest in Algeria. It takes its source from Algerian Sahara Atlas and empties into the Mediterranean Sea, across 733 km (Figure 1). We isolated five strains: for *Streptomyces* and one *Nocardia* strain, from estuarine sediment of Cheliff River, characterized using polyphasic taxonomy (using phenotypic and genotypic approaches) and investigate their potential to produce antibacterial molecules.

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2. Material and Methods

2.1. Sampling

Sediment samples from Cheliff estuary (36°02'18.8"N 0°08'04.8"E) were collected aseptically in duplicates. After *in situ* pH and temperature were measured, sediment samples were collected by sterile scraper at about 50 cm deep below the estuarine floor using sterile scrapper surface and aseptically transferred to sterile polyethylene bags. All samples were directly transferred in travel cool box for further microbiological analysis, performed immediately after arrival to the laboratory.

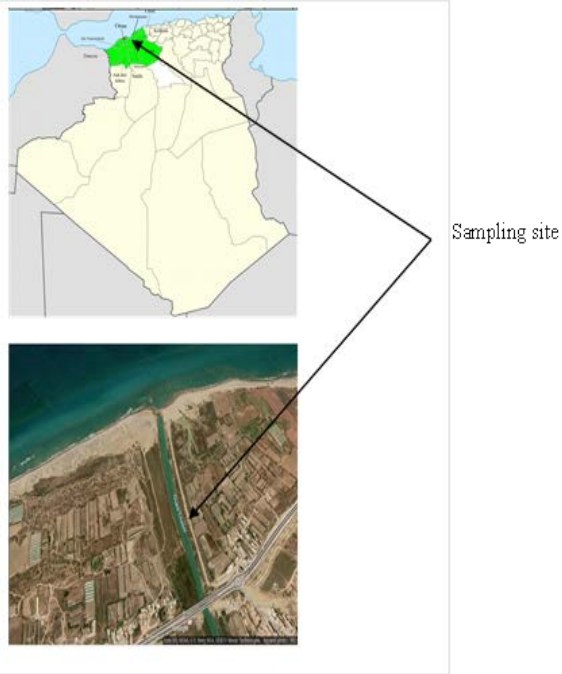

Figure 1: Cheliff River estuary geography location (Google Earth version 7.3, image © 2021 Maxar Technologies. Data SIO. NOAA. U.S.Navy. NGA. GEBCO)

2.2. Isolation and purification of actinomycetes

Prior to Actinomycetes isolation and in order to promote their growth, all the samples were oven-heated at 55°C and 1% of phenol was added for 6 min.

Actinomycetes isolation was performed using serial dilution method. One gram of sediment was suspended in 9 mL of Cheliff estuary water sterilized and diluted up to 10^{-4}. One milliliter of each dilution was spread on the surface of Petri dishes containing 20 mL of the solid culture media. Five different culture media (Starch Casein Agar, Glucose Asparagine Agar, Yeast Extract Glycerol Agar, Yeast Extract Glycerol Agar, malt extract- yeast extract agar (ISP-2: International *Streptomyces* Project-2 medium; composition: yeast extract: 4g, malt extract: 10, glucose: 4g, agar: 20g, pH 7.2) and malt extract-peptone yeast extract (GLM) were prepared using sterilized estuarian water amended with nalidix acid (10 µg/mL) and amphotericine B (25 µL/mL) sterilized on 0.22 µm filters (Ellaiah *et al.*, 1996; Cuesta *et al.*, 2010) and were tested to identify the most suitable one to study the actinomycetes. This different culture medium were amended with nalidix acid (10 µg/mL sterilized on 0.22 µm filters) and amphotericin B (25 µL/mL) to ovoid Gram-negative bacteria and fungi growth and to boost the actinomycetes development (Ellaiah *et al.*, 1996; Cuesta *et al.*, 2010). The inoculated Petri dishes have been incubated at 28°C for 2 weeks. The actinomycetes look-like colonies were then isolated and transferred on ISP-2 (Shirling and Gottlieb, 1966) agar plate medium for further characterizations. For short term preservation, monoclonal colonies on ISP-2 plates were stored at 4°C. For long term conservation, mycelia suspension was conserved with glycerol (50/50 v/v) and stored at -20°C (Sheperd *et al.*, 2010).

2.3. morphological, biochemical and physiological characterization

Actinomycetes strains were macroscopically characterized by the coloration of their areal mycelium and by the presence of diffusible pigments and morphological features. Gram staining was used both to distinguish the specific microscopic features and the Gram classification (Goodfellow *et al.*, 2004)

For biochemical characteristics, the API 20^{E} kit (bioMérieux Inc., Durham, NC) was used. Oxidase and catalase (Bactident ®Oxydase MERCK) activity were verified for *Streptomyces*-like bacteria. The effect of temperature, pH and salinity on the growth of the 5 strains was tested on Petri dishes solid ISP-2 media. Different incubation temperatures (4, 30, 37 and 45°C) and three pH values (5, 7 and 9) were tested using ISP-2 culture media. Salinity tolerance tests were carried out with ISP-2 supplemented respectively with 1, 3, 5, 7 and 10% of sodium chloride. Petri dishes were then streaked with actinomycetes monoclonal isolated strains and incubated at 30°C for 7 days. Daily visual observations were done for growth records.

2.4. Molecular characterization

Analysis of the 16S rDNA sequence and construction of phylogenetic tree using the neighbor joining method were used to confirm the actinomycetes identification. DNA extraction was done using the CTAB method associated to the boiling DNA from 3 days old strain cultures grown in 10 mL ISP-2 liquid medium (Cook and Meyers, 2003). The 16S rDNA amplification was performed using universal primers 9F (5'GAGTTTGATCMTGGCTCAG 3') and SQ6 (5'CGGTGTGTACAAGGCC3') (Weisburg *et al.*, 1991).

The final volume of the PCR reaction was 50 µL including Taq Buffer (Promega) 1/10, dNTPs (0.4 mM), $MgCl_2$ (4 mM) and 0.6 U Taq polymerase (Thermoscientific, UK), and 10 nM of forward and reverse primers. PCR was performed according to the following program: initial denaturation at 98°C for 3 min, followed by 30 cycles of reaction with denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C and final extension at 72°C for 10 min. The amplicons were sequenced on an ABI PRISM 377 genetic analyzer (Applied Biosystems, USA) with the same primers as above to generate a nearly complete gene sequence (1167 to 1250). The 16S rRNA sequences were compared to database in NCBI -GenBank using Basic alignment Search tool (BLAST) (Altschul *et al.*, 1990). Multiple sequence alignment was compared with the sequences generated in this study using CLUSTAL W program (Larkin *et al.*, 2007). Phylogenetic evolutionary tree was conducted using the neighbor-joining methods in Molecular Evolutionary

genetic Analysis (MEGA version 6.0) software based on boostrap values of 100 replications (Tamura *et al.*, 2013).

The Genbank/EMBL/DDBJ accession number of the 16S rDNA sequences of the isolate SSB1 is MH398042, for the strain SSB2: MH398493, for the strain SSG: MH398494 for the isolate SSM: MH398495) and for the strain NSR: MH398496.

2.5. Antimicrobial activity tests

The antibacterial activity of the 5 isolated actinomycete strains was tested against five human pathogen bacterial strains: *Pseudomonas aeruginosa* ATCC 27857 (*P. aeruginosa*), *Escherichia coli* ATCC 25922 (*E. coli*), *Salmonella typhi* ATCC 9289 (*S. typhi*), *Staphylococcus aureus* ATCC 6538 (*S. aureus*) and *Bacillus subtilis* ATCC 6633(*B. subtilis*) kindly provided by the Pediatric Hospital of Oran (Algeria). One milliliter of the human pathogens ATCC standardized bacterial inoculum (10^6 UFC/mL) was incorporated to melted Mueller-Hinton agar in Petri dishes. Cylinders cut from cultures of our 5 strains grown on agar medium were inverted deposited on the surface of the Petri dishes containing the human pathogens; and were then incubated at 37°C for 24 hours. Inhibition zone diameter was measured.

2.6. antimicrobial metabolites production through the cultivation time

We used wells agar diffusion assay method on the same pathogenic ATCC bacterial strains previously used for antibacterial tests to investigate the inhibitory action of the 5 isolates on the growth of pathogenic strains. Crude supernatant (100 µL) obtained from 12 days old liquid cultures of our 5 isolated strains was dispensed aseptically into 8 mm diameter agar drilled wells. In addition, Streptomycin (0.2 µg/mL) used as a positive control was placed into the agar well; and sterile distilled water was also filled into a well and served as negative control. The assays were incubated at 37°C, and the bacterial inhibition zones around the well were visually controlled every 24 hours as clear zones around the wells. Their diameters were measured daily through the 12 days of the experiment.

3. Results and discussion

3.1. isolation and characterization

The pH of sediments sample varied from 7 to 8. The temperature at the collection site was 18°C.

Based on morphological characteristics (Figure 2), five different strains of actinomycetes (named SSB1, SSB2, SSG, SSM and NSR) were isolated from sediments. The dilution factor of 10^{-4} on ISP-2 medium gave the best monoclonal well separated colonies and allowed the isolation of the *Streptomyces* and *Nocardia* strains. In addition, all the strains were isolated in ISP-2. This result may be attributed to differences between the compositions of the different media. Higher amounts of complex nitrogen and carbon sources, such as yeast and malt extract may preferably promote the growth of estuarine *Actinobacteria* strains (Ozcan *et al.*, 2013).

The isolates grown on ISP-2 medium exhibited cream to white chalky colonies, with aerial and substrate multi-colored mycelia and have slow growth rate. The colonies were elevated, convex and powdery. Under optic microscopy, hook-like structures were found only with the isolates belonging to *Streptomyces* genus. The hook-like structures are characteristic of the *Streptomyces* genus and their evidence on our colonies reinforced the morphological characterizations of the 5 isolates. *Streptomyces* strains are also known to be able to produce a large variety of pigment responsible for the coloration of aerial and substrate mycelia which can be considered as a criterion for their classification using ISCC-NBS color chart (Kelly and Judd, 1955).

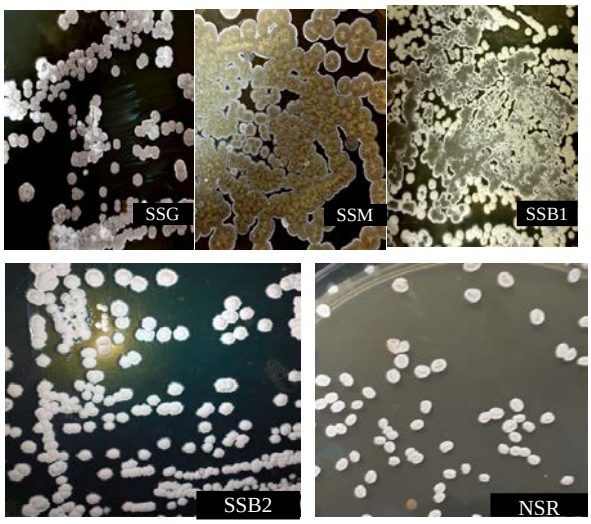

Figure2.Agar plates showing the color and morphology of the colonies of *Streptomyces* strains isolated from estuarian sediments. SSG: *Streptomyces* sp. G; SSM: *Streptomyces* sp. M; SSB1: *Streptomyces* sp. B1; SSB2: *Streptomyces* sp. B2; NSR: *Nocardia* sp. R.

3.2. biochemical and physiological characterization

API 20^E plate results (table 1) showed that all the strains were β-galactosidase producers except strain SSM. Strains SSB1, SSB2 and SSG possessed ADH and ODC enzymes and produce acetoin. SSB1, SSB2 and SSG strains were citrate positive. SSG was lysine decarboxylase positive. All strains were catalase, gelatin and indole positive but oxidase negative. Except for strain SSG, all other strains were gelatin and indole negative.

In addition, the availability of using various carbon substrates was tested, and the results varied among the isolates. All the strains used amygdalin as source of carbon. Strain SSM did not produce tryptophane desaminase and all of them were urease negative. Only strain SSG produced melanoid pigment. Comparing the five isolates, we considered that all strains were different from each other. However, the strains SSB1, SSB2 and SSG presented closely biochemical profile of carbon use (sorbitol, rhamnose, sucrose, melobiose, amygdalin and arabinose) and enzyme production (galactosidase, arginine hydrolase and ornithine decarboxylase). The use of different carbon sources by all strains indicated a wide pattern of carbon assimilation (Valan Arazu *et al.*, 2013). Most actinomycetes are known to use a wide range of organic compounds as carbon source for their growth.

Table 1. Characterization of the biochemical activity of the isolates using the API 20E test (BioMérieux Inc., Durham, NC)

Activity	Substrate	SSB1	SSB2	SSG	SSM	NSR
β-galactosidase	ONPG	+	+	+	-	+
Arginine dihydrolase	ADH	+	+	+	+	-
Ornithine decarboxylase	ODC	+	+	+	-	-
Acetoin production	VP	+	+	+	-	-
Citrate use	CIT	+	+	+	-	-
Lysine decarboxylase	LDC	-	-	+	-	-
H_2S production	H_2S	-	-	-	-	-
Urease	URE	-	-	-	-	-
Tryptophan desaminase	TDA	-	+	+	-	+
Indole	IND	-	-	+	-	-
Gelatinase	GEL	-	-	+	-	-
Glucose	GLU	-	+	-	-	-
Manitol	MAN	+	+	-	+	-
Inositol	INO	-	-	+	-	-
Sorbitol	SOR	+	+	+	-	-
Rhamnose	RHA	+	+	+	+	-
Sucrose	SAC	+	+	+	-	+
Meliobiose	MEL	+	+	+	-	-
Amygdalin	AMY	+	+	+	+	+
Arabinose	ARA	+	+	+	-	-
Melanoid pigment		-	-	+	-	-
Catalase		+	+	+	+	+
Oxydase		-	-	-	-	-

The growth temperatures tested (table 2) indicated that the strains SSB1 and NSR were able to grow from 7 to 37°C, SSB2, SSM from 28 to 37°C; while the strain SSG was able to grow from 28°C to 45°C. No growth, even moderate, was found at temperature below 28°C. All strains were mesophile except strain SSG which can be considered as thermotolerant bacteria. The thermotolerant actinomycetes play a significant role in the mineralization of nutrients and degradation of organic matter (Nayaka *et al*,. 2020).

Most of the strains were mesophiles, neutrophiles, growing in NaCl concentration ranged between 1 % and 10 %. However, some species showed a wide range of tolerance, like the eurythermal NSR strain which was psychrotolerant and mesophile. In terms of pH, the strains SSB2, SSG, SSM and NSR were acidotolerant and alkali-tolerant. Also, all the strains were halotolerant which can be explained by the fact that estuary is a transition zone between two aquatic ecosystems: fresh and marine water. Estuarine systems are affected by riverine and marine influences such waves, influx of saline water, flows of fresh water and sediment which create an unstable environment (Sobha *et al.*, 2014). Our strains developed an adaptive capacity to balance the osmotic pressure of the environment. This phenomenon was reported by many studies (Enache and Kamekura 2010; Caton *et al.*, 2009; Elshahed *et al.*, 2004). Moreover, physiological and biochemical characteristics are directly related to the enzymatic activity of the bacteria and to the regulation proteins (Li *et al.*, 2016). Morphology, metabolism and physiology of *Actinobacteria* differ from one species to another so they can be considered as strong tools for *Actinobacteria* differentiation and taxonomy (Pridham *et al.*, 1958; Wink *et al.*, 2016).

Table 2: Effects of temperature, pH and salinity on the growth of isolated strains (SSB1, SSB2, SSG, SSM and NSR)

		SSB1	SSB2	SSG	SSM	NSR
Temperature (°C)	7	++	-	-	-	++
	28	+++	+++	+++	+++	+++
	37	+++	+++	+++	+++	+++
	45	-	-	+++	+	+
pH	4.9	-	+	+	+	+
	7	+++	+++	+++	+++	+++
	9	++	+++	+++	+++	+++
NaCl (%)	1	++++	++++	++++	++++	++++
	3	+++	++++	++++	+++	+++
	5	+	++++	++++	++++	++
	7	+	+++	++++	+++	+
	10	+	+++	++++	++	+

- : No growth; +: poor growth; ++: Moderate growth; +++**:** Good growth; ++++**:** abundant growth.

3.3. molecular characterization

Following the sequencing of the entire 16S rRNA gene of the five isolated strains, the comparison with sequence available in the NCBI database the BLAST analysis showed that all the strains were related to the *Actinobacteria* phyla. The isolates SSB1, SSB2, SSG and SSM were affiliated within the *Streptomyces* genus. Indeed, the strain SSB1 (GenBank accession number MH398042) exhibited 92.50% of similarity with *Streptomyces pratensis* type strain and *Streptomyces cyaneofuscatus* type strain. The isolate SSB2 (GenBank accession number MH398493) showed 96.55% of similarity with *Streptomyces pactum* type strain, while the strain SSG presented (GenBank accession number MH398494) 96.54% of similarity with *Streptomyces olivaceus* type strain. The strain SSM (GenBank accession number MH398495) showed 95.67% of similarity with *Streptomyces intermidius* type strain. The fifth isolated strain NSR (GenBank accession number MH398496) was assigned to *Nocardia* genus, by presenting 95.29% of similarity with *Nocardia thailandica* type strain. It was placed with three strains: *Nocardia thailandica* type strain, *Nocardia neocaledonensis* type strain and *Nocardia asteroides* type strain. Comparing the data, the strain NSR was biochemically, physiologically and phylogenetically distinct from *Nocardia thailandica* type strain. Strains SSB2 and SSG were close to each other and share 97, 09% of similarity. The two other sequences related to the *Streptomyces* genus were widespread in the phylogenetic

tree and were grouped in 2 different clusters. The first group encompassed the SSB1 sequence which was closely related to *Streptomyces pratensis* type strain, *Streptomyces cyanofuscatus* type strain and *Streptomyces lavendulae* type strain, while the second group with the SSM sequence was located between *Streptomyces distaticus* (Krainsky, 1914) and *Streptomyces intermidus* (Waksman et Lechevalier, 1953) and *Streptomyces rutgersensis*16s rRNA gene sequences. The phylogenetic tree showed that the *Streptomycete* isolates (SSB1, SSB2, SSG and SSM) were distributed in different clusters. Moreover, they exhibited a significant difference with phenotypical, biochemical and physiological characteristics.

To distinguish between the closely related species of the genus *Streptomyces* and *Nocardia* cited above in tree, the polyphasic approach (Vandamme *et al.*, 1996; Goodfellow *et al.*, 2004) such as morphological, physiological, biochemical including genetic characterization were used to identify and classify the isolated species.

Then, comparing the physiology, metabolic activities and 16S rRNA gene sequences of the isolates SSB1, SSB2, SSG, SSM, with the nearest *Streptomyces* strains and NSR with the closest *Nocardia* strains. The results show that each of our isolated strains have less than 97% of 16S rRNA gene similarity from their closest relatives in addition to several differentiating features (profile of carbon use, production of ADH, ODC, LDC and tyrosinase enzymes and culture characteristics). We deduct that the isolated strains SSB1, SSB2, SSG, SSM and NSR might be new species (Janda and Abbott, 2007).

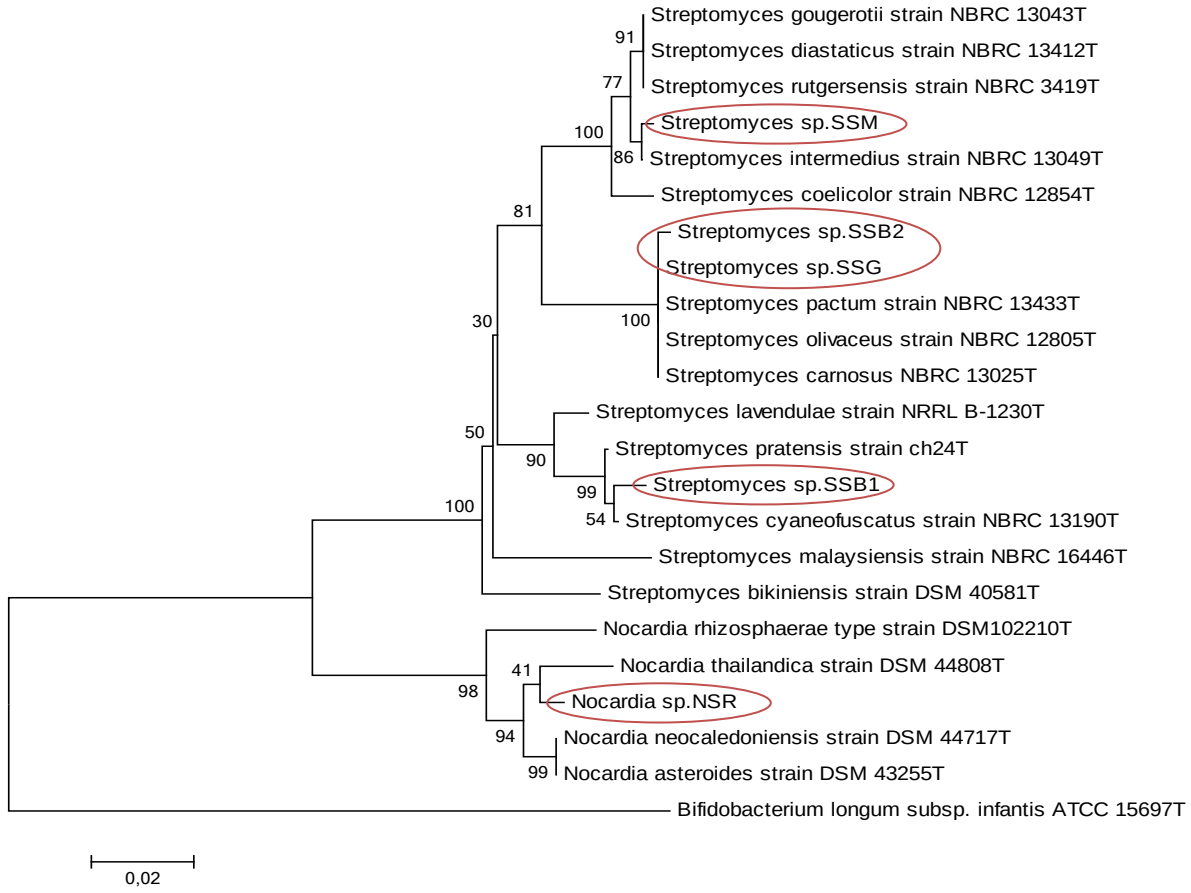

Figure 3. Phylogenetic tree based on 16S rRNA sequences of actinomycetes isolates (*Streptomyces* sp. **SSB1**, *Streptomyces* sp. **SSB2,** *Streptomyces* sp **SSG,** *Streptomyces* sp **SSM** and *Nocardia* sp. **NSR**) using the Neighbor-Joining method (Saitou and Nei, 1987). The number on branch nodes were boostrap values (from 100 replicates) (Felsenstein, 1985). Evolutionary analyses were performed in **MEGA 6.1.** (Tamura *et al., 2013*). The genus *Bifidobacterium* type strain was used as an out-group.

3.4. antimicrobial Activity

The results of the screening for the antibacterial activity were represented in table 3. Strains SSB1, SSG, SSM and NSR exhibited different spectrum of antibacterial activity (table 3). *Escherichia coli* ATCC 25922 was inhibited by four isolates (SSB1, SSG, SSM and NSR), while all strains did not show any antibiosis activity against *P. Aeruginosa* ATCC 27857. Among the 5 isolates, strain SSG exhibited the largest antibacterial activity as it inhibited the growth of all tested pathogenic strain at the exception of *Staphylococcus aureus* ATCC 6538. The antibiotic resistance shown by *S. aureus* ATCC 6538, for which the growth was inhibited by none of the tested strains, was probably due to mutations by a modification in ribosomal RNA methylase (Heelan *et al.*, 2004; Martinez *et al.*, 2018), by acquisition of resistance genes (plasmid-

mediated resistance) (Foster, 2017), by the presence of Staphylococcus biofilm's (Craft *et al.*, 2019; Saxena *et al*., 2019).

Table 3. Maximal diameter values in millimeters of the inhibition zones displayed by the 5 isolated Actinomycetes against pathogenic ATCC bacterial strains

	ISOLATED BACTERIAL STRAINS					CONTROLS	
	SSB1	SSB2	SSG	SSM	NSR	Positive	Negative
TESTED BACTERIA							
Escherichia coli ATCC 25922	8	-	8	7	10	+	-
Staphylococcus aureus ATCC 6538	-	-	-	-	-	+	-
Salmonella typhimurium ATCC 9289	4	-	11	-	-	+	-
Bacillus subtilis ATCC 6633	3	-	12	-	-	+	-
Pseudomonas aeruginosa ATCC 14028	-	-	8	-	-	+	-

The largest inhibition zones were obtained with the strain SSG against 4 bacterial pathogenic species; *B. subtilis* ATCC 6633, *S. typhi* ATCC 9289, *E. coli* ATCC 25922, *P. aerugenosa* ATCC 14028 (8-12mm, figure 4). Antibacterial activity was early observed with *E. coli* ATCC25922. In fact, after 24 hours of incubation, the maximum of bacterial growth inhibition reached and then decreased gradually, to finally disappear at day 9. *Streptomyces* strains are known to produce bacteriocin as a protein produced by ribosomes with large bactericidal spectrum activity against both Gram negative and positive bacteria (Jung *et al.*, 1992; Lee *et al.*, 2014; Hernandez-Saldana *et al*., 2020). Antibacterial activity of the strain SSG against *B. subtilis* ATCC 6633 occurs after 2 incubation days and after 4 days against *S. typhi* ATCC 9289. This bioactivity was optimal at day 7 and decreases gradually after. Likewise *P. aerugenosa* ATCC 14028 was susceptible to strain SSG bioactive compounds after 6 incubation days with maximum inhibition effect at day 10 and then decreases. Owing that the antibacterial activity of the strain SSG beginning at day 1, against only one of the bacterial strains tested (*Escherichia coli* ATCC 25922) and then through the incubation time, affecting the other bacterial strains, we suspected that a mix of bioactive metabolites was produced by the isolate SSG at different bacterial growth steps. *Streptomyces* are known to produce antimicrobial secondary metabolites, as single or mix (Macagnan *et al.*, 2006; Procopio *et al*., 2012; Song *et al.*, 2013; Song *et al.*, 2015). Previous studies showed that different strains of *Streptomyces albus* possess different gene cluster encoding the production of specific secondary metabolites (Seipke, 2015).

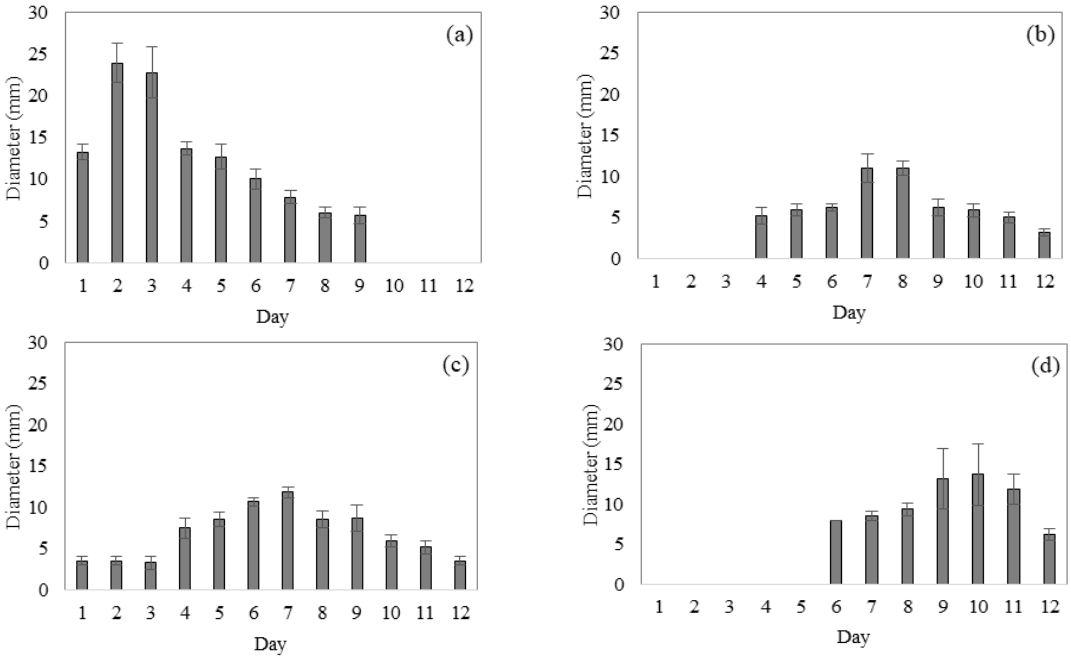

Figure 4: Antibacterial production of strain SSG through the cultivation time: **(a) against** *E. coli*.**(b) against** *S. typhi*. **(c) against** *B. subtilis*. **(d) against** *P. Aeruginosa*

4. Conclusion

This work showed that the studied estuarian actinomycete strains could be new species of the genus *Streptomyces* and *Nocardia*. On the basis of phenotypic characteristics, growth parameters (temperature, pH, salinity), metabolic and antimicrobial activity in addition to the 16s rRNA gene sequences analysis of the 5 isolated strains (SSB1, SSB2, SSG, SSM and NSR) we conclude

that these ones can be new strains belonging to *Actinobacteria* phyla. The strains SSB1, SSB2, SSG and SSM were affiliated within the *Streptomyces* genus and strain NSR within *Nocardia* genus. Owing the 16S rRNA gene sequence similarity with their closest neighbor sequence, the strains SSB1, SSB2, SSG, SSM and NSR could be considered as 5 novel species. However, more analysis, like DNA-DNA hybridization is needed to prove that. In addition, our investigation showed that the estuarine sediment harbored actinomycetes that could produce secondary metabolites with antibacterial activity.

The optimization of the culture conditions (temperature, shaking, pH and medium composition) was conducted (data not shown), and the characterization of the antimicrobial molecules is in progress. Further purification, characterization and structural elucidation of biomolecules are needed to explore the antimicrobial activity of these molecules.

5. Compliance with ethical standards

6. Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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