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Environmental Parameters Influence on the Production of Antimicrobial Substances of Actinomycetes Isolated from Lifoula Landfill Soil (Republic of Congo)

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Abstract: The influence of temperature, pH and NaCl on the growth and antimicrobial activity of nine (09) actinomycete isolates from the soil of the Lifoula landfill site was studied. The growth and antimicrobial production of the isolates was carried out on LB medium previously inoculated with pathogenic strains of *E. coli*, *S. aureus* and *A. niger* as a function of temperature, pH and NaCl concentration. The results showed that from the 9 isolates, 7 isolates (A1, A2, A3, A6, A7, A9 and A10) or 77.77% produced biosurfactants: 91.66% for A1 and 25% for A2 versus 0% for A4 and A8. Isolates A6, A7 and A9 showed significant antimicrobial activity, with growth inhibition between 11 and 21mm: A7 at 15, 20 and 16mm; A6 at 13 and 11 and A9 at 21, 11 and 12mm diameter for *E. coli*, *S. aureus* and *A. niger* respectively. Maximum growth pH for A6, A7 and A9 isolates was 9, while maximum antimicrobial production pH was 7. Maximum growth temperatures were 25 and 45°C for A6 and A9 versus 45°C for A7; maximum antimicrobial production temperatures were 37°C for A6 and A9 versus 25°C for A7. The maximum NaCl content for growth of all three isolates was 0%, and for antimicrobial production 0.5% for A6 and A9 versus 0% for A7. Extraction of biosurfactants, antibiotics and antifungals indicate that isolates A6, A7 and A9 are producers of antimicrobials inhibiting the growth of *E. coli; S. aureus*; *A. niger*, with isolate A9 showing greater performance in inhibiting the growth of target microorganisms. The use of actinomycetes could represent an alternative in the fight against antibiotic resistance.

Keywords: *Actinomycetes, antimicrobial, LB medium, soil, pathogenic strain*

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1. Introduction

Soil is a very complex living environment, long considered as a simple agricultural support, it is an interface between biomass, the atmosphere and the hydrosphere. Soil plays an important role in determining the quality of water, air and the food chain. It is also a medium of transit, storage and transport of many substances, of organic or inorganic nature, resulting from natural processes or domestic activities. Landfill soils or polluted soils result from the cumulative consequences of various human, industrial, agricultural and even urban activities. This contamination not only has environmental, health and socio-economic consequences, but also a presence of microbial biodiversity capable of cleaning up these soils through various processes [1]. The micro-organisms present in these soils play a very important role in life (animal and plant), and are currently at the origin of the development of several fields such as medical biology, pharmacology and ecology. Among this microbial biodiversity are the Actinomycetes [2]. Actinomycetes or actinobacteria are Gram-positive filamentous bacteria with a fungus-like structure and a high guanine-cytosine percentage, capable of producing or synthesizing a wide variety of metabolites (enzymes and inhibitors), degrading certain organic substances (cellulose, pectin, starches, polysaccharides, chitins, proteins), recycling other substances such as xenobiotic compounds [3,4]. However, actinomycetes, especially those with a mycelial structure, are reputed to produce antibiotics [5]. The advent of antibiotics in the world of therapeutics has helped to increase average life expectancy by some fifteen years [6]. In fact, their introduction into the treatment of infectious diseases has led to their misuse, enabling micro-organisms

to develop resistance to antibiotics and sometimes even to antifungals. This has prompted the scientific world to search for new molecules to inhibit the growth of pathogenic microbes. However, actinomycetes represent the main natural source of antimicrobial metabolites [5], capable of producing antibiotics and antifungals of different chemical classes and biological activities. The resistance of microorganisms to various antibiotics due to their overuse in humans and animals is the source of many mutations in the organism [7]. This far-reaching public health problem is driving microbiology researchers to find reliable solutions to this resistance, and many have highlighted the search for new molecules that inhibit microbial growth [8]. Indeed, new studies suggest that actinomycetes may be an important strain [9]. It is in this perspective that the aim of our work is to contribute to the knowledge of actinomycetes in the soil of the Lifoula landfill, their biology and their arsenal of different metabolites favoring the identification of new bioactive molecules against pathogenic microbes.

2. Material and Method

2.1. Biological Material

The microorganisms used in this study belong to the actinomycetes group. 9 actinomycete isolates and three strains of pathogens: E. coli; S. aureus; A. niger used in this study were preserved by freezing at -20°C in the cellular and molecular biology laboratory of the Faculty of Science and Technology, Université Marien Ngouabi. These isolates were isolated in 2023 from Lifoula landfill soil.

2.2. Demonstration of Biosurfactant Production

Biosurfactant production was demonstrated using the emulsion test, which involved taking 2 mL of gasoline and placing it in test tubes containing 2 mL of actinomycete isolate culture. The mixture was then vortexed for 5 min at maximum speed. The tubes were then incubated at 25°C for 24 h. The presence of an emulsifying zone explained the existence or production of biosurfactants by the isolate tested. Indeed, emulsification indices were calculated according to the formula below [10,11]: $E24= \frac{HE}{HT} \times 100$

With: E24: Emulsification activity after 24 hours; He: Height of emulsion formed; Ht: Total height of mixture.

2.3. Demonstration of Antibacterial Activity

Actinomycetes strains were cultured in 5 ml LB broth, then incubated for 7 days at 30°C. 6 mm-diameter wells were made in the test medium previously inoculated with a target pathogen, then 100 µL of actinomycete culture was deposited in each well. After 2 h incubation at room temperature, the Petri dishes were incubated at 30°C for 24 h. The diameters of the zone of inhibition were then measured [12].

2.4. Influence of pH on Growth and Antimicrobial Activity of Isolates

A colony of the actinomycete isolate was picked after subculturing, inoculated into an Erlenmeyer flask containing 20 mL of LB medium. After 7 days of incubation, 1 mL of this culture was removed and inoculated into an Erlenmeyer flask containing 100 mL of LB at different pH levels 3, 7 and 9. All cultures were then incubated for 24 hours at 30°C. At each pH variation, 4 mL of the actinomycete culture suspension was taken, 2 mL of to measure optical density (O.D.) at 600 nm using a spectrophotometer and 2 mL for antimicrobial activity after centrifugation. Thus, using the well technique, 50 µL of culture supernatant was deposited in 6 mm diameter wells on the surface of MH medium previously seeded with test pathogens [13]. (**Mckinney, 2004**).

2.5. Influence of Temperature on Isolate Growth and Antimicrobial Activity

First, the colony after reactivation of the isolates was sampled and inoculated into an Erlenmeyer flask containing 20 mL of LB medium and incubated at 30°C for 7 days. Next, 1 mL of the resulting culture was taken and inoculated into an Erlenmeyer flask containing 100 mL of LB, then incubated for 24 hours at different temperatures: 25°C; 37°C and 45°C. For each culture at different temperatures, 4 mL were taken, 2 mL of which were used to measure optical density (O.D.) at 600 nm using a spectrophotometer, and the other two for antimicrobial activity after centrifugation. However, 50 µL of the centrifuged culture supernatant was deposited in 6mm-diameter wells on the surface of PCA medium previously seeded with test pathogenic microorganisms. The diameters of areas showing translucent halos on the petri dish after 18 h were taken [14].

2.6. Influence of NaCl Concentration on Isolate Growth and Antimicrobial Activity

To demonstrate the growth of actinomycete isolates and their antimicrobial activity, a colony of actinomycete isolate was collected and added to an Erlenmeyer flask containing LB, then incubated in an oven at 30°C for 7 days, 1mL of the bacterial culture was collected and inoculated into Erlenmeyer flasks containing LB at different NaCl concentrations (0%, 0.5%, 1.5%, 5% and 10%), then incubated for 24 h at 30°C. In fact, 4mL of these different cultures were taken, including 2 mL for the D.O. and the other mL for centrifugation to determine antimicrobial activity. The same protocol was used for antimicrobial activity detection and halo diameter measurement [14].

2.7. Biosurfactant extraction and Antimicrobial Assay

To extract biosurfactants, we used 7-day cultures of

isolates A6, A7 and A9, 14 mL of each culture was taken and placed in Falcone tubes. These were centrifuged at 6000 rpm for 20 min, the supernatant was kept and the pellet discarded, then 1mL of hydrochloric acid was added to the supernatant, fixing the pH of the resulting supernatant at 2, the mixture was thus incubated at 4°C overnight. After incubation, the mixture was centrifuged again at 6000 rpm for 30 min. This time, the supernatant was discarded and the pellet retained; 500 µL of PBS was added to the pellet and vortexed for 3 min. 6mm wells were made on the agar plates containing E. coli, S. aureus and A. niger, then 0.5 mm of this mixture was deposited in the wells. These plates were then incubated at 30°C for 18 h, and the growth inhibition diameters of the aforementioned microorganisms were measured [15]

2.8. Antibiotic Extraction and Antimicrobial Assay

Antibiotic extraction was carried out using an ionic chemical compound called ammonium sulfate [(NH4)2SO4] as a solvent to obtain as many of the bioactive molecules produced by actinomycetes as possible. Prior to the extraction step, we prepared a culture (LB + isolate colony) of the three actinomycete isolates (A6, A7 and A9) and incubated them in an oven at 30°C for 7 days. 14 mL of each culture was centrifuged at 6000 rpm for 20 min, and the culture supernatant was mixed with 0.7 g of ammonium sulfate. The mixture was then vortexed for 2 min and incubated at 4°C overnight. After incubation, the mixture was centrifuged again at 6000 rpm for 30 min, the supernatant was discarded and only the pellet was collected. 500 µL of PBS was added to the pellet and then vortexed for 3 min. To highlight the extract obtained, we seeded our three pathogens on MH medium. Three wells were made in each petri dish to accommodate the mixture obtained after vortex mixing. After 18 h, the growth inhibition diameters of the pathogens were measured [16].

2.9. Analysis of Results

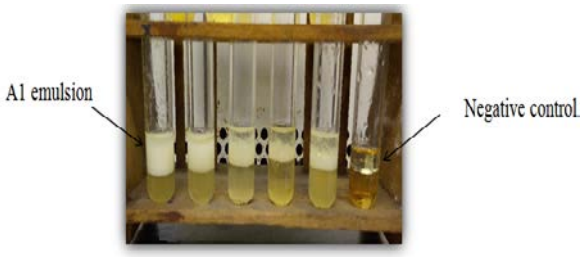
Statistical results for all classical microbiological techniques and enzyme activities were processed in Microsoft Excel.

3. Results

3.1. Demonstration of Biosurfactant Production

Figure 1 shows the biosurfactant production of the nine (9) actinomycete isolates. The emulsive profile of gasoline (hydrocarbon source) by the nine (9) isolates is revealed by the presence of an emulsive zone.

Figure 2 shows the percentages of biosurfactant production by the nine (9) actinomycete isolates. The 9 isolates tested for biosurfactant production showed that 7 isolates (77.77%) produced the emulsifier. The highest percentage of emulsification was observed with isolate A1 and the lowest with isolate A2, while isolates A4 and A8 produced no emulsifiers at all.



presence of an emulsive zone.

Figure 1. Emulsification of isolates A1, A3, A6, A7 and A9 from petrol

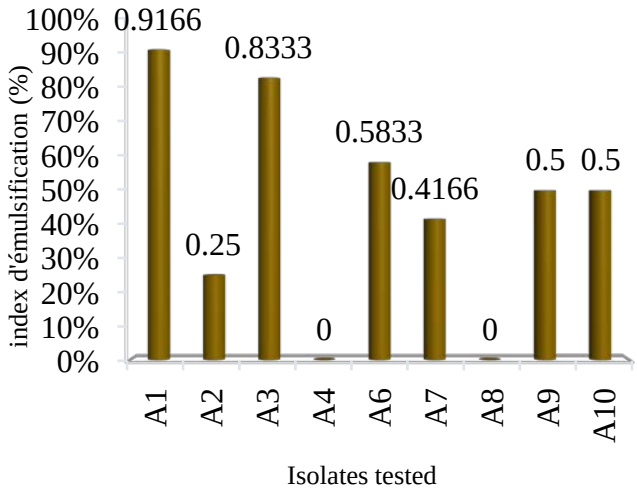


Figure 2. Percentage of biosurfactant production by nine (9) actinomycetes isolates.

3.2. Demonstration of Antibacterial Activity

Figure 3 shows the zones of inhibition of isolates expressing antimicrobial activity. Inhibition diameters range from 11 to 21mm. Three (03) isolates show variable antimicrobial activity depending on the target strain.

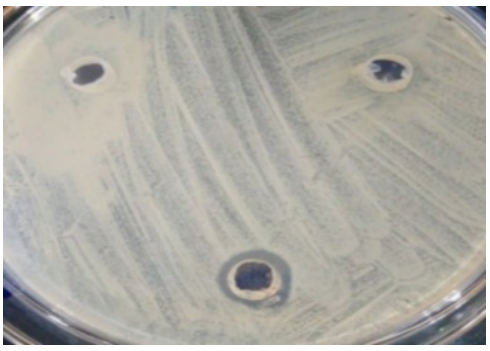


Figure 3. Growth inhibition halos of *E. coli* by isolate A9

The antimicrobial production profile of actinomycete isolates against *E. coli*, *S. aureus* and *A. niger* is shown in Figure 4. From the 09 isolates tested, isolates A3, A6 and A9 showed the ability to inhibit the growth of all three target strains: *E. coli*, *S. aureus* and *A. niger*. Isolate A3 inhibited *E. coli* at 15 mm, *S. aureus* at 20 mm and *A. niger* at 16 mm diameter. Isolate A6 inhibits *E. coli* at 13 mm, *S. aureus* at 13 mm and *A. niger* at 11mm diameter, while isolate A9 inhibits *E. coli* at 21 mm, *S. aureus* at 11 mm and *A. niger* at 12mm diameter.

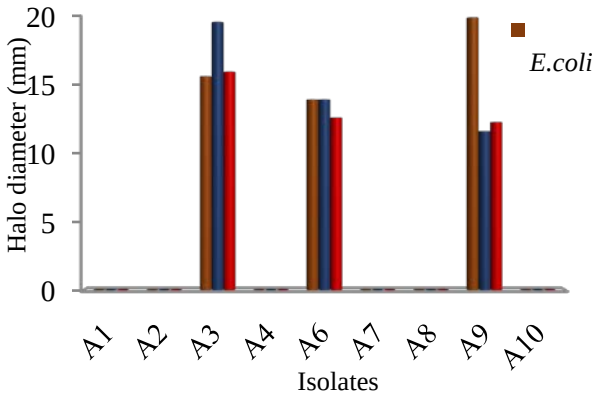


Figure 4. Profile of antimicrobial production by isolates

3.3. Influence of Environmental Parameters on Growth and Antimicrobial Activity

The influence of environmental parameters on the growth and antimicrobial activity of actinomycetes isolates was carried out with isolates that showed the ability to inhibit pathogen growth.

3.3.1. Influence of pH

3.3.1.1. Growth of Isolates at Different pH Levels

Figure 5 shows the growth of isolates A6, A7 and A9 at pH 3, 7 and 9 at 600 nm. At pH 3 isolates A7 and A9 grew slowly with optical densities of 0.173 and 0.307 respectively for A7 and A9, compared with rapid growth for isolate A6 with, an optical density of 1.01. At pH 6 average growth was observed for all three (3) isolates, with optical densities of 0.648; 0.794 and 0.660 respectively for isolates A6, A7 and A9. At pH 9 rapid growth was observed for all three (3) isolates, with optical density values ranging from 1.106 for A7 to 1.391 for A6.

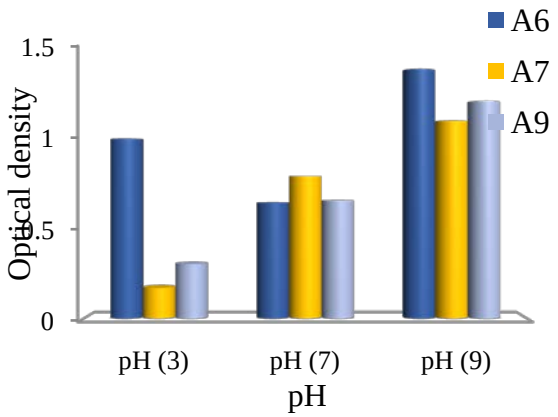


Figure 5. Growth profile of isolates A6; A7 and A9 at different pH values

3.3.1.2. Influence of pH on Isolate Production of Bioactive Substances

Figure 6 shows the growth inhibition diameters of pathogenic strains *E. coli*, *S. aureus* and *A. niger* by isolates A6, A7 and A9 at pH 3, 7 and 9. This inhibition expresses the production of bioactive substances by isolates A6, A7 and A9 at these different pH values. The Figure 7 shows that isolate A6 at pH 3, 7 and 9 inhibits the growth of pathogenic strains with inhibition diameters ranging from 9 to 15mm. With isolate A7, inhibition was only possible at pH 3 and 9 with growth inhibition diameters of the strains tested between 9 and 12mm, while at pH 3 no growth inhibition was observed on *A. niger*. With isolate A9, growth inhibition was observed at pH 3 on the *E. coli* strain, with an average inhibition diameter of 10.33mm. At pH 7, growth inhibition was observed on all strains: *E. coli*, *S. aureus* and *A. niger*. The largest inhibition diameter was 21mm for E. coli and 12mm for S. aureus and A. niger. At pH 9, diameters were very small on *E. coli* and *S. aureus*, with values of 8.66mm respectively.

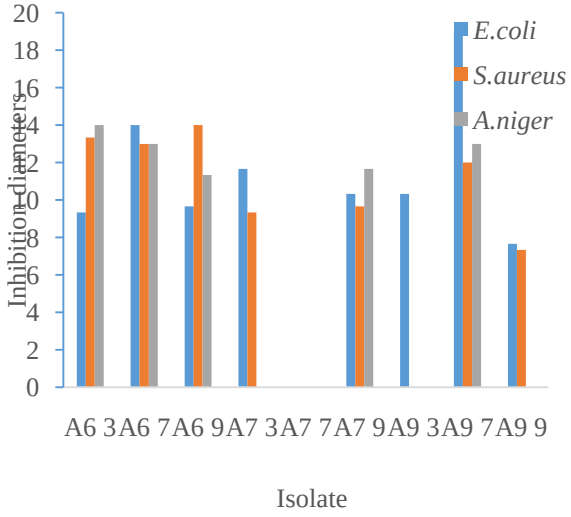


Figure 6. Pathogen inhibition diameters of A6, A7 and A9 isolates at different pH levels

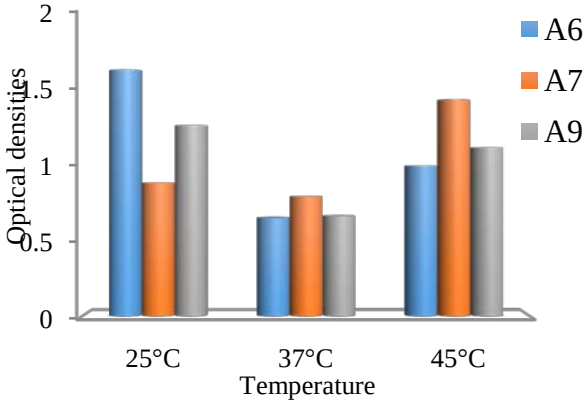


Figure 7. Variation in growth of actinomycete isolates at different temperatures

3.3.2. Growth and Bioactive Production of Isolates at Different Temperatures

3.3.2.1. Growth of Isolates at Different Temperatures

Figure 8 shows the variation in growth of isolates A6, A7 and A9 at different temperatures: 25, 37 and 45°C. Isolate A6 grew more rapidly, with optical densities of 1.636 at 25°C, 0.648 at 37°C and 1.011 at 45°C for A6. For isolate A7, rapid growth was observed at 45°C with an optical density of 1.444, whereas at temperatures of 25

and 37°C, growth was slower with optical density values of 0.888 and 0.794 respectively. With isolate A9, strong growth is observed at 25°C, expressed by an optical density value of 1.271. At 37°C growth is average, with an optical density of 0.660, and at 45°C rapid growth is observed, with an optical density value of 1.120.

3.3.2.2. Influence of Temperature on Isolate Bioactive Production

Figure 8 illustrates the variation in bioactive production by isolates A6; A7 and A9 at different temperatures. At 25°C, isolate A6 produced bioactive against *E. coli* with an inhibition diameter of 9.33mm; 11.66mm against *S. aureus* and 9mm against *A. niger*. At 37°C, the growth inhibition diameters of the three (3) strains were 14mm for *E. coli*, 14mm for S. aureus and 13mm for *A. niger*. At 45°C, bioactive production was only observed in *E. coli*, with an inhibition diameter of 12.66mm, and a total absence of inhibition zones for the other target strains. For isolate A7 at 25°C, inhibition zones were observed on the various target strains, with diameters of 10.33 mm for *E. coli*, 14 mm for *S. aureus* and 9.33 mm for *A. niger*. At 37°C, no zone of growth inhibition was observed for the target strains, whereas at 45°C, inhibition was observed with the E. coli strain, with an inhibition diameter of 11.66 mm. For isolate A9 at 25°C, but one on *A. niger*, a growth inhibition diameter of 9.66 mm was observed, marking the production of bioactive. At 37°C, the isolate showed bioactive production against all three target pathogens: for *E. coli* a diameter of 20 mm, for *S. aureus* 12 mm and for *A. niger* 12 mm. At 45°C, bioactive production is only observed on *E. coli*, with an inhibition zone diameter of 9.66 mm.

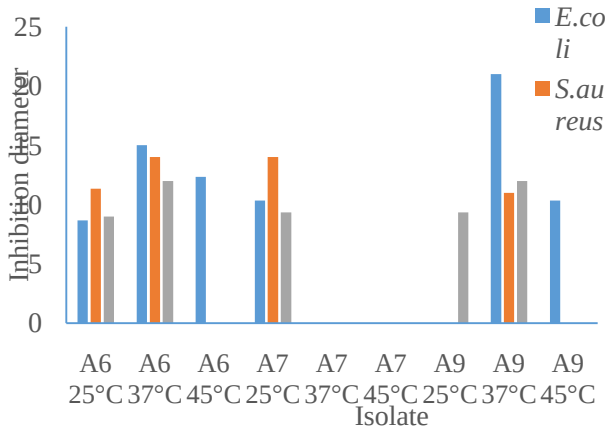


Figure 8. Pathogen inhibition diameters for isolates A6, A7 and A9 at different temperatures

3.3.3. Biosurfactant Growth and Production at Different NaCl Concentrations

3.3.3.1. Influence of NaCl Concentration on Isolate Growth

Figure 9 shows the effect of different NaCl concentrations: 0; 0.5; 1.5; 5 and 10% on the growth of isolates A6, A7 and A9. For isolate A6, rapid growth was observed at 0% NaCl, with an average optical density of 1.777. At 0.5% NaCl, the mean optical density was 0.650, while at 1.5% NaCl, a decrease in growth was observed, with the optical density value falling to 0.508. At 5% NaCl the growth and optical density is 0.377 and at 10% NaCl it rises to 0.344. Isolate A7 shows good growth at 0% NaCl, with an optical density of 1.714; this decreases at 0.5% NaCl, with an optical density of 0.793. At 1.5% NaCl, growth is weak compared with previous concentrations, with an optical density of 0.646. This growth decreases at 5% NaCl, with an optical density of 0.602, and becomes very slow at 10% NaCl, with an optical density of 0.149. As with the other two isolates, isolate A9 showed rapid growth at 0% NaCl, with an optical density of 1.301; at 0.5% NaCl, growth became average, with a density of 0.662; at 1.5%, growth increased compared with the 0.5% NaCl concentration, with a density of 0.987. This growth decreases at 5% NaCl and the optical density falls to 0.557 and then to 0.512 at 10% NaCl.

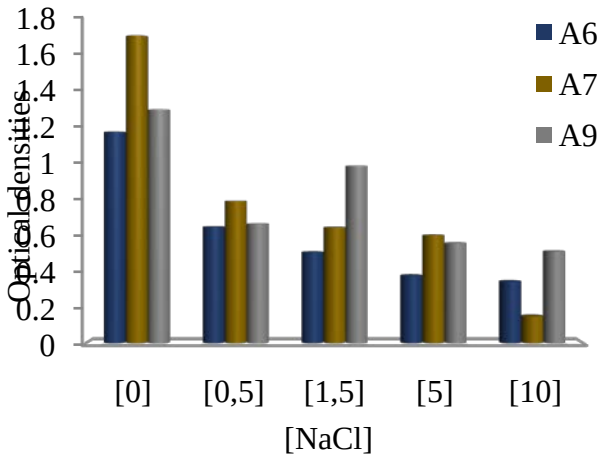


Figure 9. Effect of NaCl concentration on the growth of actinomycetes isolates

3.3.3.2. Influence of NaCl on isolate Biosurfactant Production

Figure 10 shows the influence of different NaCl at 0; 0.5; 1.5; 5 and 10% on the production of bioactive substances in isolates A6, A7 and A9. At concentrations of 0, 0.5 and 1.5% NaCl, *E. coli*, *S. aureus* and *A. niger* growth was inhibited by isolate A6, with inhibition diameters of 9-10mm, 13-15mm and 9.66-11.66mm respectively. At 5% NaCl, inhibition is only present on *E. coli* and *S. aureus* with diameters of 11 and 9.66mm respectively. At 10% we note an absence of production on *E. coli*, while on *S. aureus* the isolates produced bioactive with an inhibition diameter of 12mm and on *A. niger* with a diameter of 11.66 mm. For isolate A7, at 0% NaCl we note an inhibition of the target strains with inhibition diameters of 9mm for E. coli; 11.33 mm for *S. aureus* and 9.33mm for *A. niger*. At 0.5% no inhibition is observed and at 1.5% inhibition is only observed on the *A. niger* strain with 10.33mm in diameter. At 5% we observe an inhibition of the growth of *S. aureus* with 12mm and of *A. niger* with 11.66mm while at 10% NaCl, the inhibition of *S. aureus* presents a diameter of 11.33mm. With isolate A9 no inhibition against the target strains is observed at 0% NaCl whereas at 0.5% inhibition is observed on all strains, on *E. coli* 20mm; *S. aureus* 12mm and *A. niger* 12mm. At 1.5% no inhibition against the target strains; at 5% NaCl it

is only observed on *E. coli* at 9mm and at 10% NaCl we note an absence of inhibition of the growth of *E. coli* and *A. niger* compared to inhibition of *S. aureus* at 10mm in diameter.

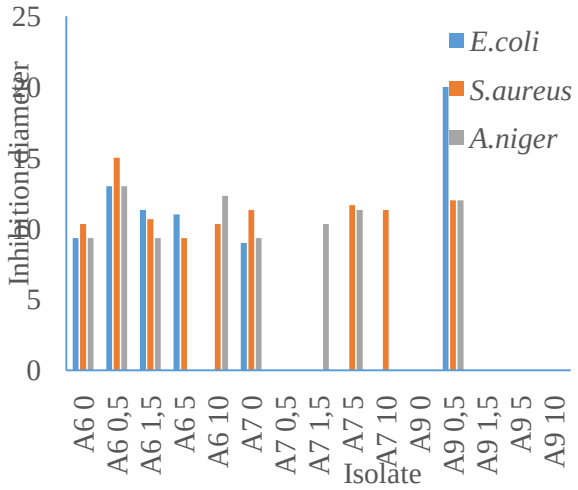


Figure 10. diameters of inhibition of pathogens by A6 isolates; A7 and A9 at different NaCl concentrations

3.4. Extraction of Biosurfactants Produced By the Isolates

Figure 11 shows the zones of inhibition of A. niger by the three actinomycete isolates selected after extraction of the biosurfactants.

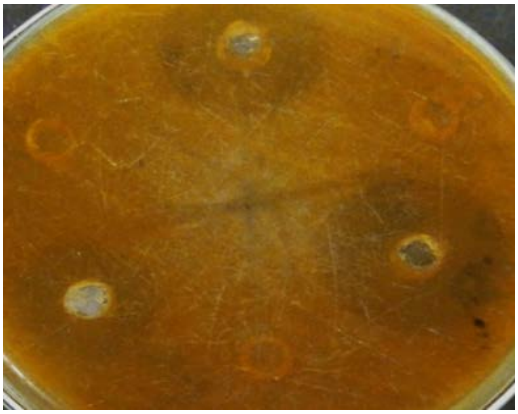


Figure 11. Halos of inhibition of *A. niger* growth by actinomycete isolates after extraction of biosurfactants

Figure 12 shows the inhibition of the growth of the target strains by the biosurfactant extract produced by the actinomycete isolates. The results obtained after the extraction of biosurfactants show that the extracts of the supernatant of isolates A6 and A9 with hydrochloric acid inhibited the growth of *E. coli, S. aureus* and *A. niger* while that of isolate A7 inhibited the growth of *E. coli, S. aureus* and *A. niger*. Presence of the inhibition zone is observed only in *E. coli* and *A. niger*. The most significant areas of lysis were observed with isolate A6.

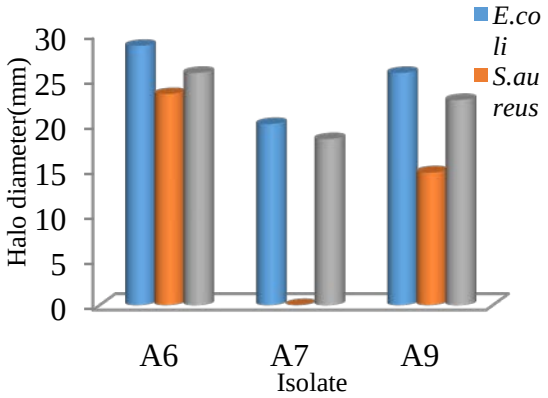


Figure 12. inhibitory effect of the extract of biosurfactants from the isolates on the growth of the target strains

3.5. Demonstration of the Extraction of Antimicrobials

Figure 13 shows the zones of inhibition of S. aureus by isolates A9 illustrated by the presence of a halo around the well.

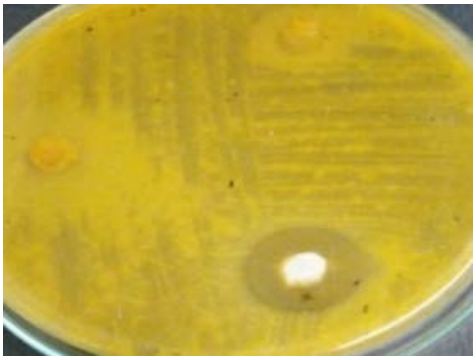


Figure 13. halos of inhibition of S. aureus growth by isolate A9

Figure 14 shows the profile of the growth inhibition halos of target pathogens after extraction of antimicrobials. These results of the extraction of antimicrobials from isolates A6, A7 and A9 show an activity of inhibiting the growth of *E. coli, S. aureus* and *A. niger* only by an actinomycete isolate which is A9.

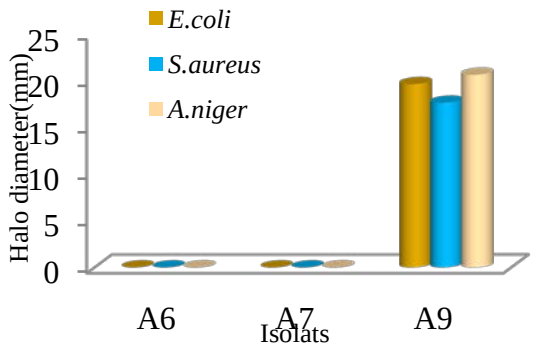


Figure 14. Growth inhibition profile of target strains by the extract of antibiotics and antifungals by actinomycete isolates

4. Discussion

The results of the influence of pH on growth and antimicrobial production showed good growth and productivity at pH 9 in the three selected isolates. These results could be explained by the fact that these types of actinomycetal bacteria develop in the group of alkalinophilic bacteria [17]. Indeed, the production of bioactive molecules was strongly observed at pH 7 for isolates A6 and A9 with respect to the target microorganisms, while it is strongly observed at pH 9 for isolate A7. Our results obtained are close to those of Lazouni and Ferhaoui (2022) who showed the influence of pH on the growth of actinomycetes. They are also similar to those of Habibeche (2013) which followed the growth and production of bioactive molecules of actinomycetes under the influence of pH at neutral and basic concentration. The influence of temperature on growth and antimicrobial production showed that the three selected isolates grew at 25, 37 and 45°C. Isolates A6 and A9 showed optimal growth at 25°C while isolate A7 did so at 45°C. A high production of antimicrobials was revealed in isolates A6 and A9 at 37°C against the target pathogens: *E. coli*, *S. aureus* and *A. niger* with inhibition diameters varying between 12 and 21 mm, for isolate A7. The high antimicrobial production is observed at 25°C against *S. aureus* and *A. niger* while, with *E. coli*, the highest inhibition diameter is observed at 45°C. These results suggest that the production temperature of antimicrobial molecules is lower than the optimal growth temperature apart from the production of bioactive in A7 with respect to *E. coli*. These results are similar to those obtained in a study where a maximum temperature for antibiotic production was at 37°C and that for growth at 30°C [19]. The results obtained could be translated by a greater development of aerial mycelium at the production temperatures of bioactive substances with respect to the target microorganisms [19,20]. The NaCl concentrations influence on the growth and production of antimicrobial substances showed that the selected isolates grow at different NaCl concentrations chosen: 0; 0.5; 1.5, 5 and 10%. All three isolates showed optimal growth at 0% NaCl concentration, and as the concentration increased growth slowed. This fact can be reflected by the fact that the different isolates, although tolerant to salty environments, grow in non-salty environments. These results are thus similar to the study carried out by Harir (2018) on the influence of NaCl on the growth of actinomycetes. They showed that the higher the NaCl concentration, the worse the growth. However, it should be noted that isolate A9 experienced good growth at 0.5 and 1.5% NaCl. This fact could be explained by the fact that the culture at 0.5% NaCl would have experienced disturbances leading to the death of the actinomycetes cells. Indeed, several scientific works have shown that the majority of actinomycetes grow abundantly at concentrations ranging from 0 to 2% NaCl compared to no growth at concentrations ranging from 6 to 7% NaCl [22,23]. However, the maximum production of antimicrobial substances of isolates A6 and A9 against E. coli, S. aureus and A. niger used in our experiment is observed at 0.5% NaCl compared to 0% for the isolate A7, concentrations where the growth is exponential. At different concentrations, variable production of antimicrobial substances is observed. These results could be explained by the fact that the rapid multiplication of cells allows the secretion of antimicrobial molecules. Our results are close to those of a previous study on the optimization of growth and production of bioactive metabolites by *Streptomyces tanashiensis* [23]. This work showed that for this strain, the maximum production of bioactive metabolites was at a concentration of 1% NaCl.

Biosurfactants extracted from the selected A6 and A9 isolates showed antimicrobial activity inhibiting the growth of *E. coli*, *S. aureus* and *A. niger*. The greatest inhibitory activity was observed in isolate A6 with inhibition diameters ranging from 22 to 30mm. Isolate A7 presented an inhibition diameter of 18 to 21 mm against *E. coli* and *S. aureus*. These results could probably be explained by the fact that the selected isolates produce biosurfactants as bioactive secondary metabolites. Our results support those of the study on the antimicrobial activity of biosurfactants produced by actinomycetes with an optimum inhibition of 30 mm against *E. coli* and *S. aureus* [24]. The results of the antimicrobial activity obtained after extraction of antibiotic and antifungal showed that only isolate A9 revealed a production of antibiotic and antifungal against the target microorganisms with diameters of inhibition ranging from 17 to 21mm. The absence of production of antibiotics and antifungals by isolates A6 and A7 in our study could be reflected by the nature of our extraction solvent because other authors have shown a difference in inhibition diameter following the activity antimicrobial after extraction of antibiotics and antifungals from several solvents [18,25,26].

5. Conclusion

This work aimed to study the influence of temperature, pH and NaCl on the growth and production of antimicrobial substances by actinomycetes isolated from the Lifoula public landfill. The obtained results showed that the growth temperature was between 25 and 45°C with an optimal antimicrobial production of 37°C for isolates A6 and A9, and 25°C for A7. The maximum NaCl content necessary for growth was 0% for the three isolates and the necessary for antimicrobials production was 0.5% for A6 and A9 against 0% for A7. The extraction of biosurfactants, antibiotics and antifungals indicate that isolates A6, A7 and A9 are producers of antimicrobials inhibiting the growth of *E. coli*, *S. aureus*, *A. niger*. Finally, isolate A9 presented more performance in inhibiting the growth of target microorganisms. This preliminary study should be continued with a molecular study in order to identify, sequence genes and purify antimicrobials substances.

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