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Characterization of Isolated Bacteria from Soils in the Likouala Peat Bog Area (Republic of Congo)

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Abstract The aims of this study were to characterized the bacteria isolated from the soils of Likouala peat bog area in Congo-Brazzaville. Three (03) composite samples were made from a total of 9 soil samples collected. 1g of soil from each composite sample was cultured on PCA, Mossel and EMB media for counting the total flora. Isolation on Mossel medium and identification of isolates was based on cultural, morphological and biochemical characters by conventional techniques. The method of Petri dishes containing the substrate relating to the search for proteolytic and amylolytic activities was used. The results showed total bacterial loads of $9.50x10^4$; $7.31x10^4$ and $6.39x10^4$ CFU/g on PCA medium; $5.81x10^4$; $5.64x10^4$ and $8.56x10^3$ CFU/g on Mossel medium versus $2.77x10^2$ CFU/g; $5.1x10^2$ and $1.16x10^2$ CFU/g on EMB medium respectively for samples 1, 2 and 3. The respective total anaerobic mesophilic flora of $3.08x10^4$; $2.93x10^4$ and $3.03x10^3$ CFU/g on PCA medium; $2.48x10^3$; $4.83x10^3$ and $3.32x10^3$ CFU/g on Mossel medium for samples 1, 2 and 3. The optical densities of the aerobic isolates were between 0.634 and 1 and the enzyme production showed diameters of translucent halos between 1 to 3.2 cm for the proteolytic enzymes and between 1.2 to 2.7 cm for the amylolytic enzymes. The evolution of enzyme production is a function of time and of the isolate. The surface part of the soils of the Likouala peat bog area is rich in aerobic and anaerobic bacteria.

Keywords: *bacteria, peatland, amylase, soil, protease*

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1. Introduction

Peat bogs are unique natural wet ecosystems accumulating an organic material called peat. They cover 3% of the earth's surface, storing a third of soil carbon, the transformation of which could, through positive feedback, aggravate the extent of climate change [1]. Their functioning is likely to be impacted by the effects of change affecting the ecosystem and the environment. The peatification process results from an imbalance between the primary production of organic matter by plants via photosynthesis, its decomposition and its mineralization by microorganisms [2]. In Congo Brazzaville, they are present in the departments of Likouala, Sangha, Cuvette and part of the Plateaux. The Congo Basin peat bog has an average thickness of 3 m, and concentrates 30 giga tonnes of CO_2, or about the equivalent of at least 15 years of CO_2 emissions from the United States. Congo's moist peat forests store almost 30% of all soil organic carbon found in tropical peatlands (about 30 billion tonnes of carbon) and, 5% of that estimated in peatlands worldwide [1,3]. The department of Likouala is made up of continental and artificial wetlands, an immense expanse of swampy forests with floodplain savannahs, floating meadows along the rivers as well as wooded peat bogs. Several studies carried out on the role of peat bogs in the carbon cycle in the face of global warming show that a rise in temperature leads to a significant increase in microbial activity through intense decomposition of organic matter and therefore a significant release of CO_2 by respiration of microorganisms. It also leads to significant carbon fixation by photosynthesis [4]. Microflora studies have revealed the presence of large quantities of molds in the peat with concentrations of molds close to 107 per gram of dry peat and up to 23 species of yeasts belonging to the genera Trichosporon, Candida, Rhodotorula and others [5] and other species of mycobacteria, pathogens such as M. fortuitum [6]. Although there is growing interest in the study of microbial diversity in peatlands, the processes maintaining this diversity and its role in the functioning and stability of

peatlands remain to date very little explored. This deficit is even greater if we consider the interaction between the effect of microbial activities and that of climate change [7]. Indeed, despite current knowledge on carbon storage, there are still considerable deficits in the microbiology of these ecosystems. It is with this in mind that we have proposed in this work to characterize the bacteria of the Likouala peat bog area.

2. Materials and Methods

2.1. Sampling

9 soil samples were taken by coring in the Likouala peat bog area in the localities of: Bondoki, Bondzale, Center, Ekolongouma, Ekondzo, Itanga, Makodi, Mbala and Moungouma [8]. The samples were transferred in sterile plastic bags in a cooler, then transported to the Cellular and Molecular Biology Laboratory of the Faculty of Science and Technology (FST) and stored at 4°C. Of the total of 9 samples, three composite samples were made up for bacteriological analysis.

2.2. Enumeration and Isolation

2.2.1. Dilution and Culture Media

1g of soil from each composite sample was taken using a precision balance in a first test tube containing 9 ml of sterile physiological water. This tube represents the stock solution of the sample from which decimal dilutions were made with PCA, Mossel and EMB media [8,9].

2.2.2. Sowing and Cultivation

Two techniques were used: on the surface of 100µl of each dilution were carried out and deposited in the centers of the Petri dishes containing previously poured and solidified media, then the dishes were incubated in an oven for 24 hours at 37°C, in function of the culture time in the oven [10,11]. And en masse, 1 ml of the solution-dilution in the Petri dishes, the incubated dishes so returned to the oven for 24 hours at 37°C at the rate of one dish per dilution.

2.2.3. Colony counting and purification

The technique consists of measuring the number of bacteria by counting only the viable and cultured cells in a culture medium was used. A significant box contains a number of colonies between (15-150) colonies for specific media and between (30-300) for ordinary media. Petri dishes showing colonies in the form of clusters were eliminated [12]. The number of colonies is given after counting by the following relationship allowing the CFU (Colony Forming Unit) to be evaluated [11,13].

$$\text{Concentration of bacteria} = \frac{\text{colonie Number}}{\text{dilution Factor x Volume ensemence}}$$

Colonies were purified on Mossel. A colony is picked with a loop or Pasteur pipette using the striation technique to obtain separate colonies. The dishes are incubated in an oven at 37°C. and then observed after 24 hours. Colonies with an identical appearance are considered pure and stored in sterile eppendorfs containing 900 µl of liquid TBS and 100 µl of glycerol, then kept cool at 4°C [11].

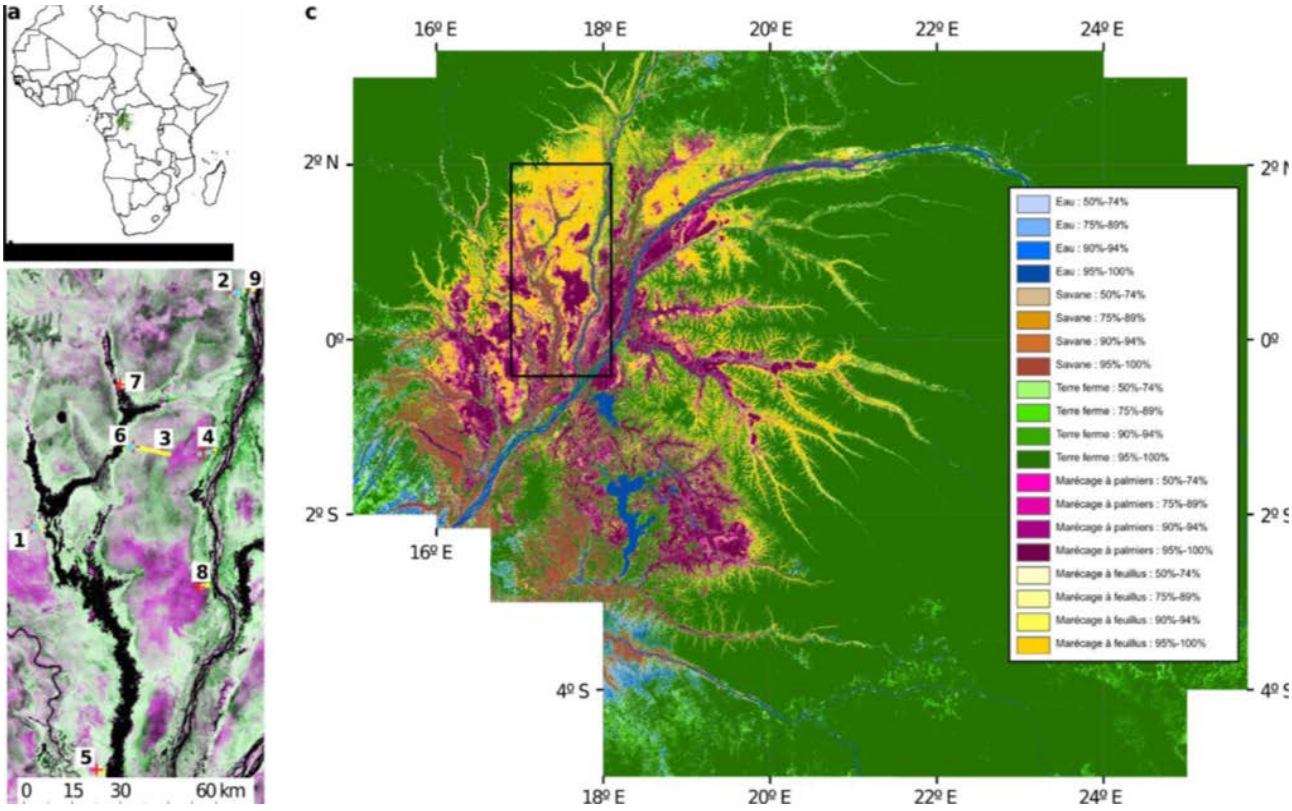

Figure 1. geographical location of the samples

Figure 2. samples of soil composites from the bog

2.3. Phenotypic Characterization of Isolates

2.3.1. Macroscopic Characterization

The macroscopic observation carried out after the isolation of the strains focused on the description of the colonies, as reported [14], in particular: the shape, the appearance, the consistency, the color.

2.3.2. Microscopic Characterization

Microscopic observation makes it possible to carry out a morphological study of the cells of a bacterial species. The microscopic study focuses in particular on the shape, mobility and arrangement of the cells. It is used to examine fresh colony samples using a 40 objective optical microscope (GX40) [15].

2.3.3. Catalase Test

Catalase is an oxidoreductase that catalyzes the dismutation of hydrogen peroxide into water and oxygen. The test consists of depositing a drop of hydrogen peroxide diluted to one tenth on the glass slide and adding, using a Pasteur pipette, an isolated colony developed on the agar. If there is effervescence or the presence of O2 bubbles, the activity of the enzyme is catalase positive (Catalase+), the opposite indicates that the bacterium is catalase negative (catalase-) [16].

2.3.4. Gram Stain

Gram staining is used to distinguish and classify bacteria into two large groups: Gram (+) are alcohol-resistant and Gram (-) are alcohol-sensitive and also have an outer membrane which takes on the color of safranin after washing. by alcohol).

2.4. Enzymatic Production of Isolates

In order to determine the involvement of isolates in the formation of peat soils, certain enzymes are sought.

2.4.1. Isolates, Culture Conditions and Optical Density

The preserved isolate is subcultured on Mossel, incubated at 37°C then the colony is inoculated in the Erlenmeyer flask containing 20 ml of liquid TSB medium then incubated at 37°C to carry out an overnight culture. Then, 4ml are taken, including 3ml to measure the optical density (O.D) which is an expression of bacterial growth using a spectrophometer calibrated at 600nm and 1ml to assess the enzymatic activity.

2.4.2. Demonstration of Proteolytic Enzyme Production

1 g of agarose powder is mixed with 1 ml of PBS in 100 ml of distilled water concentrated to 1N. The whole is brought to a boil until the agarose has completely dissolved, left to cool to 55-60°C. Then, 10 ml of skimmed milk were added, homogenize the mixture and pour into Petri dishes; after solidification, the wells are made in the gel and 100 µl of the bacterial suspension are placed in each well, followed by incubation of the Petri dish in an oven at 37°C for 12 hours. The observation of a clear translucent zone (halo) indicates that the isolate produces a proteolytic enzyme and the diameter of each translucent halo is measured [11,17,18].

2.4.3. Demonstration of the Production of Amylolytic Enzyme

1g of starch and 1.5g of agar are dissolved in 100ml of distilled water and sterilized in an autoclave at 121°C for 15 minutes; then the Petri dishes are poured and the wells are made in the gel then 100 µl of the bacterial suspension after overnight culture are placed in each well and incubated at 37°C. for 48 hours. The revelation is made with the lugol; observation of a translucent zone indicates that the strain produces an amylase enzyme and the diameter of each translucent halo is measured [11,19].

2.5. Results Analysis

The results were represented, illustrated and statistically analyzed using Microsoft Excel 2016 software.

3. Results and Discussion

3.1. Surface Seeding

Figure 3 illustrates colonies observed on PCA medium. We note the presence of different shapes colonies: whitish in color, circular, irregular and filamentous.

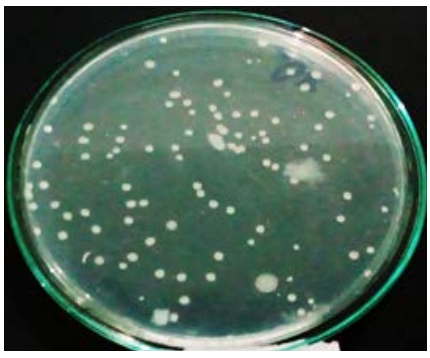

Figure 3. Colonies isolated on PCA medium after surface inoculation

Figure 4 shows the isolated colonies on Mossel : there are three distinct and visible types of colonies; yellow, pink and orange in color, circular, irregular and rhizoid in shape.

Figure 4. Colonies isolated on Mossel medium after seeding in surf

Figure 5 represents the colonies isolated on the EMB medium; two types of colonies are isolated and visible. The presence of colonies of pink and black color of circular shape.

Figure 5. Colonies isolated on EMB medium after surface seeding

Table 1 below represents the bacterial loads expressed in CFU of the total aerobic mesophilic flora of each sample on the PCA, Mossel and EMB media. On the PCA agar, the total flora of sample 1 is $9.50x10^4$ CFU/g; for sample 2 the total flora is $7.31x10^4$ CFU/g and the sample is $6.39x10^4$ CFU/g. On Mossel, sample 1 has a flora of $5.81x10^4$ CFU/g, sample 2 has a flora of $5.64x10^4$ CFU/g and the flora of sample 3 is $8.56x10^3$ CFU/g. Finally, on the EMB medium, sample 2 has a total flora of $2.77x10^2$ CFU/g and the flora of sample 3 is $1.16x10^2$ CFU/g.

Table 1. CFU/g per sample after surface inoculation

Samples / Culture media	Sample 1 CFU/g	Sample 2 CFU/g	Sample 3 CFU/g
PCA	$9.50 \pm 1.7x10^4$	$7.31 \pm 1.09x10^4$	$6.39 \pm 2.86x10^4$
Mossel	$5.81 \pm 1.08x10^4$	$5.64 \pm 1.94x10^4$	$8.56 \pm 1.19x10^3$
EMB	$5.10 \pm 1.52x10^3$	$2.77 \pm 1.06x10^2$	$1.16 \pm 0.45x10^2$

3.2. Mass Seeding

Figure 6 illustrates the colonies isolated on PCA after mass seeding. A few whitish colonies of circular and filamentous shape were obtained after mass inoculation and incubation at 37°C.

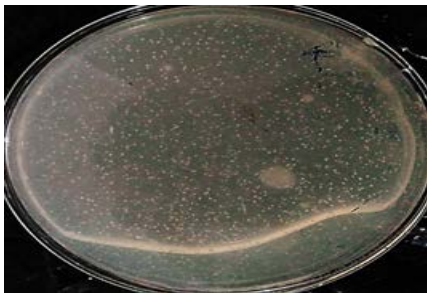

Figure 6. Colonies isolated on PCA medium after mass inoculation

Figure 7 shows the isolated colonies on Mossel after mass seeding. Note the presence of colonies of yellow and pink color, circular and irregular in shape.

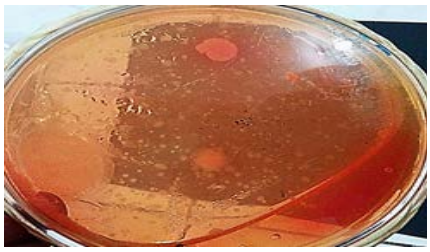

Figure 7. Colonies isolated on Mossel medium after mass seeding

Table 2 presents the results of the count expressed in CFU of the total anaerobic mesophilic flora of each sample obtained on the PCA and Mossel media. The rates of total flora depend on the sample. On the PCA, sample 1 has a loading rate of $3.08x10^4$ CFU/g, for sample 2 it is $2.93x10^4$ CFU/g and sample 3 has a loading rate of $3.03x10^3$ CFU/g. On Mossel, sample 1 has a loading rate of $2.48x10^3$ CFU/g, sample 2 has a load of $4.83x10^3$ CFU/g and sample 3 the load is $3.32x10^3$ CFU/g.

Table 2. CFU/g per sample after mass inoculation

Samples / Culture media	Sample 1 CFU/g	Sample 2 CFU/g	Sample 3 CFU/g
PCA	$3.08 \pm 1.2x10^4$	$2.93 \pm 1.06x10^4$	$3.03 \pm 2.06x10^3$
Mossel	$2.48 \pm 1.7x10^3$	$4.83 \pm 2.7x10^3$	$3.32 \pm 1.8x10^3$

3.3. Isolates and Phenotypic Characteristics

3.3.1. Cultural Characters

For purification, only aerobic bacteria were used. The different morphotypes obtained after purification of the colonies on Mossel are represented in Figure 8. A total of 51 colonies are isolated and stored in an eppendorf tube containing 900 µl of TSB and 100 µl of glycerol at 4° C. for enzymatic activities.

Figure 8. main morphotypes; (a) RE 1, (b) JE 9 isolate and (c) JE 15 isolate

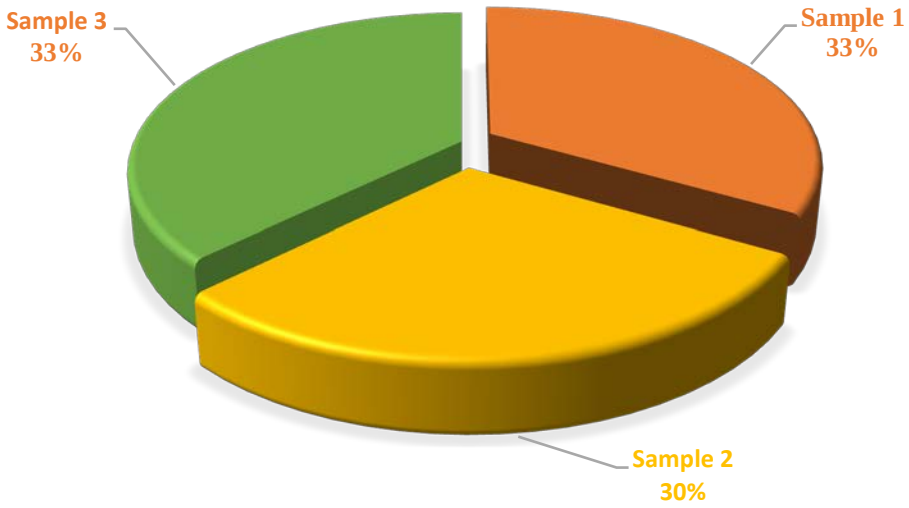

Figure 9. Percentage of isolates per sample

Figure 9 presents the results of the number of isolates from samples taken from soils in the Likouala peat bog area. A total of 51 isolates were isolated and subsequently analyzed. The distribution of results by sample is as follows: 17 isolates or 33% for sample 1; 15 isolates or 30% for sample 2 and 19 isolates or 33% for sample 3.

3.3.2. Microscopic and Biochemical Characteristics of Isolates on Mossel Medium

Figure 10 shows cell shape after fresh examination with the GX40 objective. The results show that all 51 isolates are catalases positive, aerobic, moderately motile and immobile. These cells are mostly rods, cocci and coccobacilli with a creamy and dry appearance, dominated by the presence of 25 isolates with pink colonies, 24 with yellow colonies and 2 with orange colonies.

Figure 11 shows bacterial cells after Gram staining. Of the 51 isolates; 46 isolates are Gram positive and 5 isolates Gram negative.

On sample 1, a total of 17 isolates were preserved: 9 isolates with pink colonies, 7 isolates with yellow colonies and 1 with orange colonies. Among these isolates; 11 Bacilli, 3 Cocci, 2 coccobacilli and 1 fusiform. All these bacteria are aerobic, moderately motile and Gram positive. These characteristics are shown in Table 3.

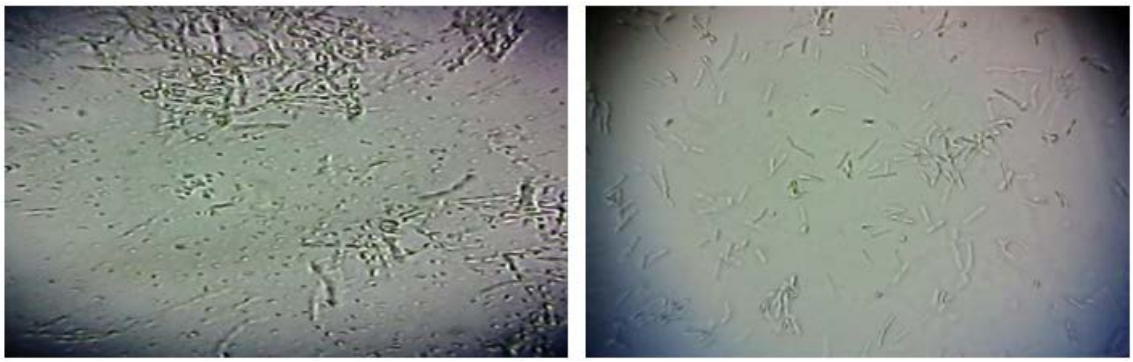

Figure 10: shape of bacterial cells in the fresh state (microscopic observation)

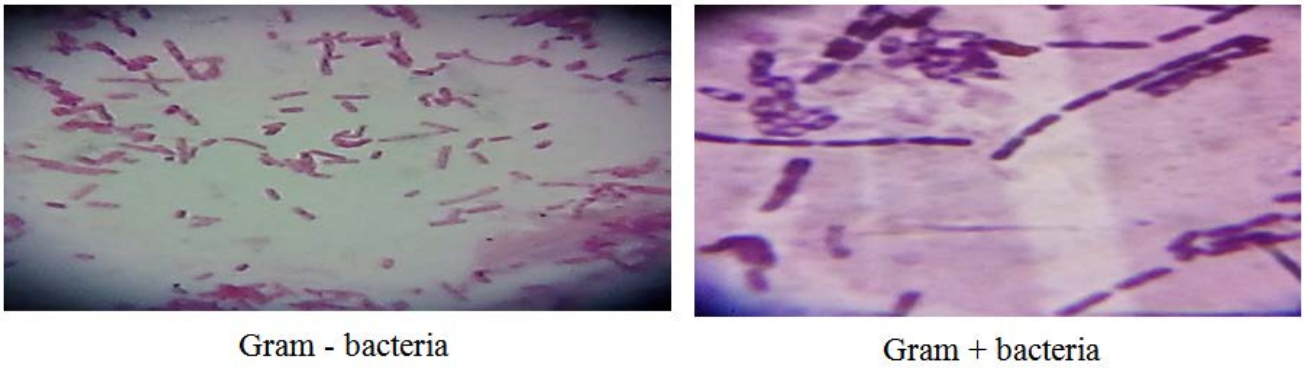

Figure 11. Bacterial cells after Gram staining

Table 3. Macroscopic and microscopic characteristics of isolates from sample 1

Isolates	Appearance, Color and Consistency of Colonies	Cell shape	Cell arrangement	Cell mobility	Gram	Catalase
RE 1	Circular, Flat Pink, Creamy	stick	isolated	+	+	+
RE 2	Circular, Flat yellow, Creamy	Fusiform	isolated	-	+	+
RE 3	Circular, Flat pink, Dry	stick	Diplobacille	+	+	+
RE 4	Circular, Flat pink, Dry	stick	chain	+	+	+
RE 5	Circular, Flat pink, Dry	Cocci	chain	+	+	+
RE 6	Circular, Flat pink, Dry	stick	chain	+	+	+
RE 7	Circular, Flat pink, Dry	stick	Diplobacille	+	+	+
RE 8	Circular, Flat pink, Dry	Cocci	isolated	-	+	+
JE 9	Circular, Flat Orange, Dry	Coccobacillus	isolated	+	+	+
JE 10	Circular, Domed yellow, Creamy	stick	isolated	-	+	+
JE 11	Circular, Domed yellow, Creamy	Cocci	isolated	-	+	+
JE 12	Circular, Domed yellow, Creamy	stick	Diplobacille	+	+	+
JE 13	Circular, Domed yellow, Creamy	stick	chain	-	+	+
JE 14	Circular, Domed yellow, Creamy	Coccobacillus	isolated	+	+	+
JE 15	Circular, Domed yellow, Creamy	stick	isolated	+	+	+
RE 16	Circular, Flat pink, Dry	stick	chain	-	+	+
RE 17	Circular, Flat pink, Dry	stick	chain	-	+	+

On sample 2, a total of 15 isolates were isolated: 8 isolates with pink colonies, 7 isolates with yellow colonies and 1 isolate with orange colonies. We have: 13 bacilli and 2 cocci. These bacteria are aerobic and 12 Gram-positive isolates and 3 Gram-negative isolates. These macroscopic and microscopic characteristics of the isolates are shown in Table 4.

Table 4. Macroscopic and microscopic characteristics of isolates from sample 2

Isolates	Appearance, Color and Consistency of Colonies	Cell shape	Cell arrangement	Cell mobility	Gram	Catalase
RE 18	Circular, Flat pink, Dry	stick	chain	+	-	+
RE 19	Circular, Flat pink, Dry	Cocci	isolated	-	+	+
RE 20	Circular, Flat pink, Dry	stick	isolated	+	+	+
RE 21	Circular, Flat pink, Dry	stick	isolated	-	+	+
RE 22	Filamentous, Flat pink, Dry	Cocci	isolated	-	-	+
RE 23	Circular, Flat pink, Dry	stick	Diplobacillus	-	-	+
RE 24	Circular, Flat pink, Dry	stick	isolated	-	+	+
RE 25	Filamentous, Flat pink, Dry	stick	isolated	+	+	+
JE 26	Circular, Domed Orange, Creamy	stick	isolated	-	+	+
JE 27	Circular, Domed yellow, Creamy	stick	Diplobacillus	+	+	+
JE 28	Circular, Domed yellow, Creamy	stick	isolated	-	+	+
JE 29	Circular, Domed yellow, Creamy	stick	chain	-	+	+
JE 30	Circulaire, Jaune bombée, Crémeuse	stick	isolated	+	+	+
JE 31	Circular, Domed yellow, Creamy	stick	chain	+	+	+
JE 32	Circular, Domed yellow, Creamy	stick	Diplobacillus	+	+	+

On sample 3, a total of 18 isolates were isolated: 8 isolates with pink colonies, 10 isolates with yellow colonies. 10 bacillus, 7 cocci, 1 coccobacillus and 1 fusiform. These bacteria are aerobic and 16 Gram-positive isolates and 2 Gram-negative isolates. The characteristics of isolates from sample 3 are shown in Table 4.

Table 5. Macroscopic and microscopic characteristics of sample isolates 3

Isolates	Appearance, Color and Consistency of Colonies	Cell shape	Cell arrangement	Cell mobility	Gram	Catalase
RE 37	Rhizoid, Flat Rose, Dry	stick	chain	-	+	+
RE 38	Circulaire, Rose plate, Sèche	stick	chain	+	+	+
RE 39	Circular, Flat pink, Dry	stick	chain	+	+	+
RE 40	Circular, Flat pink, Dry	stick	chain	+	+	+
RE 41	Irregular, Flat pink, Dry	stick	Diplobacillus	+	+	+
JE 42	Irregular, Flat yellow, Dry	Cocci	isolated	+	+	+
RE 43	Circular, Flat pink, Dry	stick	isolated	+	-	+
JE 44	Circular, Domed yellow, Creamy	stick	isolated	-	+	+
JE 45	Circular, Domed yellow, Creamy	stick	isolated	+	+	+
JE 46	Circular, Domed yellow, Creamy	Cocci	isolated	+	+	+
JE 47	Circular, Domed yellow, Creamy	Cocci	isolated	+	+	+
JE 48	Irregular, Flat yellow, Dry	Fusiform	isolated	-	+	+
JE 49	Circular, Domed yellow, Creamy	Cocci	isolated	+	+	+
JE 50	Circular, Domed yellow, Creamy	Cocci	isolated	+	+	+
JE 51	Circular, Domed yellow, Creamy	Cocci	isolated	+	-	-
JE 52	Circular, Domed yellow, Creamy	Stick	isolated	+	+	+
JE 53	Circular, Domed yellow, Creamy	Cocci	isolated	+	+	+
JE 54	Circular, Domed yellow, Dry	Stick	isolated	+	+	+
RE 55	Filamentous, Flat pink, Dry	Coccobacillus	isolated	+	+	+

3.4. Enzymatic Production of Isolates

3.4.1. Measurement of Bacterial Growth

Table 5 represents the optical density (OD) values which expresses the bacterial growth of some isolates after 24 h of culture on the TSB liquid medium. It varies in different ways. The O.D. measurement was carried out on 22 isolates and the values are between 0.634 and 1. The value 1 represents the largest O.D. value or the cutoff value of the isolates. The different values of the optical density are the different Figure 13 and Figure 15.

3.4.2. Production of Proteolytic Enzyme

Figure 12 represents the diameters of the translucent halos used as an indicator of proteolytic enzyme production. Diameters vary from 1 to 3.8 cm.

Figure 13 shows the variation in optical density and protease production of each isolate. From 22 isolates tested, 18 isolates produce proteases. The representative value is 3.8 cm. The highest diameter is attributed to the RE 2 isolate with a diameter of 3.8 cm while the lowest diameters are observed at the level of the RE 21 and JE 22 isolates at a height of 1 cm and the RE 8, JE 10, JE 42 and JE 43 showed an absence of proteolytic enzyme production.

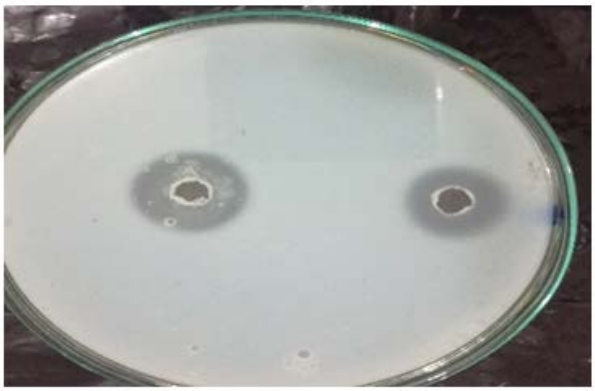

Figure 12. Translucent halos indicating casein digestion

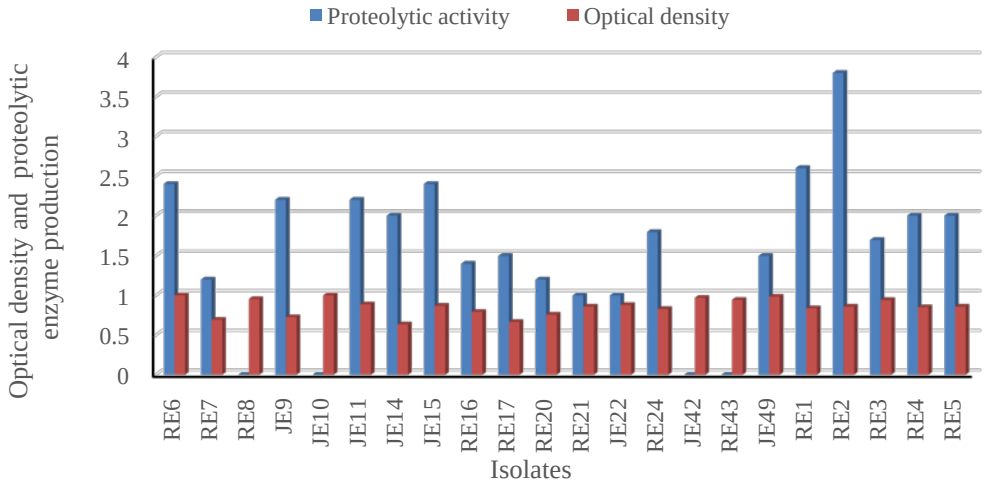

Figure 13. Production of proteolytic enzyme and optical density according to isolates

3.4.3. Amylolytic Enzyme Production

Figure 14 highlights the halos used as an indicator of amylolytic enzyme production. On the whole the values of the diameters vary from 1.3 to 3.2cm.

Figure 14. halos indicating starch digestion

Figure 15 below illustrates the variation in optical density and amylolytic enzyme production of each isolate. From 22 isolates tested, 16 isolates produced enzymes capable of degrading starch. 3.2 cm is the representative value and is attributed to isolate RE 3 while isolate RE 4 has the smallest diameter: 1.3 cm. However, with isolates RE 6, RE 7, RE 8, JE 10, RE 2 and JE 22 there is no enzyme production.

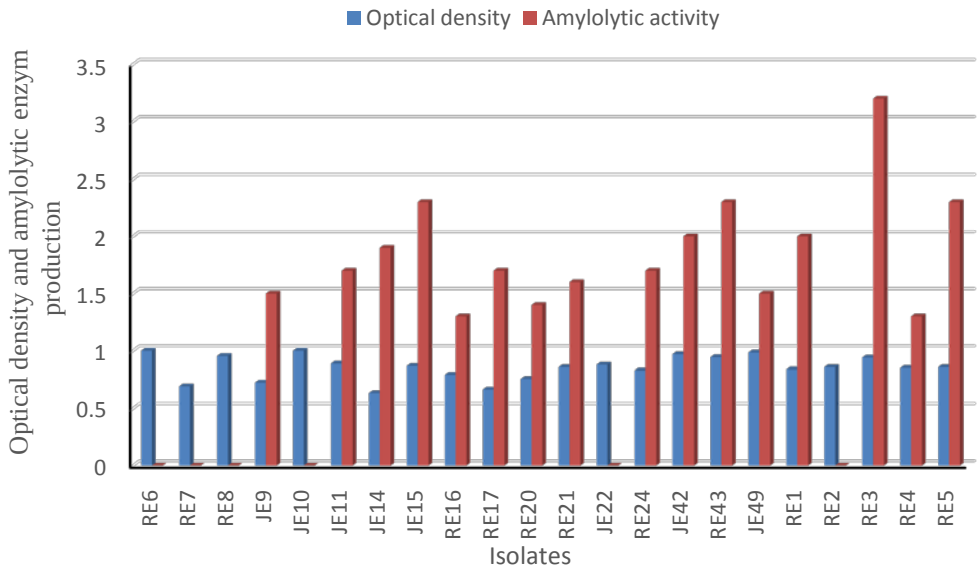

Figure 15. Optical density and production of amylolytic enzyme according to the isolates

3.5. Optimization of Growth and Enzyme Production as a Function of Time

Optimization of growth and enzyme production as a function of time is carried out using isolates RE 20, RE 21 and RE 24 in the growth and enzyme production figures.

3.5.1. Growth of Isolates Over Time

Figure 16 represents the growth kinetics in three Mossel isolates RE 20, RE 21 and RE 24 as a function of time. The variation of the curves obtained presents the same pace, the same phases with different amplitudes at the level of the 3 isolates. There is an exponential growth phase, a slowing phase, a stationary phase and finally a declining phase. At the level of isolates RE 20, RE 21 and RE 24, from t0 to t1 (0 to 24 hours), the increase in growth is progressive and proportional to time; which corresponds to the exponential phase of growth. From t1 to t2 (24 to 48 hours) this is the slowing down phase. From t2 to t4 (48 to 96 hours), despite the increase in time, a stabilization of growth is observed, thus corresponding to the stationary phase. Beyond 96 hours, the growth (D.O) becomes inversely proportional to the time, it is the phase of decline.

3.5.2. Enzyme Production as a Function of Time

Figure 17 represents the results of the study of the production of amylase and proteases. The production of amylase is variable depending on the time and the isolate. At RE 21, from t0 to t2 (0 to 48 hours), production is average and reaches its optimum at t2 (48 hours). Beyond t3, it becomes average and vanishes at t5. In isolate RE 21, low amylase production is observed from t0 to t1 (0 to 24 hours). It becomes maximum and beyond t3 and inversely proportional to the increase in time and so long is canceled at t5. At RE 24, from t0 to t2 (0 to 48 hours), the production of amylase is zero; it reaches its optimum at t3 (72 hours). Beyond t3, it decreases and vanishes at t5 (Figure 17a). Figure 17b shows the kinetics of proteolytic enzyme production. It is noted that, at RE20 and RE24, from t0 to t1 the production is average, this production reaches its optimum at t3. Beyond t3, it slows down and tends to vanish t5 after. Whereas, in RE21, from t0 to t2 (24 to 48 hours), there is no absence of enzyme production; from t2 to t3 (48 to 72 hours) production becomes average and reaches its optimum at t4 (96 hours). Beyond t4, it decreases and vanishes at t5 (120 hours).

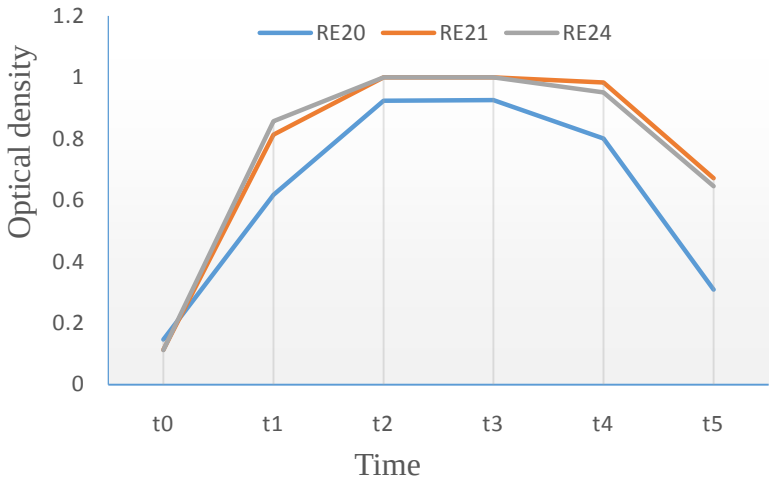

Figure 16. Growth curves of isolates on Mossel as a function of time

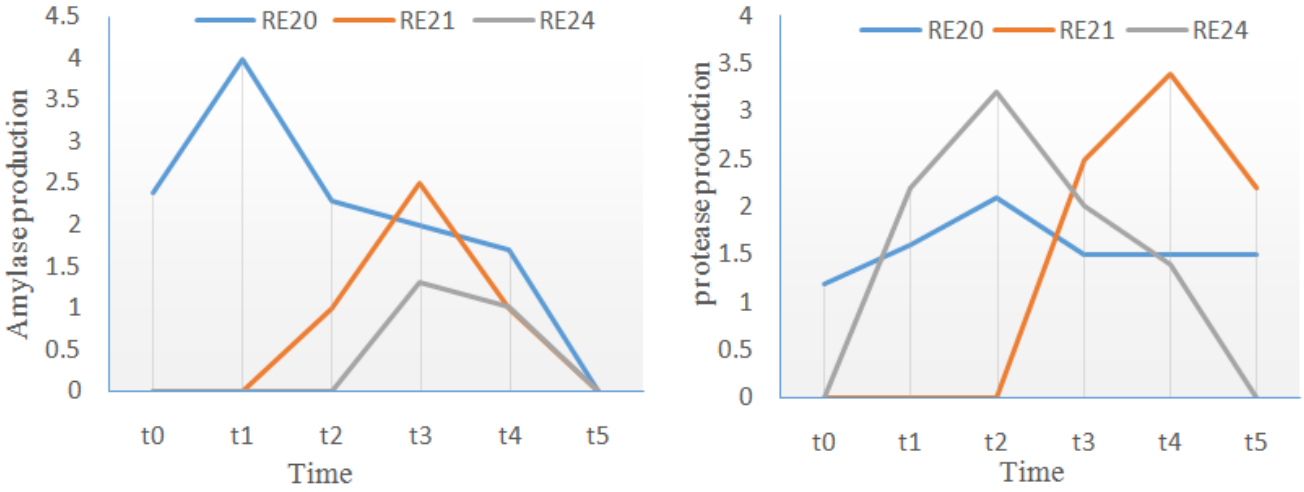

Figure 17. Amylase and protease production of isolates RE 20, RE 21 and RE24

3.5.3. Evolution of Bacterial Growth and Enzyme Production

3.5.3.1. Growth and Amylase Production

Figure 18 shows the evolution of growth and enzyme production (amylase) as a function of time (120 h). The evolution depends on the isolate; optimal production of amylase is observed with isolate RE 20 during the exponential phase of growth. This production slows down from the stationary phase of growth of the isolate to cancel out in the phase of decline. However, with the RE 21 and RE 24 isolates, during the exponential phase of growth, the production of amylase is average and only reaches its optimum in the stationary phase to cancel out in the decay phase.

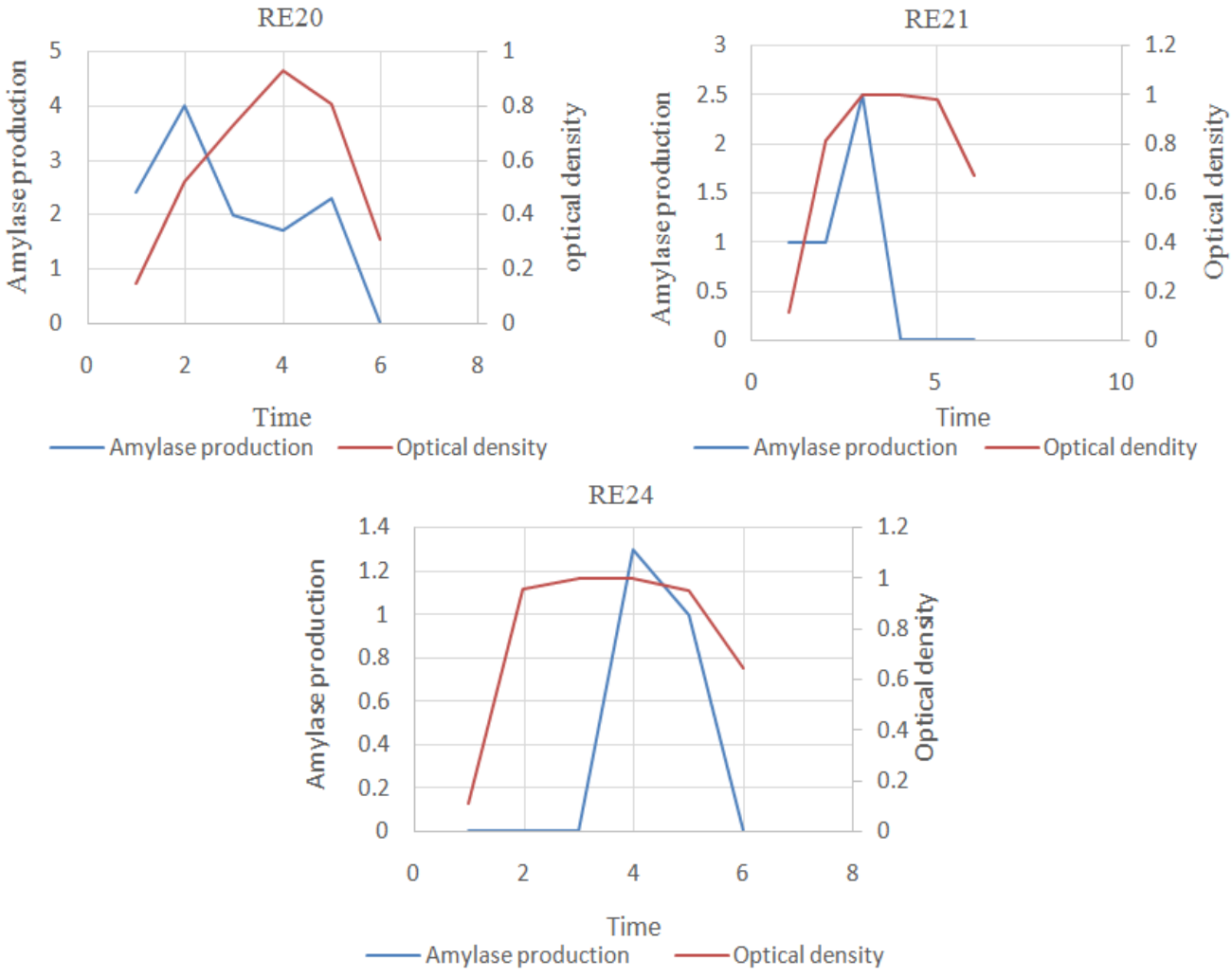

Figure 18. Evolution of bacterial growth and amylase production as a function of time

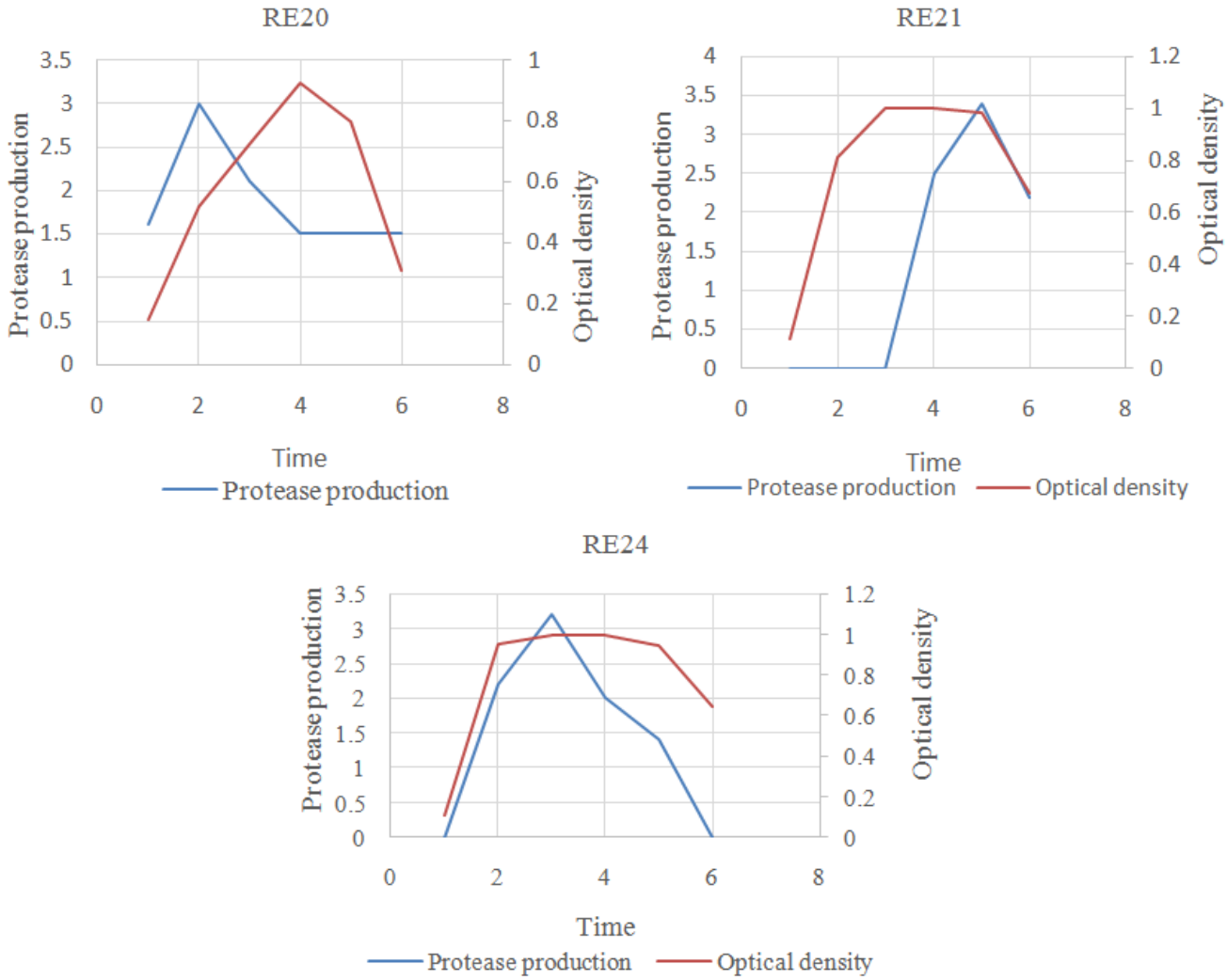

Figure 19. Evolution of bacterial growth and production of proteases over time

3.5.3.2. Growth and Proteases Production

Figure 19 represents the development of the growth and production of proteases of isolates RE 20, RE 21 and RE 24 as a function of time. Protease production is time and isolate dependent. A maximum production of protease with RE 20 is observed during the exponential phase of growth, it decreases in the stationary phase and remains stable; against an average production during the exponential phase with RE 21 and RE 24. The production of proteases becomes optimal with isolates RE 21 and RE 24 in the stationary phase of growth and slows down with the phase of decline in growth.

4. Discussion

The count of aerobic bacteria on the PCA medium showed a total flora of $9.50x10^4$ CFU/g in sample 1 compared to samples 2 and 3 which were respectively $7.31x10^4$ CFU/g and $6.36x10^4$ CFU/g. These results are weak compared to those obtained [19,20,21] which were of the order of $3.4x10^5$ to $5.5x10^7$ CFU /g for areas of natural bogs. However, these values were obtained in Europe and the United States where the vegetation, climatic and geological conditions are different from those of Congo-Brazzaville. These factors would certainly explain this difference because these factors are likely to influence the microflora and therefore the counts of microorganisms [22]. In our study, the total anaerobic mesophilic flora depends on the sample. Sample 1 has a bacterial load of $3.08x10^4$ CFU/g, sample 2 has a load of $2.93x10^4$ CFU/g while it is $3.03x10^3$ CFU/g for sample 3. The comparison of total aerobic and anaerobic mesophilic loads leads to the conclusion that the aerobic flora is greater than the total anaerobic flora. These results can be justified by the fact that the nature of the samples used consisted of superficial peat soils. Our results are consistent [19,23] who states that the microbial population is abundant on the surface of peatlands, because there is more oxygen and where carbon is readily available and decomposable. The counting of aerobic bacteria on Mossel (selective medium without polymyxin) shows a high concentration of bacteria of the *Bacillus* and *Staphylococcus* genus, always varying per sample. With sample 1 the flora is $5.81x10^4$ CFU/g, followed by sample 2 at $5.64x10^4$ CFU/g then sample 3 with $8.56x10^3$ CFU/g. These values still remain below those obtained [24] with a count of the order of $9.3x10^4$ CFU/g of bacteria of the *Bacillus* genus with soil and pepper samples from Brazzaville (Congo). Several studies have already shown that *Bacillus* can be isolated from food and soil [25,26]. Compared to counting on EMB medium where the sample loads are $2.77x10^2$ CFU/g; $5.1x10^2$ and $1.16x10^2$ CFU/g respectively for samples 1, 2 and 3. Whereas, with

anaerobic bacteria, sample 1 at a rate of $2.48x10^3$ CFU/g preceded by samples 2 and 3 with respective rates of $4.83x10^3$ CFU/g and $3.32x10^3$ CFU/g. The morphological, biochemical and cultural characterization of the isolates made it possible to highlight a diversity of colony forms in bacteria. The isolates purified on the different media showed the presence of various Gram + and Gram- bacteria which were catalase positive in accordance with the media used. Of the 51 isolates obtained, 22 were tested for their enzymatic activity. It appears that, out of the 22 isolates, 4 isolates do not produce proteases, namely RE 8, JE 10, JE 42 and RE 43 against 18 isolates which produce proteases with the best performance attributed to isolate RE 2 with a diameter of 3.8 cm while the smallest diameters are observed at the level of isolates RE 21 and JE 22 with 1 cm in diameter. 6 out of 22 isolates do not produce amylase: RE 6, RE 7, RE 8, JE 10, JE 22 and RE 2 against 16 which produce amylase with 3.2 cm in diameter attributed to the RE isolate 3 while isolate RE 4 has the smallest diameter with 1.3 cm. These results therefore suggest a significant production of proteases compared to amylases in the soils of peatland areas. This is why the follow-up of the evolution of the production of proteases and amylase during growth was carried out according to time with the isolates RE 20, RE 21 and RE 24 in a random manner. From the results of the experiment, it appears that the growth curves in the 3 isolates show the same phases: the exponential phase, the slowing phase, the stationary phase and the declining phase; these curves do not show a latency phase. During the exponential phase of growth, a normal multiplication of bacteria. The growth rate is maximum. The stationary phase results in a balance between the number of bacteria resulting from the multiplication and the number of those which die following the depletion of nutrients, accumulation of toxic waste, environmental conditions becoming unfavorable. During the decline phase, there is more growth, the bacteria die and are decomposed more or less quickly by the enzymes released at the time of their death (web). In the 3 isolates, it is after 48 h (t2) that growth is optimal. The absence of the latency phase is due to the time interval used; DO measurements were taken every 24 hours. Note, however, that the production of enzymes does not depend on bacterial growth, they are two distinct phenomena depending on bacterial metabolism. In fact, in isolates RE 21 and RE 24, the enzyme production reaches its optimum during the stationary phase, whereas with isolate RE 20 the optimum is reached during the exponential phase of growth. These results show that our isolates produce more proteases than amylases with diameters greater than those obtained [10,18].

5. Conclusion

The objective of this study was to characterize bacteria isolated from the soils of the Likouala peat bog area in Congo-Brazzaville from three (03) composite samples consisting of a total of 9 soil samples collected in the area of Likouala peat bog. It appears from this study that the surface part of the soils of the Likouala peat bog are richer in aerobic than anaerobic bacteria. These bacteria were characterized phenotypically with a dominance of bacteria of the genus *Bacillus*. 22 isolates showed protease and amylase production capacities over time.

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