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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/22625
DOI URL: http://dx.doi.org/10.21474/IJAR01/22625

RESEARCH ARTICLE

CHARACTERIZATION OF PRODUCING-BIOACTIVES COMPOUNDS BACTERIAL ISOLATES COLLECTED FROM SOIL AT BRAZZAVILLE

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Manuscript Info

Manuscript History
Received: 10 November 2025
Final Accepted: 12 December 2025
Published: January 2026

Key words:-
Hydrolytic enzymes, biosurfactants,bacterial genus, soils, Brazzaville.

Abstract

In the objective of understanding the microbial diversity of soil and the production of related bioactives compounds, an exploration of samples soil have been hold in Brazzaville, Republic of Congo. Four soil samples were collected in fours sites of Brazzaville has listed : at Faculty of Science of Marien NGOUABI University two sites 300m away (plantation soil and ordinary sol), at Massamba Debat stadium (landfill, soil) and at Bacongo district (garage soil). Temperature were ranged from 33° to 41°and the pH of soils from 4,7 to 7.Enumeration of microrganismes vary from a group to another and from a category of soil for each site as followed the most important were groups Staphylococci, Bacillus, enterobacteria, pseudomonas, streptomyces, yeast, The quantity of microorganisms varies according to group or genus. The total aerobic mesophilic flora is estimated at 3.4 x 10^6 CFU/g, actinomycetes at 1.8 x 10^5 CFU/g, and Bacillus at 1.3 x 10^5 CFU/g. Fungi are present at 1.4 x 10^4 CFU/g, while enterobacteria account for 9 x 10^3 CFU/g. Staphylococcus and Pseudomonas show values of 1.18 x 10^4 CFU/g and 2 x 10^2 CFU/g, respectively. According to the hydrolytic enzymes production, 34% of isolates are lipase-producing, 34% are cellulase isolate producers and 32% of the isolate produce amylolytic enzymes. All the Bacillus isolate are biosurfactants producers.

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Introduction:-

Soil results from the weathering of parent rocks due to chemical and biological forces[1]. It is also a medium for the transit, storage, and transport of many substances, regardless of their nature, organic or inorganic[2]. Soil is a living environment, serving as an interface between biomass, the atmosphere, and the hydrosphere.The structure of the soil can change significantly in a short time, especially under the influence of human activities. This characteristic is

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continually evolving, depending on soil texture, the quantity and quality of humus, acidity, soil lifespan, climate, and the abundance of certain minerals[3].Soils are an essential element of continental biotopes; they are ecosystems containing microorganisms that represent the majority of living organisms and constitute an important part of the planet's genetic diversity[4].Soil is a major reservoir of microbial diversity[5]. Among soil microorganisms are bacteria, fungi, and actinomycetes, which interact with each other. However, the most representative are bacteria[6,7, 8]). Microorganisms perform essential functions such as the biodegradation of organic matter, nutrient production for plants, nitrogen fixation, and pollutant degradation. Microorganisms play an important role both in soil formation and its functioning[9]. Bacteria play important roles in the nutrient cycle; many live in symbiotic association with plants, promoting their growth and increasing soil fertility[10].

Some bacteria, notably those of the genus Bacillus, are ubiquitous. They are found in diverse environments such as plants and soils, hydrothermal vents, extreme environments, and seawater [11]. Soil bacteria produce several bioactive substances of interest, including hydrolases, biosurfactants, antibiotics, and many others. In Brazzaville, Republic of Congo, several studies have been conducted on the microbial diversity of Brazzaville soils. Examples include[12,13,14]. All of these studies explored the microbial biodiversity of Brazzaville soils, revealing a diversity of genera and species of microorganisms. This diversity varies from one area to another in Brazzaville in terms of abundance and also of UFC, with a consistency that the different species produce bioactive substances of interest.In this study, soil samples were collected from different locations than in previous studies, with the difference that the soil type is specified. These locations included the landfill soil from the Massamba-Debat stadium, ordinary soil from the Faculty of Science and Technology, planting soil from the Faculty of Science and Technology, and garage soil in Bacongo. Microbiological and biochemical analyses were performed, and the production of bioactive compounds was tested

Materials and Methods:-

Collection Sites, Sampling, and Measurement of Physical Parameters:-

Soil samples were collected from four (4) sites, the GPS coordinates of which are recorded in Table I. At each sampling point, a small amount of soil was collected from a depth of 0.5 to 1 cm using a sterile spatula. Soil samples were collected from three points at each site. The soil samples from the three points at each site were mixed to create a composite. Physical parameters were measured using a multiparameter analyzer. The different samples were then transported to the laboratory for microbiological analysis.

Table I: Sites, Sample Collection Points, and GPS Coordinates

SITES	Type of soil	GPS coordinates of points		
		Point 1	Point 2	Point 3
FST1	Plantation sol	S :04,28672° E :015,25464° A :304m	S :04,28677° E :015,25479° A :316m	S :04,28659° E :015,25491° A :320m
FST2	Ordinary soil	S :04,28672° E :015,25461° A :329m	S :04,28782° E :015,25419° A :314m	S :04,28787° E :015,25404° A : 312m
Stade	Landfill soil	S :04,27206° E :015,24929° A :317m	S :04,27190° E :015,24958° A :318m	S :04,27178° E :015,24951° A :317m
Bacongo	Garage soil	S :04,28821° E :015,25475° A :307m	S :04,28824° E :015,25477° A :312m	S :04,28808° E :015,25472° A :312m

Identification of genera and enumeration:-

The NF EN ISO 6887-1, 2017 standard, which defines the general rules for the preparation of the stock suspension and decimal dilutions for microbiological testing, was used.The stock solution was prepared according to[12]. Briefly, 10 grams of each soil sample were weighed under sterile conditions using a balance and then placed in an Erlenmeyer flask containing 90 ml of physiological saline. The mixture was homogenized for two minutes to obtain a stock solution. The stock dilutions were prepared according to[12].. Five test tubes, each containing 9 ml of

previously sterilized physiological saline, were prepared. One ml of the stock suspension was taken and introduced into the first test tube. After mixing, one ml was withdrawn from this tube and placed in the next tube, and so on, up to the fifth tube. Decimal dilutions from 1/10 to 1/100,000 were successively performed. Surface inoculation was carried out, and 100 microliters of suspension from each of the five tubes were taken and spread into Petri dishes previously filled with different culture media specific to each genus to be identified and enumerated. Three Petri dishes were inoculated for each suspension. The Petri dishes were incubated at 37°C for 24 h according to[15,16,17].The plates were finally removed, the colonies counted, and the following relationship allowed us to evaluate, in CFU/g, the microorganisms belonging to the genera to be identified[15].

CFU/ml = N/Vd
N = number of colonies, V = inoculated volume (ml), d = dilution considered.

Isolation and phenotypic characterization of isolates:-
Isolated colonies observed in each of the plates for different culture media were collected and then subcultured in these media. Each colony was subcultured three times using the striations until homogeneous colonies were obtained. Colony selection was done randomly. After three successive subcultures, the pure isolates were placed in Eppendorf tubes containing a mixture of liquid TSB and glycerol and then kept at 4°C for pure culture[13,14]. Microscopic observation of live bacteria and determination of their morphology, grouping patterns, and motility were performed after preparing a microscopic slide from each purified colony[18,19], Gram staining was used to distinguish Gram-positive from Gram-negative bacteria[15]. The catalase test was performed according to[20,21].

Capacity of Isolates to Produce Hydrolase and Biosurfactant Substances:-
Use of the Cellulose Plate:-
The base medium supplemented with 0.5% cellulose was used to select bacterial strains with cellulolytic activity. Cellulolytic activity was assessed using cellulose-agar medium, the composition of which per 100 ml was 0.5 g of cellulose and 1.5 g of agar. This mixture was then autoclaved at 121°C for 15 min. After this, the medium was poured into sterile Petri dishes. After solidification, 6 mm diameter wells were made in the agar. 100 µL of each culture was then placed in each well. The plates were then incubated at 30°C for approximately 48 h. After the addition of Lugol's iodine, the absence of staining around the colonies indicated that the cellulose had been hydrolyzed. The size of the light yellow zone was used to assess the degree of cellulose hydrolysis and thus cellulase production[12,22]

Use of Starch Plate:-
The same technique was used, replacing cellulose with starch. After incubation, the starch agar medium was covered with Lugol's solution for a few minutes, followed by rinsing with distilled water. The presence of amylolytic activity is indicated by the appearance of a clear zone around the colonies [23,24]; .

Use ofTween80-Agar Plate:-
Lipase production was performed on Tween 80-Agar medium. The composition per 100 ml was: 1 ml of Tween 80 and 1.5 g of agar. The mixture was then placed in an autoclave at 121°C for 15 minutes. After incubation, the plates containing Tween 80-Agar were flooded with Lugol's iodine for a few minutes and then decolorized with NaCl. However, the absence of color change around the colonies indicated that the Tween 80 had been hydrolyzed. The formation of a light yellow zone around the colonies in blue medium indicated the hydrolysis of Tween 80 and confirmed lipase production[25].

Calculation of the Emulsification Index and Biosurfactant Production:-
In tubes containing 2 ml of a hydrocarbon solution, 2 ml of the supernatant from the culture of the different isolates were added. The tubes were vortexed at high speed for 5 minutes and then incubated at 25°C for 24 hours. The presence of an emulsified zone explained the existence or production of biosurfactants by the tested isolate[26]. The emulsification index (E24) was calculated as the percentage of the height of the emulsified layer (mm) divided by the total height of the liquid column (mm).
Emulsification indices were calculated using the formula below:

E24 = HE/HT × 100
With: E24: Emulsification activity after 24 hours; He: Height of the emulsion formed; Ht: Total height of the mixture[27]

Results and Discussion:-

Results:-

analyzed Physical parameters:-

The soil samples taken generally consist of sandy soil, rich in humus, dark brown in color tending towards black, with a predominantly acidic pH (varying between 4.7 and 7) and temperatures between 33°C and 41°C.

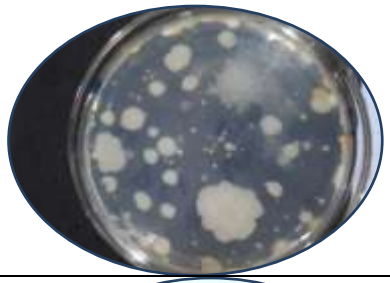
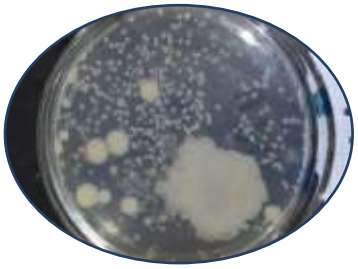
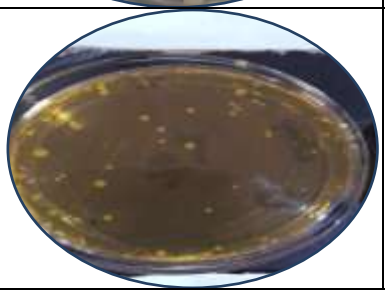
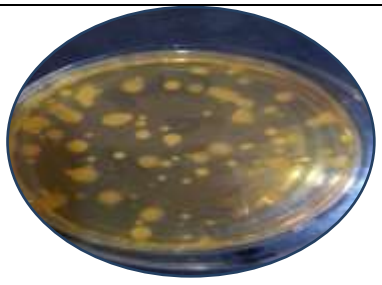
Tableau I:Physical paramerters of each site

Sites	Type of soil	Température et PH				Composite
		Point 1	Point 2	Point 3	moyenne	
FST	Planting soil	pH :7 T° : 36°C	pH :7 T° : 35°C	pH :6,9 T° : 35°C	pH :6,96±à,01 T :35,33±0,02	C1
	Ordinary soil	PH :7 T° : 33°C	PH :7 T° : 33°C	PH :7 T° : 34°C	pH : 7,00±0,00 T :33,33±0,02	C2
Stade	Landfill soil	PH :5,8 T° : 38°C	PH :4,9 T° : 37°C	PH :4,7 T° : 38°C	pH :5,13±0,01 T :37,66±0,02	C3
Bacongo	Garage soil	PH :7 T° : 38°C	PH :7 T° : 39°C	PH :7 T° : 41°C	pH : 7,00±0,00 T :39,33±0,02	C4

Identified and Quantified Microorganism Genera

Several groups, including FMATs, actinomycetes, yeasts, molds, and four bacterial genera, were identified in the soil samples. The results are presented in Table VI.

Table III : Different Groups of Microorganisms and Bacterial Genera present in the Soils

Genre mis en evidence	Representation sur la boite	
Total aerobic mesophilic flora		
Staphylococci		

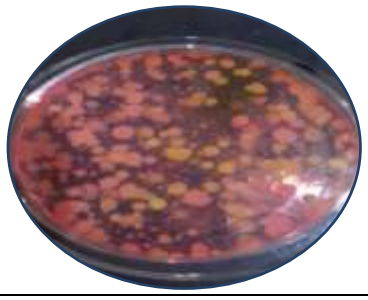
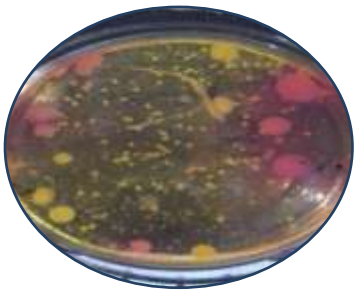
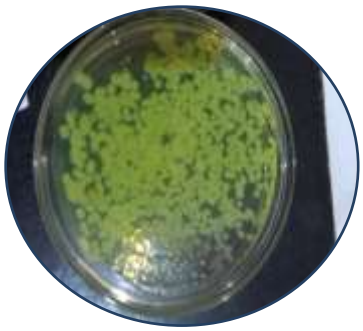
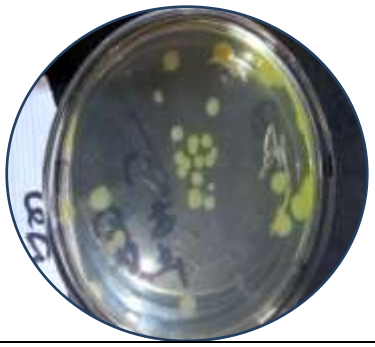
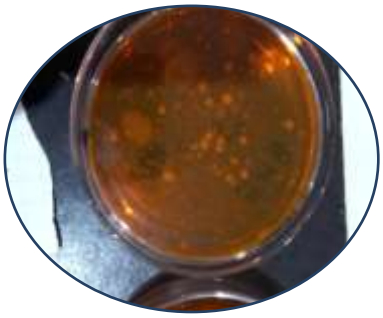
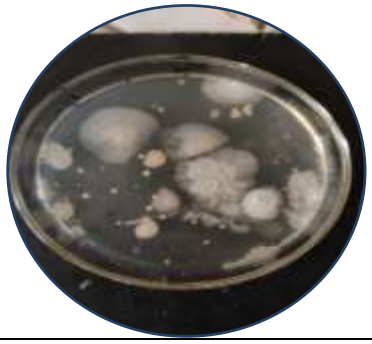
Bacillus		
Pseudomonas		
Enterobacteriaceae		
Actinomycetes		
Mold and yeast		

Figure 2 shows the distribution of colony-forming units (CFU) per gram in the landfill soil composite. This distribution varies according to the bacterial group or genus. The total aerobic mesophilic flora is estimated at 1 x 10^6 CFU/g. This is the most abundant group. Actinomycetes are present at 1 x 10^5 CFU/g, Bacillus and Fungi at 1 x 9 x 10^5 CFU/g each, while Enterobacteriaceae are present at 1 x 7 x 10^5 CFU/g. Staphylococcus and Pseudomonas show levels of 9 x 4 x 10^4 CFU/g and 3 x 4 x 10^4 CFU/g, respectively.

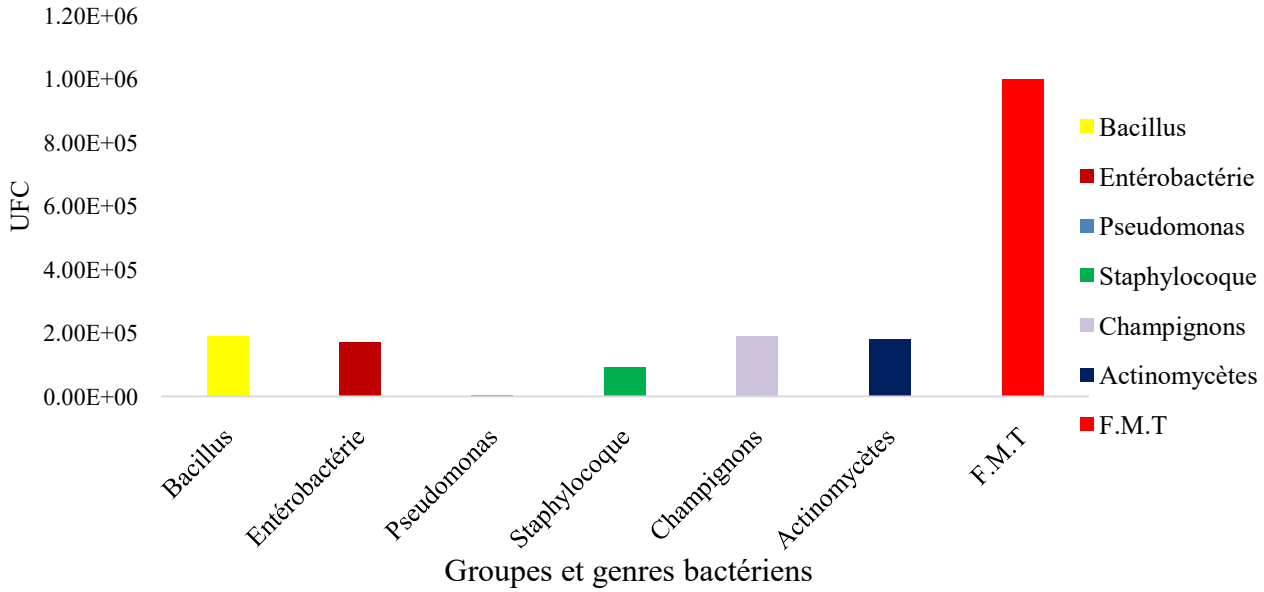


Figure 2: Enumeration of microorganisms in landfill soil

La figure 3 illustre le denombrement en UFC/gdes microorganismes de l'echantillon de sol Ordinaire en fonction des genres et groupes . Il est constate une flore mesophileaerobie totale de 1,6.10^6 UFC/g ; des Actinomycètes avec en UFC/g de 1,8.10^5 ; les Bacillus presentent une en UFC/g de 1,4.10^5 ; il est note en UFC/g de 5,4.10^4 pour les Champignons et 1,1.10^4 des Enterobacteries ; les Staphylococcus et les Pseudomonas ont des UFC/g respectives de 5,4.10^3
et 2.10^2.

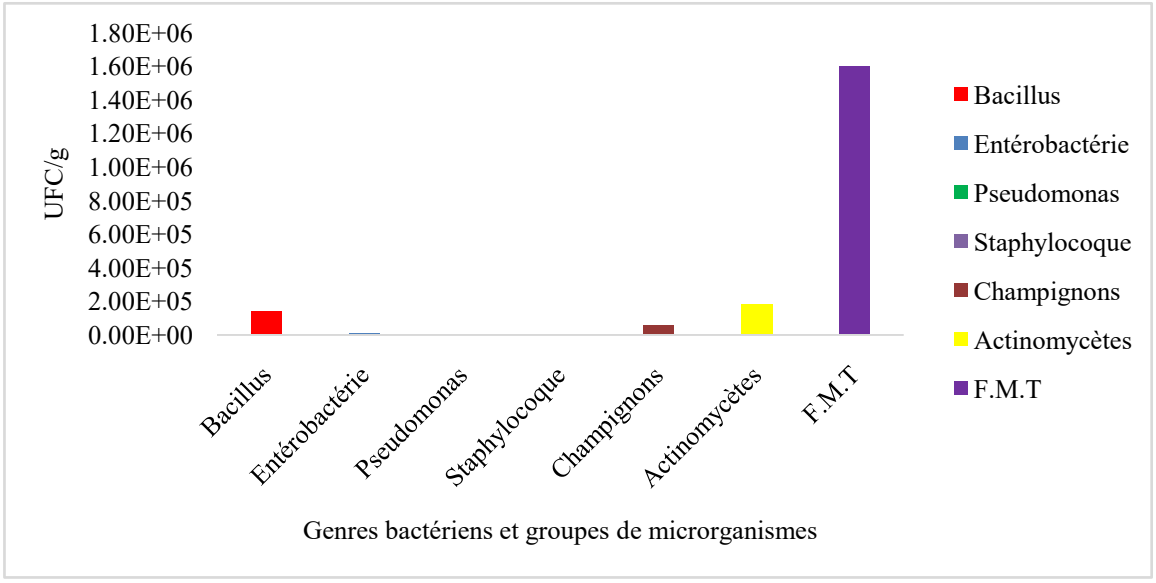


Figure 3:denombrement des microorganismes en UFC de sol Ordinaire

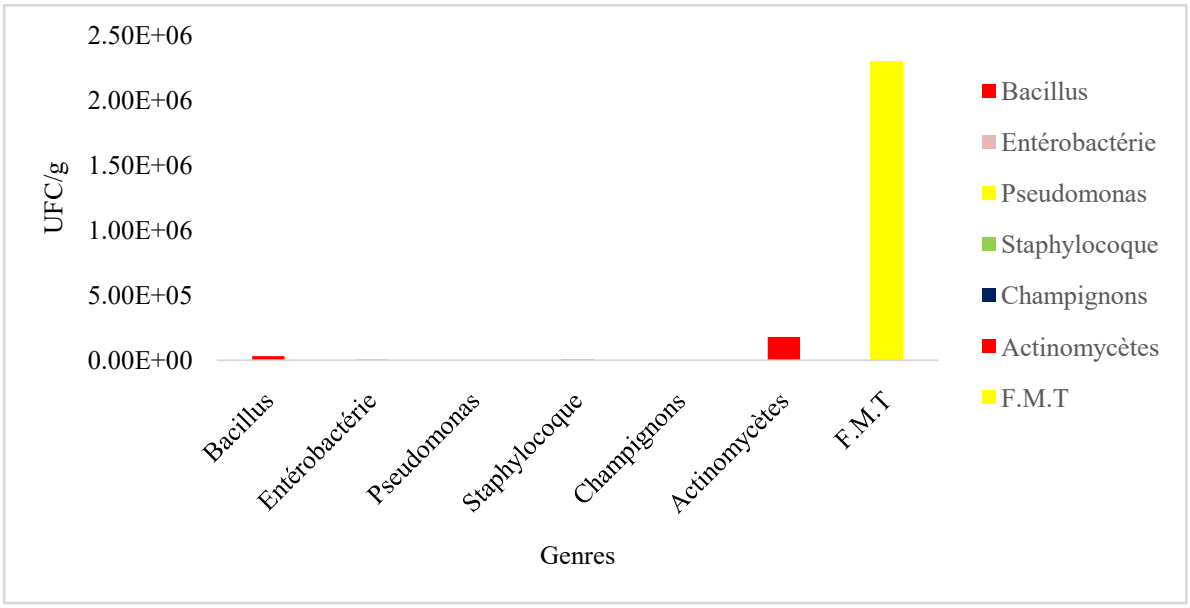


Figure 4:denombrement en UFC des microorganismes de sol de plantation

Figure 4 shows the quantification of microorganisms in the planting soil sample, expressed in colony-forming units (CFU) per gram. The total aerobic mesophilic flora is estimated at 2.3 x 10^6 CFU/g, with actinomycetes dominating at 1.8 x 10^5 CFU/g, followed by Bacillus at 3.3 x 10^4 CFU/g. Fungi account for 4 x 10^3 CFU/g, while enterobacteria represent 1.4 x 10^4 CFU/g. Staphylococcus represents 8.7 x 10^3 CFU/g.

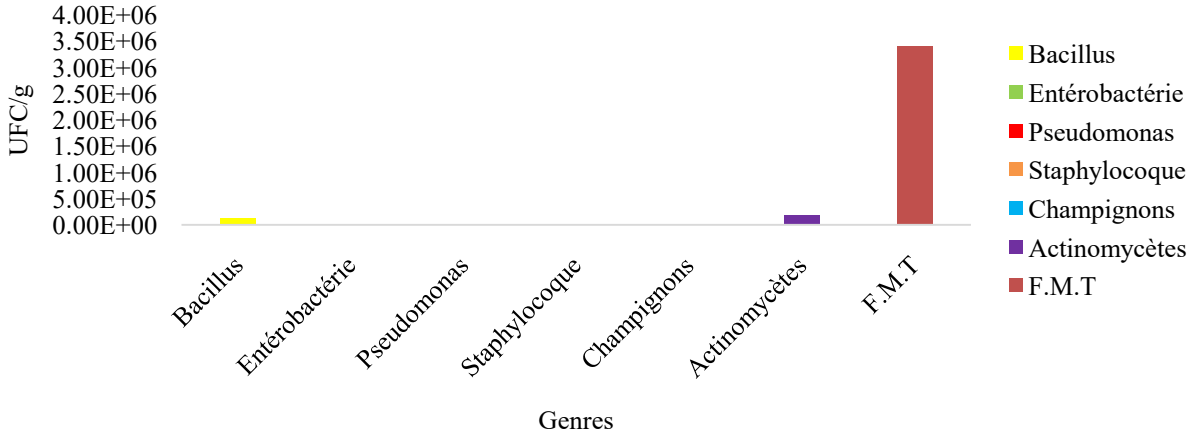


Figure 5: denombrement en UFC des microorganismes de sol de garage en fonction des genres et groupes

Figure 5 illustrates the quantity of microorganisms in the garage soil sample, expressed in CFU per gram. The quantity of microorganisms varies according to group or genus. The total aerobic mesophilic flora is estimated at 3.4 x 10^6 CFU/g, actinomycetes at 1.8 x 10^5 CFU/g, and Bacillus at 1.3 x 10^5 CFU/g. Fungi are present at 1.4 x 10^4 CFU/g, while enterobacteria account for 9 x 10^3 CFU/g. Staphylococcus and Pseudomonas show values of 1.18 x 10^4 CFU/g and 2 x 10^2 CFU/g, respectively.

Morphotypes of bacterial isolates observed in soil samples:-

A total of 59 isolates were obtained, including 19 isolates of the genus Bacillus; 8 isolates of the genus Pseudomonas; 14 isolates of the genus Staphylococcus; and 18 isolates of the genus Enterobacteriaceae. The distribution according to each soil category was as follows: 12 isolates from plantation soils; 17 isolates from

landfill soils; 17 isolates from garage soils; and 13 isolates from ordinary soils. All were catalase-positive and some were motile and others non-motile. These cells were predominantly rod-shaped. The colors and appearances were variable. Figures 6 and 7 represent the colors and appearances of the colonies in plantation soil (Figure 6) and landfill soil (Figure 7).

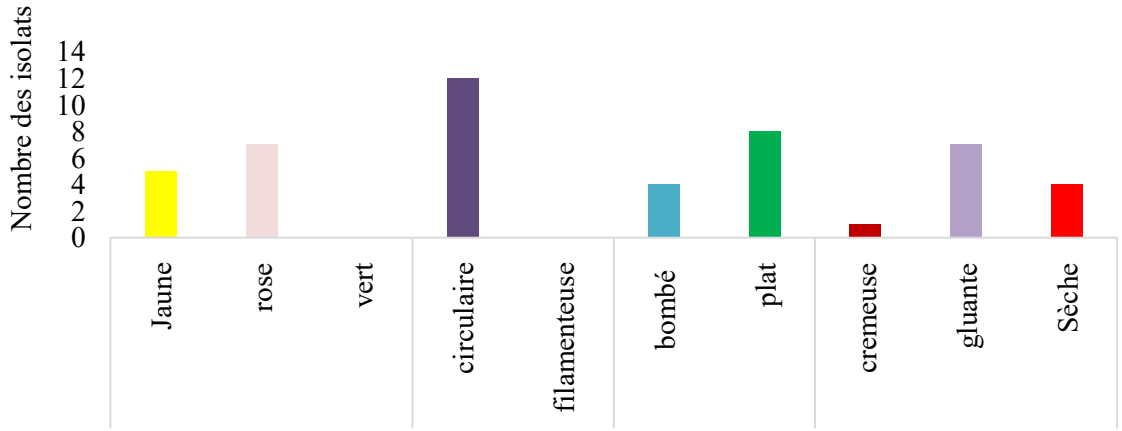


Figure 6: Caracteristiques phenotypiques des isolats bacteriens des sols de plantation

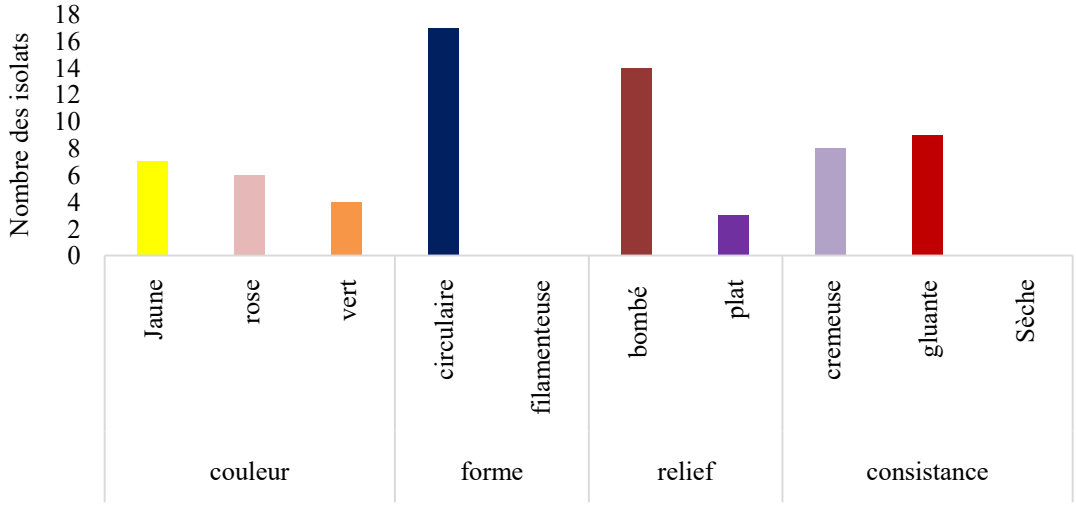


Figure 7:Caracteristiques phenotypiques des isolats bacteriesdes sols de decharge

Hydrolases produced by bacterial isolates:-

Cellulase-producing isolates:-

Figure 8 shows two cellulose plates, C1 and C2, where zones of cellulose degradation (halos) can be observed around the wells by the supernatants of the different culture isolates.

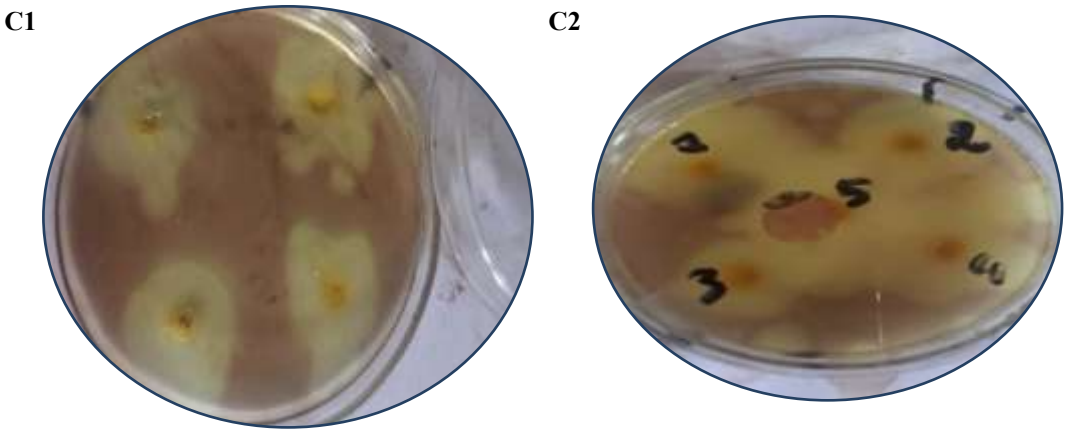


Figure 8: Halos showing cellulose degradation

Figure 9 shows the cellulase production profiles of different isolates; cellulase production varies numerically depending on each isolate. The highest-producing isolates are: MO3, MO5, MG2, and MO4. The lowest-producing isolates are: MD1, MD2, and MD3.

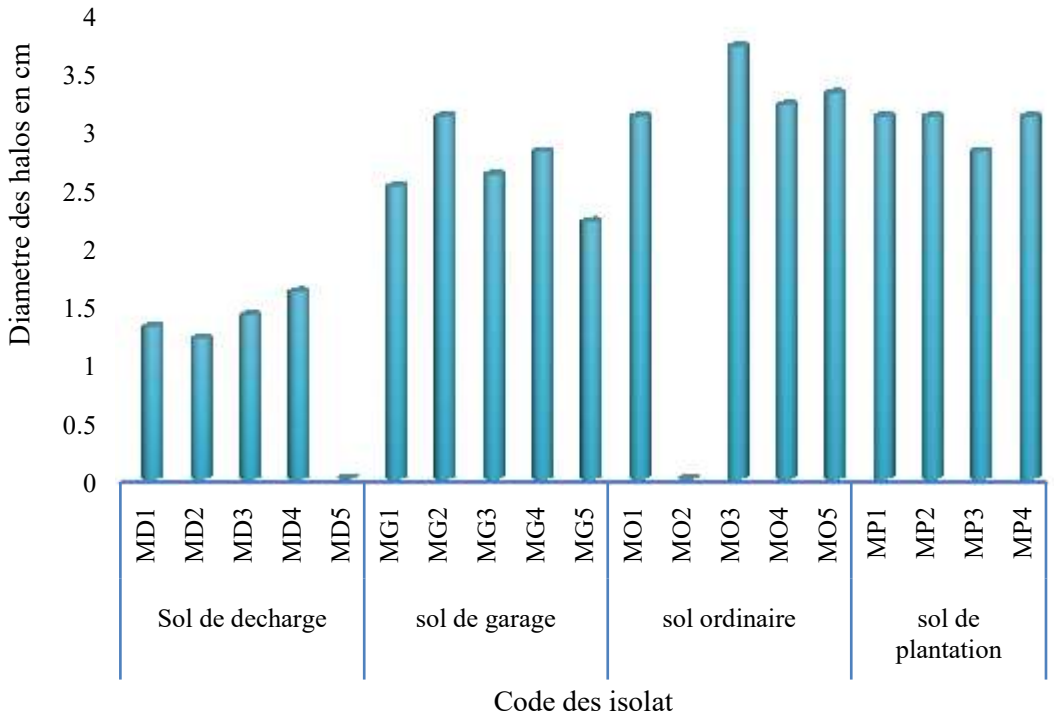


Figure 9 : Profil de production de cellulase par les isolats de Bacillus

Amylase-producing isolates:-

Figure 10 shows, in two starch plates, halos indicating the digestion of starch by the isolates used. These halos are around the wells that received the supernatant of the different isolates.

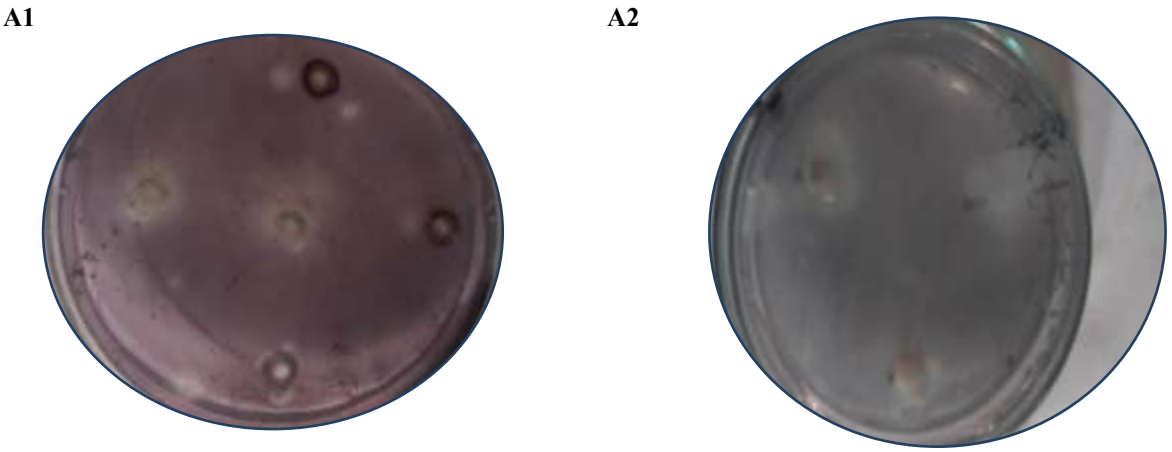


Figure 10: Halos montrant la degradation de l'amidon par les isolats des Bacillus

Figure 11 shows the profiles of amylolytic enzyme production by the studied isolates; the profiles differ from one isolate to another. Isolates MP1, MP2, MG5, MP3, MO3, MD4, and MP4 produce a significant amount of amylase, while isolates MD1, MD2, and MO4 produce quantitatively less amylase.

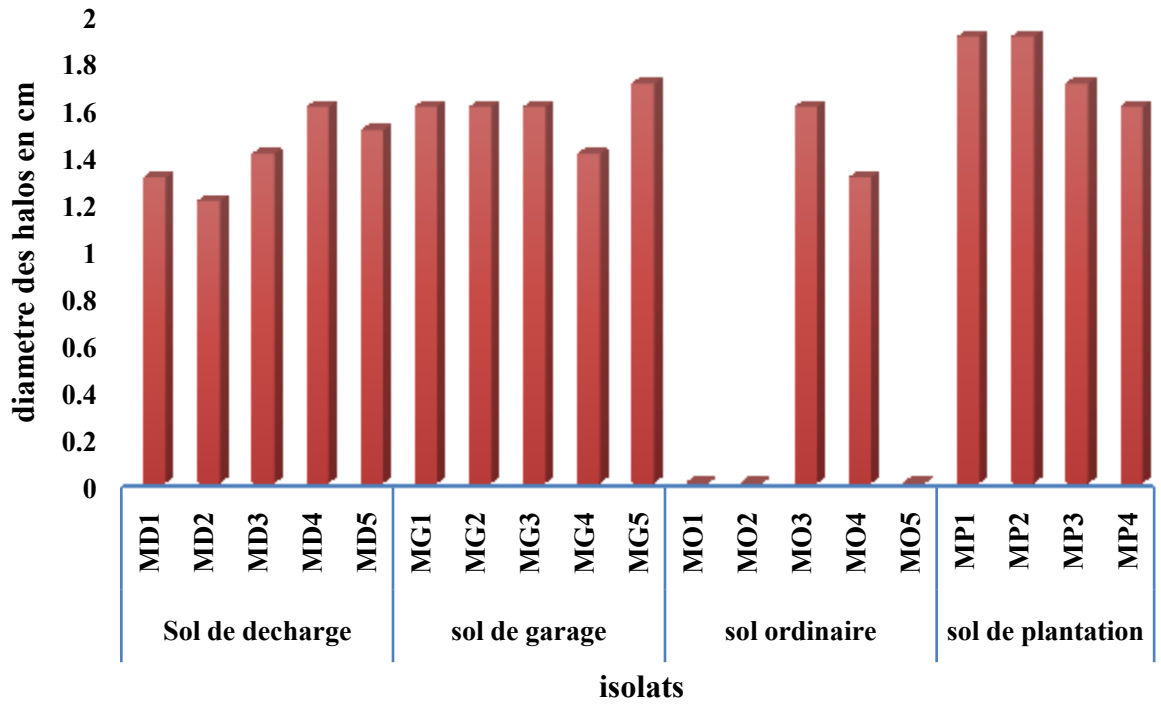


Figure 11: Profil de production de l'amylase par les isolats de Bacillus

Lipase-producing isolates:-

Figure 12 shows in two tween-80 plates the degradation of lipid through halos. The isolates in question produce lipases.

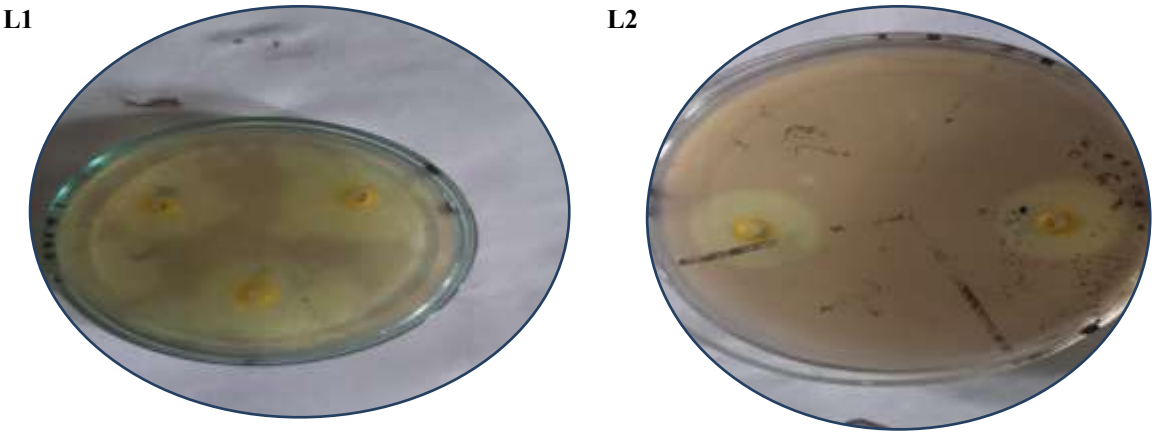


Figure 12: Halos montrant la production des lipases par les isolats des Bacillus

Figure 13 shows the production profile of lipases by Bacillus isolates; the lipase degradation ability varies depending on each isolate. The highest producers are isolates M03, MP1, MP3, MP4, MD3, and MG2. The lowest producers are MO1, MD2, and MD4.

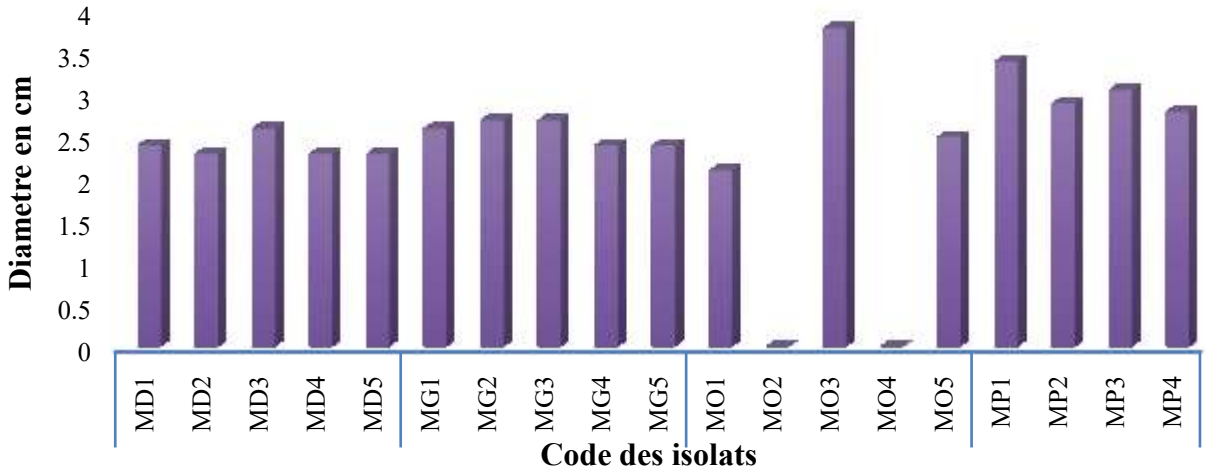


Figure13: Profil de production de lipase par les isolats de Bacillus

Figure 14 shows the distribution of the percentage of enzymatic productions according to the 4 categories of soil. It is observed that the percentage of amylase production is much higher in landfill soils compared to garage, ordinary, and plantation soils. As for the percentage of cellulase production, it is noted that ordinary soils contain more cellulase compared to the others. Finally, it is observed that the percentage of lipases in landfill soils is higher than in garage, ordinary, and plantation soils.

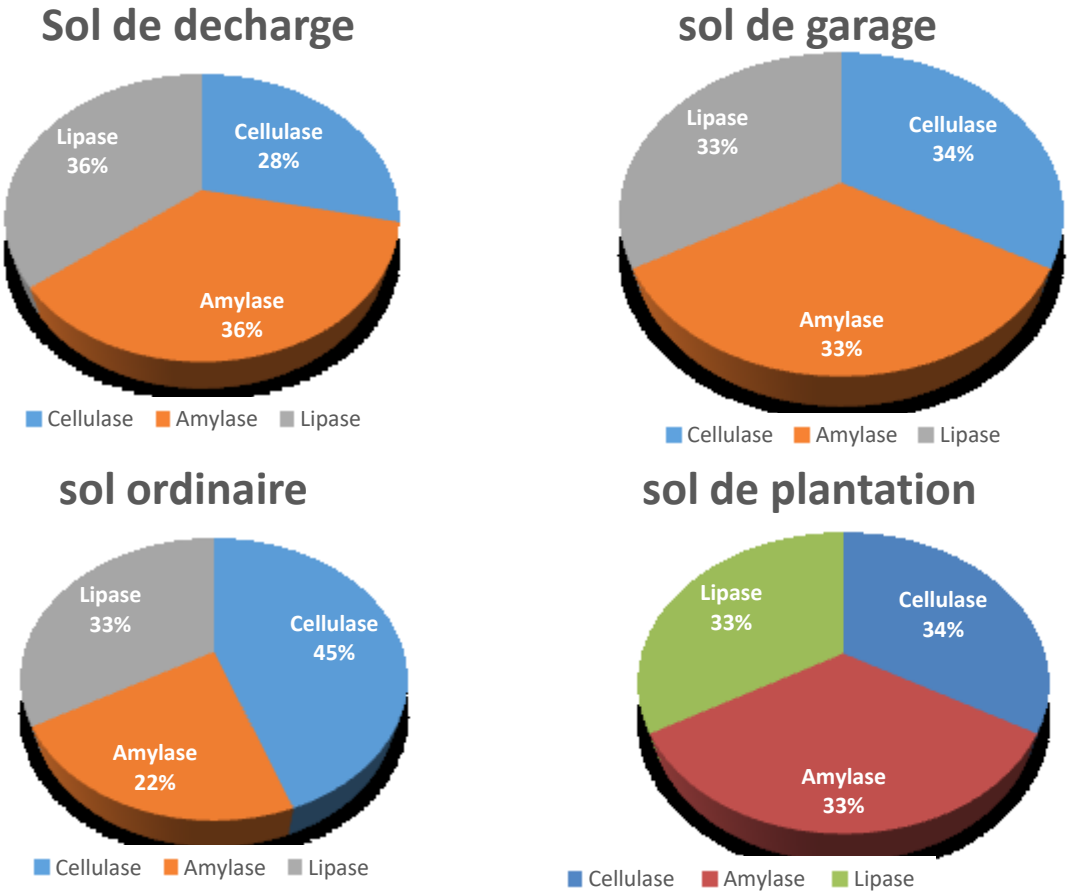
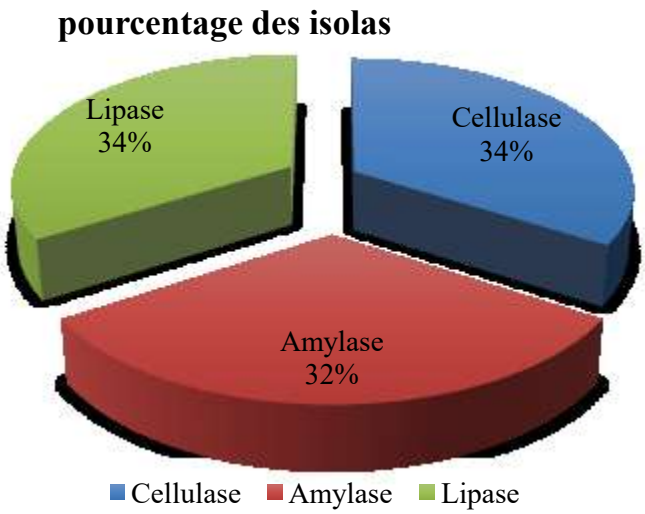


Figure14: Repartition de la production enzymatique en fonction des sols

Figure 15 shows the distribution of isolates based on enzymatic activities. An equal percentage of isolates was observed for cellulolytic and lipolytic production, while a considerable percentage was noted for amylases.



Biosurfactant-Producing Isolates:-

Figure 17 illustrates the biosurfactant production profile of the nineteen (19) Bacillus isolates, expressed as a percentage. All nineteen isolates showed significant emulsification indices, indicating biosurfactant production. Indeed, the highest emulsification percentages were observed in isolates MG5, MP4, MD2, and MD5, while low production was noted for isolates MD1, MD4, MG3, MO1, and MO5.

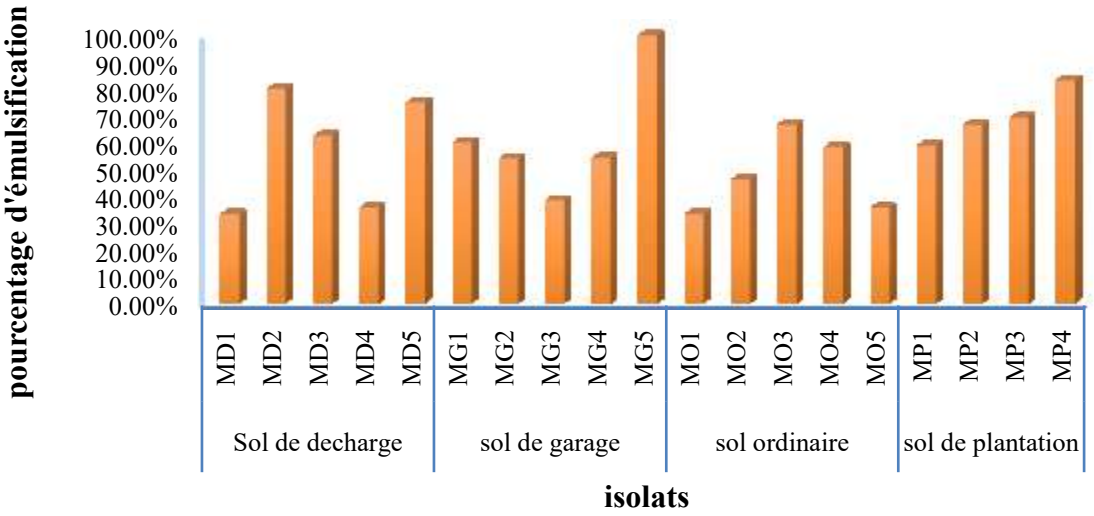


Figure 1: Profils de Production des biosurfactants par les isolats des Bacillus des differentes categories des sols

Discussion:-

The objective of this work was to characterize the microbial isolates obtained from the soils of Brazzaville. To this end, four sites representing four categories of soils were selected and targeted. Two physical parameters were analyzed: temperature and pH. For all soil samples collected, these soils had a sandy texture, were rich in humus, had a dark brown color tending towards black, with a mostly acidic pH approaching neutrality (ranging from 4.7 to 7) and temperatures between 33°C and 41°C. Two previous studies were conducted in Brazzaville by[12,13,28] The sampling in these studies did not take place in the same locations in Brazzaville as in this study. Both studies yielded similar results in terms of pH and temperature. This suggests that soils in Brazzaville can be acidic, tending towards neutrality in terms of pH.

Four bacterial genera, namely Pseudomonas, Enterobacteriaceae, Bacillus, Staphylococcus, and other different groups such as FMAT, actinomycetes, yeasts, and molds, were identified in samples from the four categories of soils collected. In terms of counting, FMAT were the most representative in the four soil categories; however, the FMAT rate showed significant variations between the four samples studied. The presence of all these groups and genera of microorganisms has already been reported by[12,13,14]. The other groups also showed variations in CFU/g across different sites, thus in different categories of collected soils.The results also revealed the presence of bacteria of the genus Bacillus from Mossel medium, with two main color trends: yellow and pink. In terms of shape, the colonies were mainly circular, with predominantly dry, creamy, and sticky appearances. Most of the cells were Gram-positive, catalase-positive rods.

These results are very close to those found by[13,14],. The experimentation to demonstrate enzymatic activity involved nineteen Bacillus isolates, all of which were capable of producing three types of hydrolytic enzymes: cellulases, amylases, and proteases. The production of these enzymes varied from one isolate to another, showing a distinct specificity for each isolate, regardless of the origin of the isolate. These results are similar to those reported by[12].
The results obtained demonstrated that the nineteen isolates are capable of producing biosurfactants. Strong emulsification was observed in isolates MG5, MD2, MD5, and MP4, indicating high biosurfactant production, while isolates MD3, MG1, M03, M04, MP2, and MP3 showed weaker emulsification. The third category of isolates includes all the others whose emulsification, based on their index, is low [29]Nguimbi-Tsati et al. (2023) found that the presence of an emulsifier with neutral pH in the mixture of gasoline and bacterial culture suggests that the isolates

secrete surface-active substances capable of degrading hydrocarbons used as nutrients, which could contribute to bioremediation. These results are very similar to those of the aforementioned authors. Several Bacillus species producing biosurfactants are in agreement with[29].which also showed that bacteria can be classified into three categories based on their emulsification index, that is, according to their production of biosurfactants: the most efficient, the medium, and the weak.

Conclusion:-

The soils of Brazzaville, with a pH ranging from 4 to 7, include acidic and neutral soils. Several types of microorganisms belonging to various groups and multiple bacterial genera are found there. These bacteria are capable of producing several types of hydrolytic enzymes as well as other substances of interest, such as biosurfactants.

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