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Enzymatic Activity of Actinomycetes Isolated from the Soil of the Public Landfill of Lifoula (Republic of Congo)

Mboukou Kimbatsa Irène Marie Cecile[1,2], Ignoumba Evariste Mesmin[1,3], Gatsé Elgie Viennechie[1,3,*], Nkounkou Bendo Yvestha Hanselme[1,3], Morabandza Cyr Jonas[1,3], Nguimbi Etienne[1,3]

[1]Laboratory of Microbiology, Infectiology and Immunology, ENS-UMNG, B.P 69 Brazzaville
[2]Laboratory of Remote Sensing and Forest Ecology, ENS-UMNG, B.P 69 Brazzaville
[3]Laboratory of Cellular and Molecular Biology, FST-UMNG, B.P 69 Brazzaville
*Corresponding author: venagatse@gmail.com

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Abstract Actinomycetes are Gram-positive filamentous bacteria that have colonized even the most hostile ecosystems. To date, they remain the main source of secondary metabolites and are currently at the origin of the development of several fields such as medical biology, pharmacology and ecology. This work aimed at the characterization of actinomycetes isolated from the soil of the public landfill of Lifoula (Brazzaville); with a view to the possible discovery of rare strains or strains with high enzymatic production potential for industrial use. Soil samples were taken from 2 different points of the landfill, between 5 and 10 cm deep. Inoculation on specific media was followed by enumeration, isolation, phenotypic identification of isolates and enzyme production using an agar well technique. The results showed loads of 70 and 73.30 x 104CFU/g on starch-casein medium compared to 53.30 and 56.60 x 104CFU/g on starch-casein enriched with yeast extract for samples 1 and 2, respectively. 20 isolates were recovered including 9 purified isolates: 6 isolates from sample 1 and 3 from sample 2. The skim milk clotting test was positive for 8 isolates and negative for isolate A2. Enzyme production diameters were amylase, 20 mm for isolates A1 and A7 compared to 14 mm for A4, A9 and A10; proteases, 38 to 50 mm for isolates A1, A2, A7 and A8 compared to 19 mm for A3, A4 and A6 and no production in A9 and A10; Cellulase, 20 mm for A3, A4, A6, A7, A8 and A10 compared to 15 mm for A1, A2 and A9; Lipases 30 mm for A6, A9 and A10 compared to 20 mm for A1, A2 and A8 and 15 mm for A3 and A4. These results suggest that the soil of the Lifoula landfill is rich in enzyme-producing actinomycetes, which can be used in a number of fields, including the food, textile and pharmaceutical industries.

Keywords: *actinomycetes, enzymes, starch-casein, yeast extract, Lifoula*

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1. Introduction

Contaminated soils are the result of the cumulative effects of various human activities, whether industrial, agricultural or urban. This contamination has environmental, health and socio-economic consequences, as well as microbial biodiversity, which can purify the soil through various processes [1]. The microorganisms present in these soils play a very important role in life and have been responsible for the development of several fields such as medical biology, pharmacology and ecology [2]. These microorganisms are capable of producing metabolites (enzymes, antibiotics, biosurfactants, antifungals), representing a large source of structurally diverse compounds with very significant and extensive biological potential. In practice, it has been shown that the ability to produce different compounds depends on the groups of eukaryotic. Among the latter are actinomycetes, Gram-positive filamentous bacteria with a structure similar to that of fungi and a high percentage of guanine-cytosine. However, the survival of living organisms depends on catalysts called enzymes in anabolic reactions and the synthesis of chemical compounds [3]. While other enzymes are used in catabolic reactions to facilitate the breaking of chemical bonds and the breakdown of certain biochemical compounds, these enzymes accelerate these reactions millions of times. About twenty enzymes are currently known to be available on an industrial scale. In the food sector, extracellular enzymes are mainly used for the degradation of natural polymers [4]. Due to the increasing demand for enzymes in industry, the results of several studies indicate that actinomycetes are important producers of amylases, cellulases, proteases and lipases. The Lifoula landfill is the largest in Brazzaville, and it is important to note that the distribution of waste in public landfills over many years has led to soil contamination

and the establishment of a wide variety of microorganisms. In this sense, we wanted to contribute to their understanding by characterizing the actinomycetes isolated from the soils of the Lifoula landfill (Brazzaville), their biology and their enzymatic arsenal.

2. Materials and Methods

2.1. Sampling

4 soil samples were taken with a sterile spatula from a depth of 5 to 10 cm in the underlying layers, while removing large debris and miscellaneous waste (stones, roots, plastics) at 2 different locations in the landfill. Two composites were prepared from these 4 soil samples, which were immediately transported in a cool box and stored in sterile plastic packaging at 4°C in a refrigerator. Analyses were performed at the Laboratory of Cell and Molecular Biology, Faculty of Science and Technology, Marien Ngouabi University, Republic of Congo.

2.2. Enumeration

For enumeration, 10 g of soil was diluted in 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask. The resulting suspension was shaken vigorously for 5 minutes and then allowed to stand for 10 minutes [5]. A series of decimal dilutions from 10-1 to 10-4 were prepared from the stock solution, and 0.1 mL of each dilution was applied to starch-casein media and yeast starch-casein. Petri dishes were incubated at 30°C for 5 days and the colonies were counted.

2.3. Isolation, Purification, Revivification and Conservation of Isolates

Twenty (20) colonies were selected from the soil samples based on the characteristic appearance of the actinomycetes and nine (9) of these were the subject of our work. The colonies were subcultured on the same starch-casein media and yeast starch-casein extract used for isolation and purification. The starch-casein media were supplemented with chloramphenicol at 50 µg/ml to eliminate bacteria.

2.4. Phenotypic Characterization

Phenotypic characterization was based on macroscopic observation: color of colonies, consistency, contour and filamentous appearance, colony size was not measured; microscopic observation with Gram stain test. Isolates with positive Gram staining and filamentous appearance were stored at 4°C. Biochemical analysis was based on the skim milk clotting test by mixing skim milk with actinomycete colony culture followed by incubation for 7 days, and on the catalase test followed by Gram staining. These tests were repeated twice on the same isolate [6].

2.5. Demonstration of Enzymatic Activity

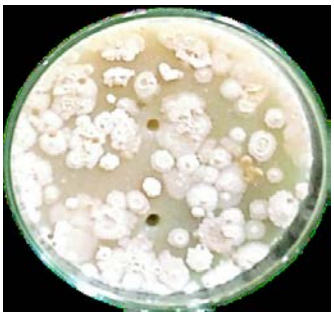
Enzymatic production of the isolates is performed from the culture of each isolate after 7 days of incubation at 30°C. 1 mL of each culture is taken to measure the optical density, which is an expression of microbial growth, using a streptophotometer at 600 nm to evaluate enzymatic production [7]. The demonstration of enzymatic activities is carried out using the well technique, from the media (starch agar, casein agar, Tween 80 agar, cellulose agar) poured into sterile Petri dishes, after solidification then 100 µL were made on these different agar plates. 50 µL of each actinomycete culture, centrifuged at 6000 rpm, was added to each well. The plates were then incubated at 30°C for approximately 48 hours. After incubation, plates containing starch agar, cellulose agar, and Tween 80 agar were rinsed with Lugol for a few minutes and the diameters of the halos were measured in cm, the absence of staining around the colonies indicating hydrolysis [8,9,10,11].

3. Results

3.1. Enumeration

Figure 1 shows the actinomycete colonies on starch-casein and starch-casein media enriched with yeast extract after 5 days of incubation. These colonies were recognized by their macroscopic criteria, they appear dry and creamy and adhere to the agar, generally white in color, the seeded cultures were countable and especially observed on the starch-casein medium.

a



b

Figure 1. Actinomycete colonies after seeding (a= Yeast Starch Casein.; b=Starch-Casein)

Figure 2 shows the actinomycete colony loads in CFU/g × 104. We observe loads of 70 and 73.30 x 104 CFU/g on the starch-casein medium compared to 53.30 and 56.60 x 104 CFU/g on the casein-starch medium enriched with yeast extract. On both media, the load was higher in sample 2 and it was the casein-starch medium that showed more load.

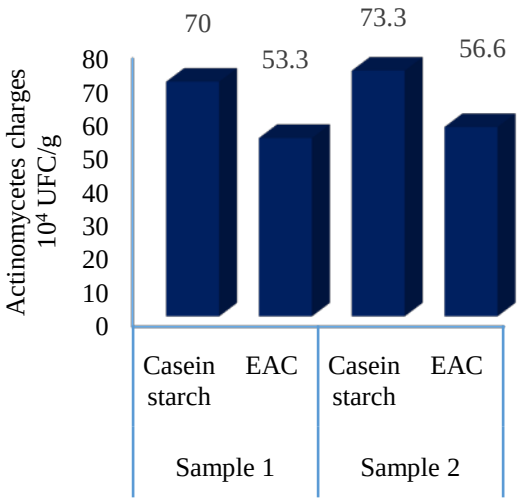


Figure 2. Loads in CFU/g of actinomycetes of landfill soil samples 1 and 2 on starch-casein and EAC media

3.2. Isolation

The different morphotypes obtained after the purification of the colonies on the starch-casein medium after 3 days (a) and 7 days (b) are shown in Figure 3; we note that on the 3rd day the colonies are white and small, on the 5th day the colonies become yellow-green and their size increases. A total of 20 isolates presenting the macroscopic criteria of actinomycetes were selected.

3.3. Phenotypic Characteristic

Of the 20 isolates obtained, 09 isolates were retained and showed phonotypic characteristics of actinomycetes: 6 isolates from sample 1 (A1 to A6) and 3 isolates from sample 2 (A7 to A10). These different isolates were coded as follows: A1, A2, A3, A4, A6, A7, A8, A9, A10. Table 1 shows some macromorphological characteristics observed in the 9 isolates.

Macroscopic characterization led to the observation of different isolates. Table 2 below presents the results of macromorphological images; micromorphological and microscopic observations of the 9 selected actinomycete isolates.

Table 3 shows the pictures of the result of coagulation of skimmed milk by the actinomycete isolates. The skim milk coagulation test of the 9 Actinomyces strains showed a positive test for eight (8) isolates with a negative test for isolate A2.

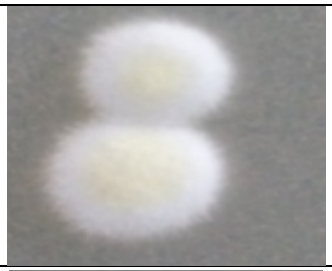
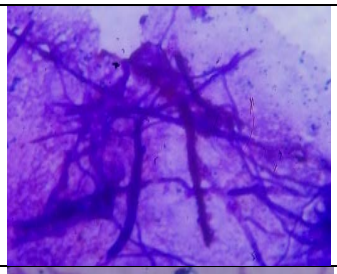
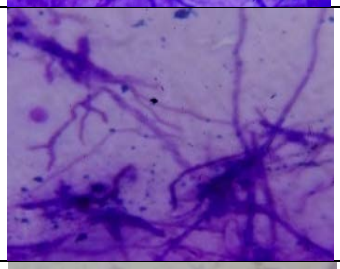
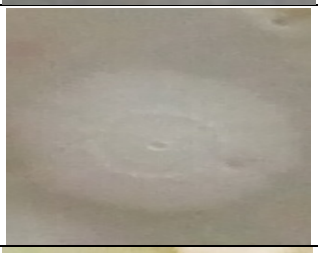
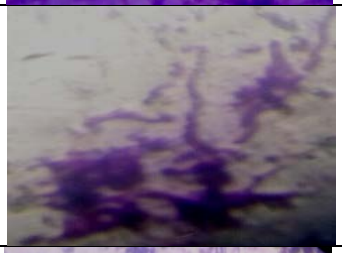
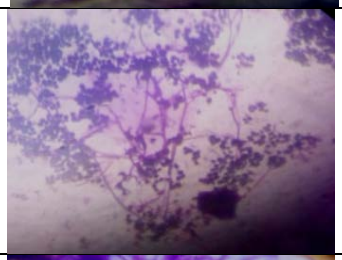
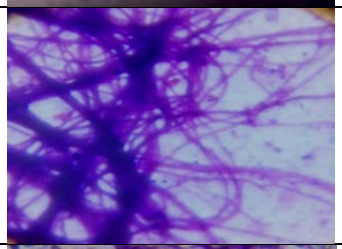
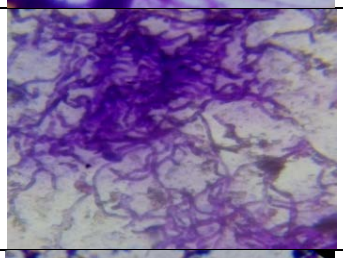
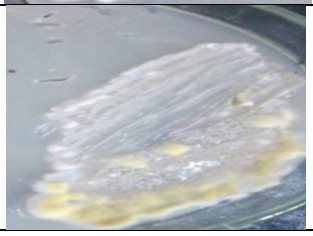
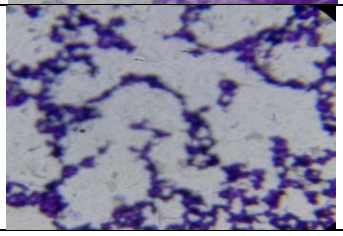
a b

Figure 3. different morphotypes obtained after purification Isolates: a) after 3 days and b) after 7 days

Table 1. The macromorphological and cultural characteristics of the 9 isolates

Isolates	Color	Outline	Relief	Shape	Consistency	Mycelium
A1	Yellow white outline	Regular	Bomb	Circular	Dry	+
A2	Yellow white outline	Regular	Bomb	Circular	Dry	+
A3	White	Regular	Plat	Circular	Dry	-
A4	White	Regular	Plat	Circular	Dry	+
A6	Yellow white outline	Filamentous	Moderately rounded	Irregular	Dry	+
A7	Yellow white outline	Filamentous	Moderately rounded	Irregular	Dry	+
A8	Yellow white outline	Regular	Plat	Irregular	Creamy	-
A9	White	Filamentous	Bomb	Circular	Dry	+
A10	White	Filamentous	Moderately bomb	Circular	Dry	+

Table 2. phototypic characteristics of isolates

Isolates	Macroscopy	Microscopy	
		Gram stain	Observation
A1			Mycelium made up of more or less fine branched and fragmented filaments.
A2			Mycelium made up of more or less fine branched and fragmented filaments
A3			Mycelium made up of more or less fine branched and fragmented filaments
A4			Mycelium made up of more or less fine branched and fragmented filaments
A6			Mycelium long, well developed, curved in a zigzag shape
A7			Mycelium long, well developed, curved in a zigzag shape
A8			Cockles in the form of chain-shaped clusters

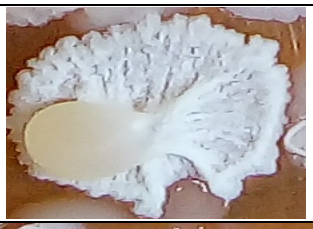
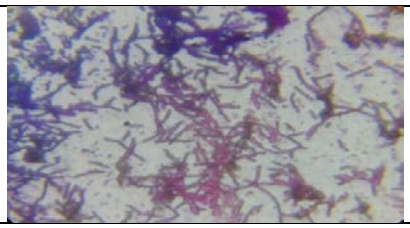
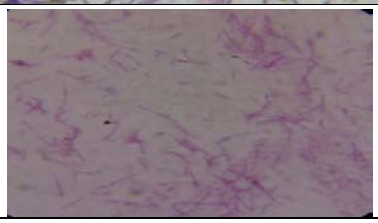
A9			Medium mycelium, poorly developed, not curved into a cluster shape.
A10			Mycelium consisting of isolated unbranched filaments

Table 3. Effect of isolates on coagulation in skimmed milk

Isolats	Réaction
A1	+ -
A2	-
A3	+
A4	++
A6	+
A7	+ -
A8	+
A9	+
A10	+

++: Positive; +: moderately positive; +-: more or less positive; -: negative

3.4. Demonstration of Enzymatic Activities

3.4.1. Optical Density

Isolates A10, A9, A8, A4 and A6 showed good growth performance under the study conditions with an average density of 0.9 each. However, isolates A1, A3 and A7 showed less significant growth with optical densities varying between 0.4 and 0.6, and finally A2 showed less significant growth with an optical density of 0.2. In conclusion, the selected isolates show growth under our culture conditions.

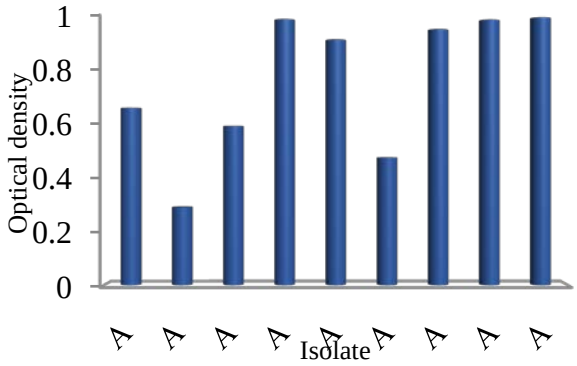


Figure 4. Variation in optical density depending on the isolates

3.4.2. Demonstration of Amylolytic Activity

Figure 5 shows the different starch hydrolysis profiles of the isolates studied after three tests. All the selected actinomycete isolates produced amylase. Diameters greater than 15 mm were observed for isolates A1, A2, A3, A6, A7 and A8, with 20 mm for isolates A1 and A7 compared to 14 mm for A4, A9 and A10.

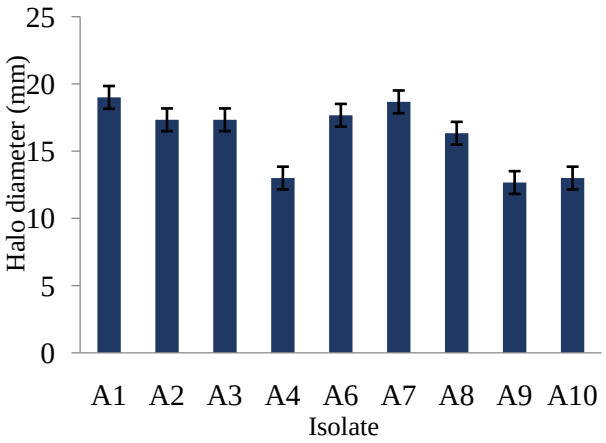


Figure 5. Profile Of amylase production by actinomycete isolates

3.4.3. Demonstration of Proteolytic Activity

Figure 6 shows the different casein degradation profiles of the isolates after three trials, with each isolate producing proteases differently. The highest production of proteases was obtained with isolates A1, A2, A7 and A8, with homozygous diameters varying between 50 and 38 mm. The least productive isolates are A3, A4 and A6. In fact, A9 and A10 do not have the ability to hydrolyze casein.

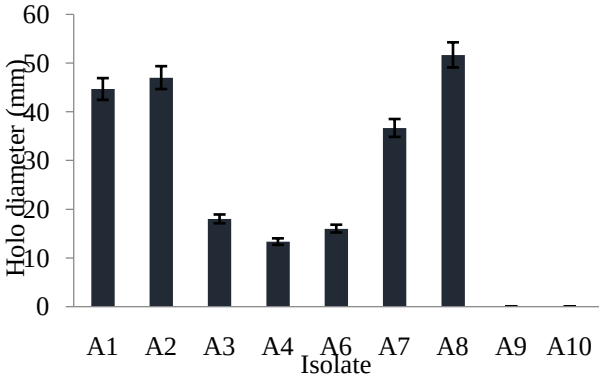


Figure 6. Casein degradation profile by actinomycete isolates

3.4.4. Demonstration of Cellulolytic Activity

Figure 7 shows the cellulase production profiles by the different actinomycete isolates after three tests; cellulase

production varies quantitatively depending on each isolate, there is a significant difference between the isolates. Cellulolytic activity was observed with all isolates: A3, A4, A6, A7, A8 and A10 with a diameter greater than 20 mm compared to 15 mm for A1, A2 and A9.

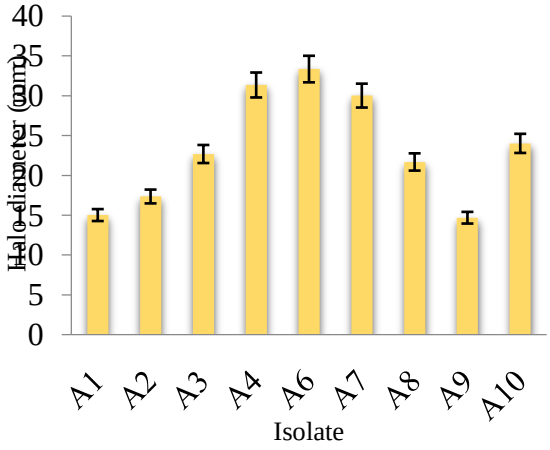


Figure 7. cellulase production profile by actinomycete isolates

3.4.5. Demonstration of Lipolytic Activity

Figure 8 shows the lipase production profiles by actinomycetes isolates after three tests, the degrading power of lipase varies depending on each isolate, there is a significant difference between isolates. Lipase production was observed at diameters greater than 30 mm for A6, A9 and A10 compared to 20 mm for A1, A2 and A8 and 15 mm for A3 and A4.

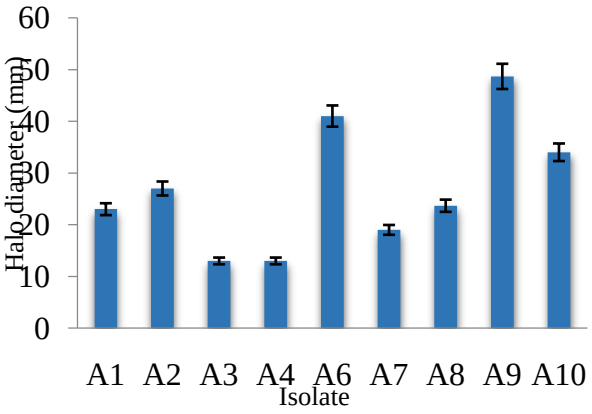


Figure 8. Lipase production profile by actinomycete isolates

4. Discussion

The objective of this work was to identify and evaluate the enzymatic production of actinomycetes from two (2) soil samples from the Lifoula landfill; sample 1 was collected from the center of the landfill and sample 2 was collected from the entrance of the landfill. This area is currently a public landfill for the city of Brazzaville (Congo). The results showed an abundant load of countable actinomycetes after dilution in our samples.

The actinomycete load in the two soil samples showed a slight difference in the levels of microorganisms in CFU/g with a predominance of sample 2: 73.3 x 104 CFU/g on the starch-casein medium against 56 x 104 CFU /g for EAC. This could be explained by the fact that sample 2 was taken at the entrance and contained less waste, but the sample was contaminated by several types of waste that can influence the pH and temperature of the environment. Our results are consistent with those obtained by Boucheffa (2011) [12], on the screening of actinomycete strains from contaminated soils of the Boulimat public landfill in Bejaia in Algeria, and those of Saci and Safane (2015) [13], on the isolation of actinomycete strains. of contaminated soils in Algeria. Actinomycete colonies are well observed on media containing starch and casein, which favors selective isolation.

However, the starch-casein medium supplemented with chloramphenicol and griseofulvin was more effective in isolating actinomycetes. This indicates that the antibiotic and antifungal added to the medium reduced the invasive bacterial and fungal load, thereby increasing the actinomycete load. These results are in agreement with those of Belferkh, 2016; Boudjella et al., 2007 [14,15]. From our observations, it appears that of the 20 isolates phenotypically characterized, only 9 isolates showed cultural characteristics of actinomycetes and the presence of mycelium. These observations were also made by other authors [16,17,18]. After microscopic observation, 9 isolates were selected based on their filamentous appearance and specific cocci of actinomycetes, in accordance with the conclusions of other studies focused on the isolation of antibiotic-producing actinomycetes [19,20].

The experiment focused on nine (09) isolates selected after microscopic observation. From this study, it appears that of the 9 isolates tested, all were able to degrade starch, which reflects the ability of our isolates to produce amylases, with isolate A1 (19 mm) from sample 1 being the best producer and isolate A9 (12 mm) from sample 2 being the least productive. Our results are therefore in agreement with the conclusions of other authors who carried out the same experiments [21,22]. In the case of protease production, 7 isolates showed the ability to degrade casein with inhibition diameters reaching 54 mm, the highest value attributed to isolate A8 from sample 2 and the lowest value was that of isolate A4 from sample 1 with 13.33 mm. This result indicates that our isolates have the ability to secrete proteases including caseinases; our results are close to previous works [23,24,25]. Regarding cellulase production, all 9 isolates showed translucent halos around the wells, indicating cellulase production by all selected isolates. Isolate A6 was the best producer with a diameter of 33.33 mm and isolate A9 was the best producer with a diameter of 14.66 mm. These results corroborate those of studies that have demonstrated the ability of actinomycete strains to synthesize cellulases [26]. The same observations were made on Tween 80 agar medium. In fact, the results showed the presence of halos around the 9 actinomycete isolates selected, thus expressing their ability to excrete lipases into the extracellular environment, with isolate A9 (41mm) from sample 2 being the best producer and isolates A3 and A4 (13mm) from sample 1 being the least productive; thus joining the results of previous studies that have worked on the capacity of actinomycetes to produce lipases [27]. It should be noted that out of the nine (9) isolates, 7 isolates including A1, A2, A3, A4, A6 and A7 significantly produced most of the enzymes tested and

showed more potential in extracellular secretion of enzymes. To increase the production of enzymes, it will be important to optimize the parameters of the environment that can influence the production of enzymes, such as determining the optimal pH and temperature, the concentration of NaCl, the best sources of nitrogen, carbon, mineral salts and ions.

5. Conclusion

This work focused on the characterization of actinomycetes isolated from the soils of the Lifoula landfill (Brazzaville). It appears after enumeration, isolation and purification that the phenotypic and biochemical characteristics of the isolates obtained confirm that they are actinomycetes. The evaluation of biological enzymatic activity showed significant production of four different enzymes: amylase, protease, lipase and cellulase with a predominance of lipases. These preliminary results show that the soil of the Lifoula public landfill is rich in actinomycetes that produce enzymes of industrial interest. A molecular identification of the different species of actinomycetes and a quantification of the different enzymes should be carried out.

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