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Isolation, characterization and antimicrobial screening of bioactive substance-producing actinobacteria isolated from Algerian soil samples

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Abstract

The emergence of multidrug-resistant bacteria has necessitated the search for novel bioactive compounds in unexplored and natural environments. Actinobacteria contain important bioactive substances. Actinobacteria are Gram-positive, facultatively anaerobic, filamentous, fungus-like bacteria that remain among the leading producers of natural antibiotics. Consequently, in this work, 50 actinobacteria were isolated from 5 soil samples collected from the Algerian provinces of Medea and Blida in searching for untapped producers of antimicrobial compounds. All the isolates were further subjected to antimicrobial screening against pathogenic bacteria.The obtained results indicated that sixteen of the isolates showed antimicrobial activities against most of the tested pathogenic microorganisms. Therefore, these promising isolates, previously identified as *Streptomyces* by morphological, biochemical and physiological methods, were selected for antibiotics susceptibility, kinetic growth production of antibiotics, evaluation of pH and metabolite production such as biomolecules.These results pointed out that actinobacteria from Algerian provinces of Medea and Blida soils suggest that these selected isolates could be excellent candidates,especially BN01, BN20, BN17, CH2,OUZ 5 and TAM 47,for the discovery of antibiotics and could be potential sources of antimicrobial bioactive compounds. For antibiotics susceptibility,all the isolates showed a sensitivity of 100% to Amoxicillin, Neomycin and Chlortetracycline. After fermentation in ISP2 medium an extraction was done, the results revealed that the maximum zone of inhibition was recorded against all the tested pathogens.This finding may be significant for future research aimed at developing broad-spectrum antibiotics for therapeutic purposes.

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Introduction

Since the widespread use of antimicrobial chemotherapy, resistance monitoring has revealed that nearly all pathogenic microorganisms have developed resistance to chemotherapeutic agents, largely as a result of their misuse. Many pathogenic microorganisms have developed resistance to the chemotherapeutic agents' penicillin and sulfonamide, which were the first widely used (Madigan et Martinko, 2007).Faced with the emergence of antibiotic resistance, the discovery of new molecules is a necessity that can only be met by the extraction of new derivatives or analysis of the fermentation products of new bacterial or fungal species isolated from ecosystems that have been little or no explored, such as soil (Kitouni, 2007).

Soil hosts different types of microorganisms, among which actinobacteria is defined as one of the major groups of the soil population. They constitute between 10 and 50 percent of the total microbial community, making them less dominant than bacteria but more dominant than fungi (Silini, 2012). Actinobacteria are the most promising candidates for the production of antibiotics based on previous research methods.

The main source of antibiotics is microorganisms, which have been the subject of extensive research for decades and have enabled and continue to enable the discovery of interesting secondary metabolites that can be utilized by humans (Breton *et al.*, 1989).

Actinobacteria are the primary source of anticellular metabolites with a 43 percent activity rate. Due to the development of multi-drug resistance in the majority of pathogenic microorganisms, the search for bioactive metabolites, such as novel antibiotic compounds, from microbial sources for potential use in pharmaceutical and industrial applications has become more important. Researchers scour the globe for novel, potent, long-lasting, and broad-spectrum antimicrobial compounds derived from various sources, including microbes (Berdy, 2005; Praveen *et al.*, 2008; Singh and Tripathi, 2011).

In this regard, the natural soils of Algeria, which are exposed to a range of climatic conditions, represent specific ecosystems that require further investigation. Therefore, the isolation of various actinobacteria strains from natural soils in Algeria (Medea and Blida provinces), their antimicrobial activity against pathogenic microorganisms, their characterization using conventional methods, and the extraction of metabolites from potential isolates were studied.

Material and methods

Sample collection

During the research, five soil samples were collected from various regions of the Algerian provinces of Medea and Blida (organically cultivated fields and riverbanks). In order of their prelevement, samples were labeled with the alphabet A, B, etc. Similarly, the isolates from each sample were additionally labeled with the numbers A1, A2, etc... 5–15 cm of soil samples were collected, then approximately 50 grams were placed in sterile bags and refrigerated (4°C) for transport to the laboratory.

Isolation of Actinobacteria

Soil samples were placed in clean, empty Petri dishes for a week and then dried at 55°C for 15 minutes to reduce the number of gram-negative bacteria, as recorded by Baskaran *et al.* 2011. One gram of soil was then treated for one week with 0.1g of $CaCO_3$ at 28°C.

Using distilled water as a diluent, 1g was taken and serially diluted up to 10-5. The mixture was vigorously shaken using a vortex; 0.1ml of each dilution was placed on different culture media ISP2.

The inoculum was distributed using a sterile glass spreader on SCA medium. Seven days of plate incubation were conducted at 28°C. Colonies of the resulting actinobacteria were collected, streaked, and recultivated on two medium culture plates before being re-incubated at 28°C for seven days to verify their purity. For subsequent studies, the pure actinobacteria isolates were kept on ISP2 and SCA agar plates at 4°C.

All chemicals, media, media components and other reagents were purchased from Sigma-Aldrich (USA).

Screening of the actinobacteria isolates for antimicrobial activity

The screening of actinobacteria isolates was conducted on a solid medium. The antimicrobial activity was evaluated using the agar cylinder method (Audrey, 2007) against selected pathogens (Taechowisan *et al.*, 2005): the pathogens were obtained from the laboratory of Microbiology, Saidal antibiotical group in Medea (Algeria): *Staphylococcus aureus* (ATCC 6538), *E. coli* (ATCC 8739), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 9027), *Candida alibicans* (ATCC 10231) and *Salmonella typhimurium* (ATCC 14028).0.1 ml of test bacteria were streaked on the sterile agar medium. On the surface of the inoculated medium in each plate, six identical stainless steel cylinders were positioned. Antimicrobial activity was determined by measuring the zone of inhibition of the test organism after 24 hours of incubation at 28°C (Rai *et al.*, 2016). The average diameter of the inhibition zone is used to categorize the antimicrobial activity of actinobacteria isolates. In this instance, the diameter of the inhibition zone was classified as follows: excellent activity (≥18 mm), good activity (12-15 mm), moderate activity (10-12 mm), and weak activity (≤9 mm). The isolates with the highest antimicrobial activity were chosen for additional research.

Cultural characteristics from the selected cultures

In accordance with Bergey's Manual of Systematic Bacteriology, 2nd Edition, Vol. 5, The Actinobacteria, Part A, morphological, biochemical, and physiological tests were used to identify actinobacteria isolates.

Macroscopic and Microscopic observation: The actinobacteria isolates were observed for aerial or submerged mycelium, size, shape, color, and diffusible pigments. Gram staining was utilized to conduct the microscopic examination. Biochemical and physiological tests:For all of the isolates, a loopful of pure culture colony was placed in both ISP2 and SCA broth and incubated for 3 days at 28°C. The culture suspension was utilized for testing catalase and sugar utilization. Additionally, numerous hydrolysis tests, including starchhydrolysis, caseinhydrolysis, lipidhydrolysis, and lecithin hydrolysis tests, were conducted.

Antibiotics susceptibility

To determine the sensitivity of the actinobacterial isolates to various standard antibiotics, Muller Hinton agar (MHA) medium was utilized. Actinobacterial isolates were initially grown in ISP2 broth at 28°C for 2 days. On MHA plates, the adjusted actinobacterial culture was seeded, and various standard antibiotic discs were placed. The plates were then incubated at 37C for 24 hours. Using zone of inhibition measurements, the sensitivity of antibiotics against different actinobacterial isolates was determined (Williams *et al.*, 1989). Actinobacterial strains were classified as antibiotic-sensitive (S), intermediate (I), or resistant (R) (Zothanpuia *et al.*, 2016).

Kinetic of growth, production of antimicrobial substances and evolution of pH

In liquid SCA medium, the kinetics of growth, production of antibiotics, and monitoring of pH evolution of selected actinobacteria isolates were examined. Precultures were prepared on tubes containing 3 ml of SCA broth for each isolate. After 48 hours of incubation at 28°C with constant shaking at 250 rpm, the preculture is inoculated into 500 ml flasks containing 100 ml of the same liquid medium (Reghioua *et al.*, 2008).

Growth kinetics

On the second day of culture incubation, two milliliters of each selected isolate's broth was withdrawn daily in Eppendorf tubes. According to the method, the dry weight of the mycelium (biomass) was determined (Pfefferle *et al.,* 2000).

After centrifuging the samples at 7000rpm for 20 minutes, the resulting mycelial pellet is washed three times with distilled water and dried at 105°C. The dry

weight of the mycelium is then calculated by subtracting the tare weight of the eppendorffs (Reghioua *et al.*, 2008).

Kinetics of antibiotic production

Daily monitoring of antibiotic production was conducted by removing 2 ml of each culture from SCA broth. The evolution of the antimicrobial activity is detected by the method of diffusion in wells (200 µl of culture filtrate per well of 6 mm in diameter) against the employed target microorganisms (Jihani *et al.*, 2012).

Evolution of the pH

The evolution of the pH was also monitored daily, and residues of culture supernatants used to determine antimicrobial activity were utilized for pH measurement (Reghioua *et al.*, 2008).

Extraction of bioactive substances

Production of crude extract:The primary screening isolates with the highest antimicrobial activity were subjected to 7 days of submerged fermentation in ISP2 medium at 250 rpm on 28 °C in a shaker incubator. 10 minutes were spent centrifuging the extract at 13000rpm to separate it. The supernatant was mixed with butanol in equal volume and left overnight. The uppermost layer of butanol was subsequently separated and evaporated at a temperature of 40°C. The concentrated extract was utilized for further assay.

Disc diffusion assay

So as to evaluate the potential of crude extracts, the disc diffusion method was used. It means the use of a sterile disc made from filter paper; it depends on the adsorption of a known quantity of antimicrobial agents. The discs containing crude extracts were placed on an agar plate seeded with adjusted bacteria inoculum. After incubation at 37°C for 24h, the zone of inhibition found is estimated and interpreted according to regulated standards (Balouiri *et al.*, 2016).

Results

In the current research, screening antimicrobial activity and extraction of bioactive substances of actinobacteria isolated and identified from the soil of Algerian provinces of Medea and Blida against various pathogenic bacteria were investigated.

Table 1. Repartition of isolates according to the region.

Soil sample	Isolat	Total of isolates
Tamesguida (Medea province)	TAM36,TAM37,TAM38,TAM39,TAM40 ;TAM41,TAM42, TAM43,TAM44,TAM45,TAM46,TAM47 ,TAM48,TAM49, TAM50	15
Chiffa 01 (Blida province)	CH2,CH3,CH9,CH12,CH13,CH14,CH21,	7
Chiffa 02 (Blida province)	CH23,CH24,CH25,CH26,CH27,CH28,CH29,CH30	8
Ben chicao (Medea province)	BN1,BN4,BN10,BN11,BN15,BN16,BN17,BN18,BN19,BN20,BN22,BN31,BN33,BN34,	14
Ouzera (Medea province)	TAM5,TAM6,TAM7,TAM8,TAM32,TAM35	6

Sampling and isolation of actinobacteria

Five samples were collected from two distinct locations in the provinces of Medea and Blida (Algeria). The selection of sampling locations is determined by the fact that actinobacteria are chemoorganotrophs. They utilize organic matter and can degrade lignin, the primary component of wood. The distribution by region revealed that both the Medea and Blida provinces (Algeria) had approximately the same number of isolates (among 15

isolates), with the exception of the Ouzra region in the Medea province, which may be attributable to a less organic soil sample component (Table 1). From a total of 5 soil samples, 50 distinct actinobacteria isolates were obtained in various culture media; both ISP2 and SCA showed significant actinobacteria isolate growth.

Screening of the actinobacteria isolates for antimicrobial activity

Using the Agar cylinder technique, antibacterial activity was demonstrated. During the primary screening, 46 of the 50 actinobacteria isolates (92%) obtained from the Algerian provinces of Medea and Blida exhibited antimicrobial activity against the test organisms (gram-positive and gram-negative bacteria). While the four strains (8 percent) possessed no antimicrobial properties against the target pathogens (Fig. 1). Only 16 actinobacterial isolates with antibacterial activity have active potential; these isolates are designated as BN1, BN17, BN20, BN33, CH2, CH13, CH24, CH28, OUZ5, OUZ8, OUZ35, TAM 37,TAM42,TAM43,TAM47, and TAM50.

92% of total isolates were found to be active against both Gram-positive and Gram-negative test organisms. Among 46 active isolates, only 16 isolates were more active and showed a zone of inhibition against the test organisms. Allofthe 16 isolatesshowed activity against *Staphylococcus aureus* (ATCC 6538), *E. coli* (ATCC 8739), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 9027), *Candida alibicans* (ATCC 10231) and *Salmonella typhimurium* (ATCC 14028) (Fig. 2).

Table 2. Physiological and biochemical properties of isolates.

	BN01	BN17	BN20	BN33	CH2	CH13	CH24	CH2	OUZ5	OUZ8	OUZ35	TAM43	TAM37	TAM42	TAM47	TAM50
catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glu	+	+	+	-	+	-	-	+	+	-	-	+	+	+	+	+
TSI H2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lac/Sac	+	-	+	-	-	+	-	-	+	+	-	-	-	+	-	-
Caseinhydrolysis	+	-	+	-	-	+	-	+	+	+	+	+	-	+	+	-
Lipidhydrolysis	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Strachhydrolysis	+	-	+	-	-	-	-	+	+	+	-	-	-	+	-	-
Lecithinhydrolysis	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+

+ Positive growth - negative growth.

63% are antibacterial against Gram-negative pathogens, including *E. coli* (ATCC 8739), *Pseudomonas aeruginos* (ATCC 9027), and *Salmonella typhimurium* (ATCC 14028), whose maximum diameter is given by the CH2 isolate (30mm).In contrast, only 37 percent demonstrate antibacterial efficacy against Gram-positive germs, such as *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228) and *Candida alibicans* (ATCC 10231), whose greatest diameter is represented by the isolate BN 20 (20 mm).

Morphological, biochemical and physiological characterization of the isolates

The colonies that, upon observation with the naked eye and under an optical microscope, displayed the characteristic morphology of actinobacteria, specifically the formation of the aerial mycelium and the mycelium of the substrate with extremely fine filaments, are considered to be actinobacteria. In fact, the majority of isolates that grew for more than fourteen days on various culture media exhibit powdery colonies of whitish or mixed colors, small sizes, regular or irregular, flattened or rounded, and an earthy odor. This characteristic is unique to isolates producing an aerial mycelium; others are pigmented and sporulated(Fig. 3).

The results of our staining confirm that these isolates have branched, Gram-positive filaments. Each actinobacterium isolate was Gram-positive and catalase-positive. On the majority of organic media used, actinobacteria colonies grew well and were

convex and smooth. Except for BN01, BN17, BN20, and CH13, which had white colonies, the major strains' aerial mycelium was yellowish-gray,Which indicates that *streptomyces sp* is the predominant presence among isolates. Numerous compounds, such as casein and sugar, were hydrolyzed by the isolates, as demonstrated by casein and sugar utilization tests. Also different hydrolysis tests, namely, starchhydrolysis, caseinhydrolysis, lipidhydrolysis and lecithinhydrolysis (Table 2).

Table 3. Sensitivity of actinobacteria isolates to different antibiotics.

isolates	Inhibition zone diameter (mm)									
	Amoxicillin		Penicillin		Neomycin		Tetracyclin		Nystatin	
	S/R	DIM	S/R	DIM	S/R	DIM	S/R	DIM	S/R	DIM
BN 01	S	29.6	R	00	S	12	S	18.8	R	00
BN17	S	28.4	R	00	S	13	S	21.2	R	00
BN20	S	31.8	RR	00	S	12	S	18	R	00
BN33	S	35	R	00	S	13	S	22.6	R	00
CH02	S	21.8	R	00	S	12	S	28	S	22
CH13	S	26.2	S	16.2	S	12.4	S	27	R	00
CH24	S	35	S	16	S	11.4	S	21.4	R	00
CH28	S	31.6	R	00	S	12.4	S	21.6	R	00
OUZ5	S	35	S	29.7	S	14.6	S	21.6	R	00
OUZ8	S	35	S	35	S	35	S	35	R	00
OUZ35	S	35	S	35	S	35	S	35	R	00
TAM37	S	35	S	20.8	S	11.8	S	21.8	R	00
TAM42	S	35	S	12.6	S	19.4	S	24.4	R	00
TAM43	S	34	S	12.3	S	17	S	22	R	00
TAM47	S	32.4	S	10.2	S	13.2	S	18.4	R	00
TAM50	S	35	S	30	S	12.8	S	31.8	R	00

S: Sensitive, R: Resistant, DIM: Diameter.

Antibiotics susceptibility

The susceptibility and resistance of the isolates were evaluated according to the following criteria: Presence or absence of the zone of inhibition, Diameter ≥ 10 mm = susceptible isolate; ≤ to 10 mm = resistant isolate. The results of the antibiotic susceptibility tests carried out on the 16 isolates are shown in Table 3.

The 16 Isolates which showed antimicrobial activity against almost all the tested microorganisms were selected and screenedfor their antibiotic sensitivity pattern against 5 standard antibiotics Amoxicillin (Amp^{10}), Neomycin (N^{10}), tetracycline (TE^{30}), Penicillin (P^{2}) and Nystatin (NS50). According to the results, all the isolates were sensitive to Amoxicillin, Neomycin and tetracyclin, whose zone of inhibition varies between 11.4 up to 35 mm in diameter.

Only the isolates CH13, CH24, OUZ5, OUZ8, OUZ35, TAM37, TAM42, TAM43, TAM47, TAM50 were sensitive to Penicillin, the zone of inhibition varies from 10.2 up to 35 mm. concerning Nystatin all the isolates showed resistance excepting CH02 strain that had an inhibition zone of 22 mm.

Kinetic growth, production of antibiotics and evolution of pH

The isolates that showed the highest antimicrobial activity were selected for studying the growth kinetics to determine the optical density and the measurement of pH and dry weight.

The results obtained are presented in Fig. 4. The latency period is almost nonexistent. During the growth phase, the dry weight increases rapidly during the first 24 hours of fermentation and then at a slower rate between the second and third day (Fig.4). This phase is characterized by acidification of the culture medium, which can be attributed to the inoculum's adaptation to the culture medium (preculture).

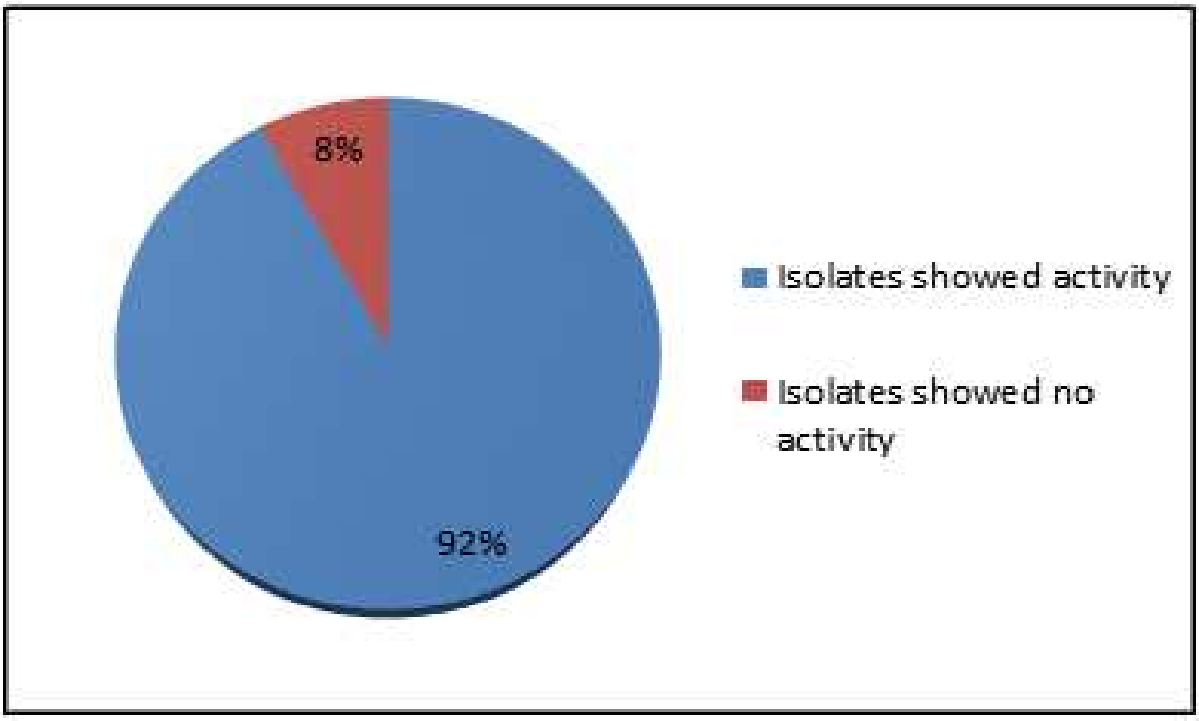

Fig. 1. Graphic representation of actinobacteria isolates with antibacterial activity.

Extraction of bioactive substances Metabolite production

The crude extract of isolates BN20, CH2, OUZ 5 and TAM 47 after fermentation in ISP2 medium was tested by Disc diffusion assay on MHA.

The results revealed that the maximum zone of inhibition was recorded against *C. albicans* and *Pseudomonas aeruginosa*(19 mm), followed by *E. coli*(15 mm) and *Staphylococcus aureus*(15 mm). CH2 showed the most potent activity, especially against *Pseudomonas aeruginosa* (36 mm), *S. aureus* (25 mm) and *S. epidermidis* (25 mm) (Fig. 5).

Discussion

Actinobacteria continue to be one of the most promising and potential sources of useful bioactive compounds, such as secondary metabolites with an unfathomable range of important biological activities, antimicrobials, and valuable enzymes from distinct classes.

In the present study, 50 isolates of actinobacteria were obtained from the soil of the provinces of Medea and Blida (Algeria). $CaCO_3$ treatment is, therefore, more effective for the isolation of actinobacteria since it not only allows the greatest number of actinobacteria to be obtained but also a low number of bacteria and fungi compared to their contribution. Respective numbers were obtained without preprocessing; this result is in perfect agreement with that obtained by El-Nakeeb and Lechevalier, 1963.

The results of antimicrobial activity are intriguing due to the presence of Gram-positive and Gram-negative bacteria-causing broad-spectrum antibiotics. On the other hand, several strains generate intentions with a narrow spectrum that act against a single target microorganism. The spectrum of action of three strains against *Staphylococcus epidermis* and *Candida albicans* was restricted. Four isolates possess a limited spectrum of action against *E. coli* and *Candida albicans*. Two isolates's spectrums of

activity were limited to *Salmonella typhimurium*. Three isolates show limited activity against *Pseudomonas aerogenosa* and *E. coli*. Three strains have limited activity against *Candida albicans* and *Pseudomonas aerogenosa*. Only one isolate showed limited activity against *Staphylococcus aureus* and*E .coli*.These findings are consistent with those of Sateech *et al*., 2011, who isolated actinobac"teria from Karwar on the west coast of India, where 54 strains of actinobacteria and 28 isolates of active strains against the target microorganisms were found. Jihani *et al*.,2012 isolated six strains from an old Moroccan house that are active against Gram-positive and Gram-negative bacteria and are related to *Streptomyces sp*. Additionally, Ali Alharbi *et al*., 2012 isolated strains from Saudi Arabian soil that are active against Gram-positive and Gram-negative bacteria.

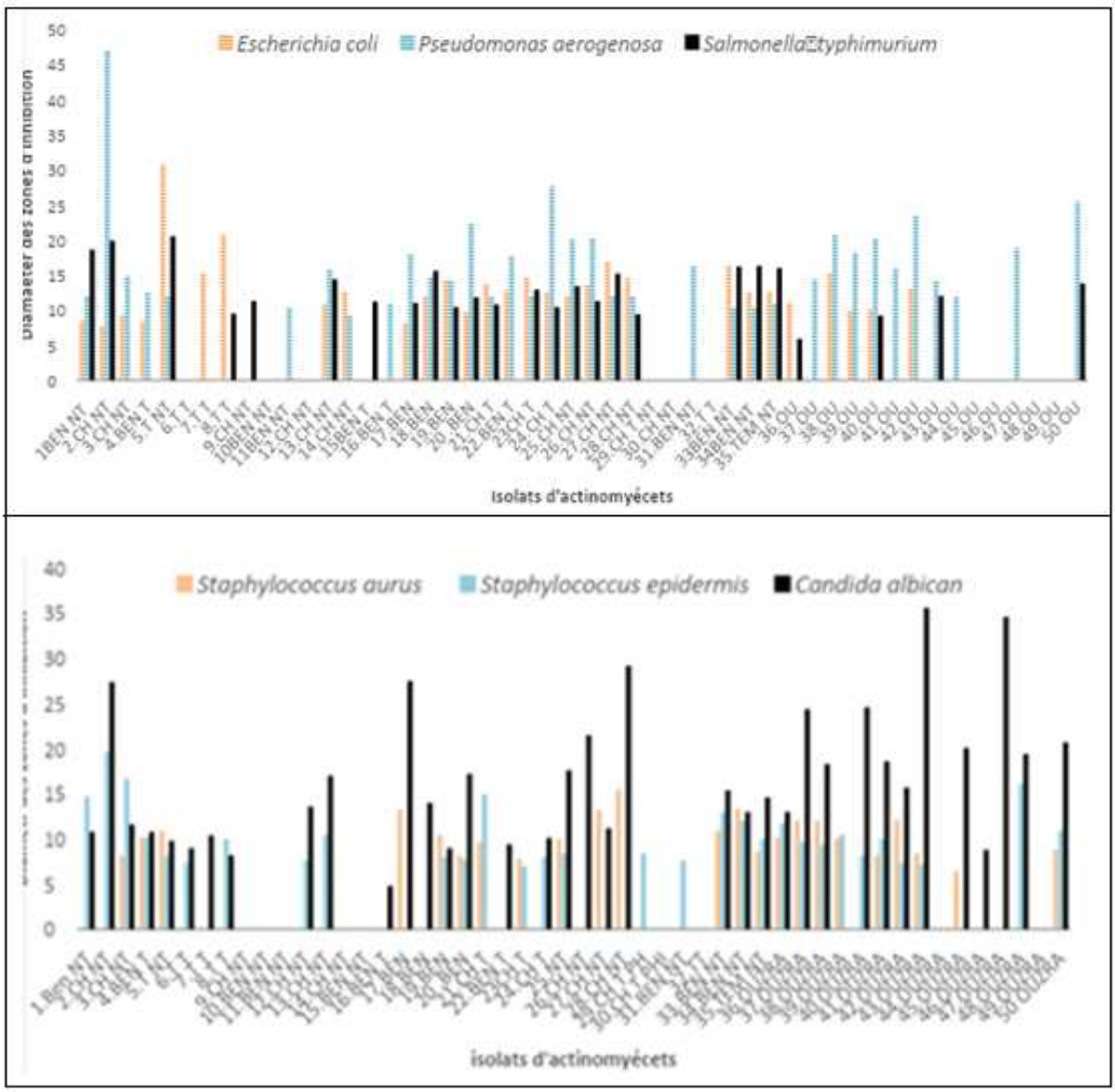

Fig. 2. Antimicrobial activity of actinobacteria against Gram positive and Gram negative bacteria.

After selection and purification, actinobacteria colonies are recognized on the basis of their macro and microscopic morphological aspects, which express a genetic polymorphism of the isolates. Variations visible to the naked eye are observed; the colonies obtained have different sizes, small, medium and large. Domed forms are observed. On the other hand, there are flattened forms, with a vegetative mycelium surmounted by an aerial mycelium of different colors: brown, gray, greenish, whitish, and

beige. According to Palaniyandi, 2013, the colors of the aerial mycelia of actinobacteria are different from those of the substrates and; most of them have a whitish color and others have different colors like gray, beige, black, brown, green, yellow, which confirms the observed results. Examination for catalase shows a positive result, all the isolates were Gram-positive.

According to some authors, the universal technique for isolating actinobacteria does not exist. Therefore, it is recommended to vary the methods and isolation media in order to achieve significant isolation of the flora of the actinomycete order (Boudemagh, 2007).

Fig. 3. Isolation of actinobacteria on different culture media.

The 16 selected strains from the antimicrobial activity were evaluated for their antibiotic resistance pattern using 5 standard antibiotic discs.

All the isolates showed the sensitivity of 100% to Amoxicillin, Neomycin and Chlortetracyclin. 6 of 16 isolates were resistant to penicillin so 37.5%, BN20 isolate showed very high resistance to penicillin.In contrast, 93.75% of isolates were resistance to Nystatin.In accordance with the present study, Gousterova *et al.*,2014 also reported the biosynthetic potential of twenty-six actinobacteria and their antibiotic sensitivity profiling against twelve antibiotics. According to (Passari *et al.*, 2017),these results suggest that these isolates could be excellent candidates for the discovery of antibiotics.During the first three days of culture, the DO rises rapidly to 0.23 for strain 1, 0.13 for strain BN20, 0.48 for strain BN17, and 0.11 for strain BN33. This is significant in terms of the kinetics of growth, antimicrobial substance production, and pH. The current phase is exponential.Due to the release of organic acids, the two culture media become more acidic during this phase. On day two, the strains enter the decline phase. Due to the degradation of organic nitrogen sources, such as the amino acids in yeast extract, which are deaminated to release ammonium, the DO of the culture decreases and the pH rises gradually to approach neutrality by the end of the culture (Strub, 2008).

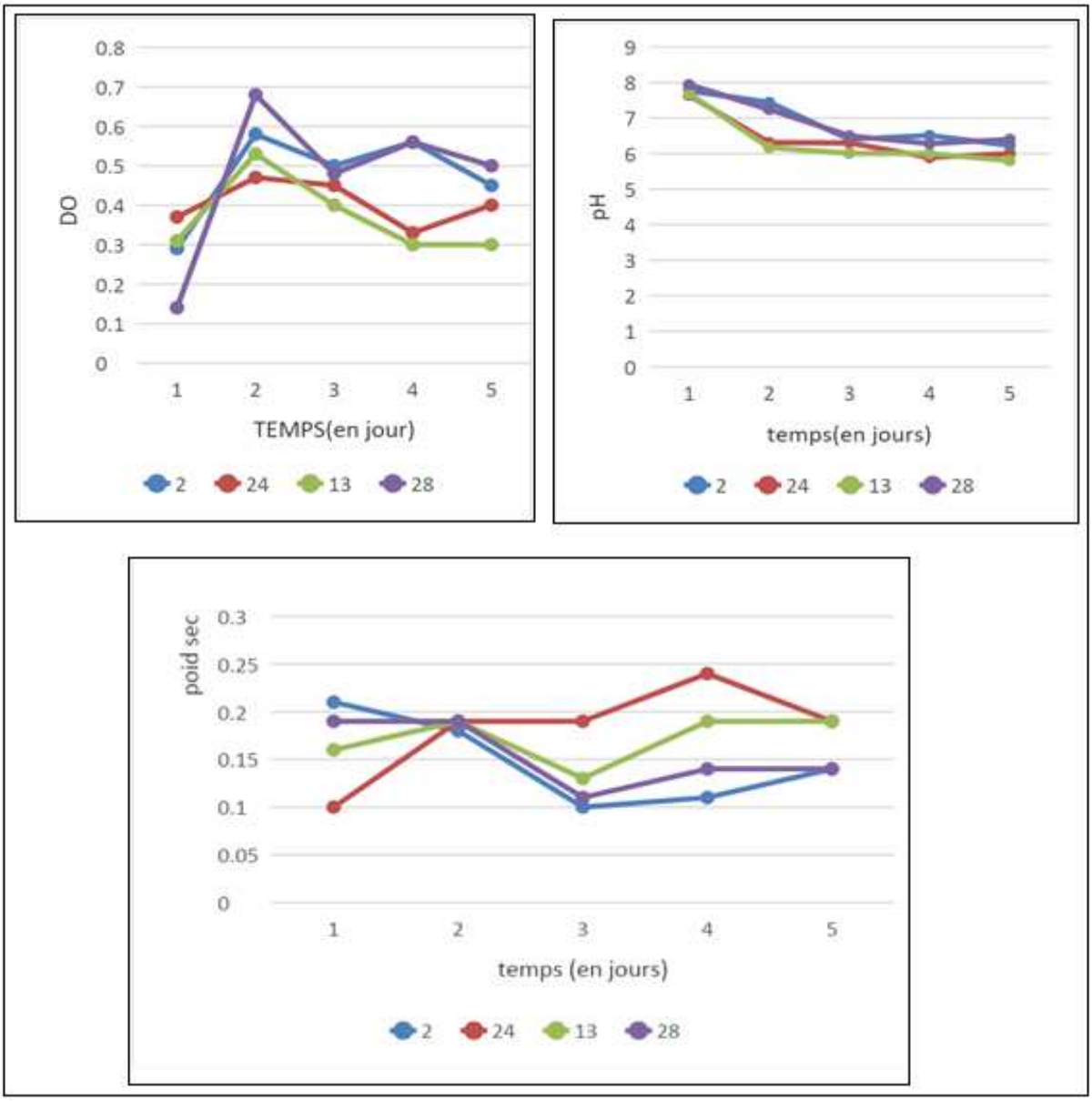

Fig. 4. Kinetic of growth, production of antibiotics and evolution of pH of actinobacteria isolates.

On the third day for strains (BN01, BN20, and BN17) and the second day for strain 24, it observed an increase in dry weight for strains, which can be explained by the technical's low precision. According to Martin and Mcdaniel (1975), this is the case; the determination of nucleic acids is one of the most advantageous techniques for tracking the growth of filamentous microorganisms with greater precision.

All isolates exhibited a broad spectrum of antimicrobial activity for the crude extracts; Gram-negative bacteria are generally more resistant to antimicrobial compounds than Gram-positive bacteria, so it is noteworthy that the crude extracts from the isolates exhibited antimicrobial activity against them. Lee *et al.* 2014 reported that actinobacteria isolated from soil samples of the Malaysian mangrove forest of Tanjung Lumpur exhibited inhibitory activity against Gram-negative bacteria; the same observations were done by Lamari *et al.*, 2002 and Djinni*et al.*, 2019about actinobacteria isolated from Algerian Sahara Soils and Kumala *et al.*, 2017 using Indonesian sample soil.

In contrast, RabiaBoukhalfa *et al.*, 2017 detected no activity against Gram-negative bacteria by a halotolerant actinobacterium isolated from a sample of salt lake soil in the Algerian Sahara.

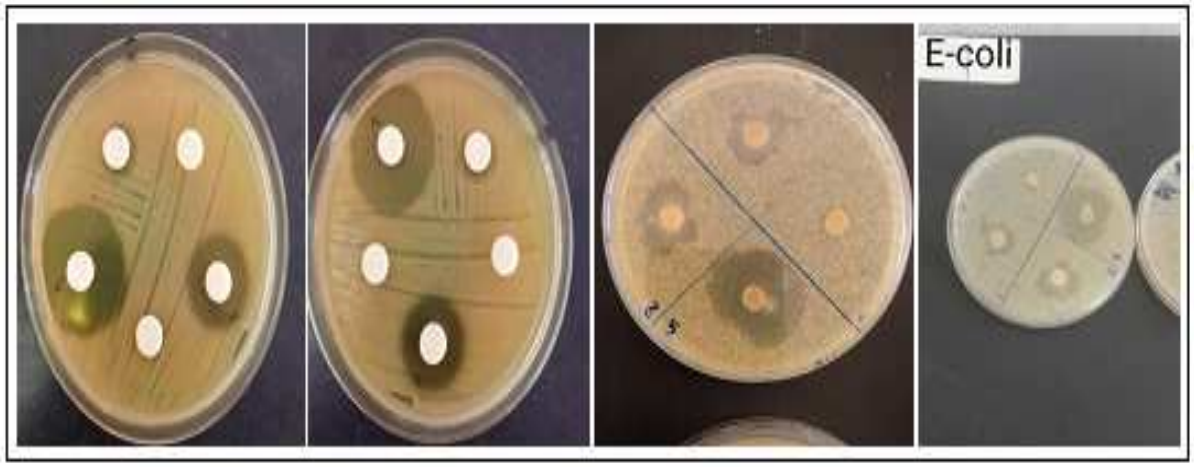

Fig. 5. Antimicrobial activity of the crude extract of actinobacteria isolates against different pathogenic microorganism.

Conclusion

It's concluded from the current research that actinobacteria isolated and screened from various regions of the Algerian provinces of Medea and Blida expressed strong antimicrobial activity against Gram-negative bacteria than Gram-positive, knowing that it's one of the most frequent bacterial infections. The isolates that showed antimicrobialactivity possessed an inhibitor substance as a potential bioactive molecule. According to our *In vitro* assays, these isolates demonstrated clearly a very interesting strategy in the treatment of different pathologies. We can show the significant impact of actinobacteria considered as potential biomolecule producers, especially against Gram-negative. Further studies of the *in vitro* selected candidates having antimicrobial biomolecules are encouraged to be continued.

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