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Screening and Isolation of Lipase Producing Bacteria from Contaminated Soils from the Littoral-Region of Cameroon and Partial Study of the Fermentation Conditions of the Crude Enzyme Produced

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A B S T R A C T

Lipases are enzymes that catalyze the transformation reactions of triglycerides in to fatty acids and glycerol. They can be produced by animals, plants and microorganisms. This article presents the isolation of lipase-producing bacteria from soil samples collected from sites contaminated with waste from palm oil production in the littoral region of Cameroon. These permit to isolate a multitude of bacteria capable of producing lipase from Rhodamine B-agar olive oil culture medium. Of the 35 isolates obtained from the 66 soil samples, one was selected as the best based on its enzymatic activity, the DI_1A isolate. After production of the crude enzyme, the influence of temperature and pH on it was studied, followed by the impact of some physicochemical parameters on the fermentation medium. The DI_1A isolate produced a crude enzyme showing better activity of 5.63±0.31 IU/ml with optimal activity at a temperature of 55°C and a pH of 8. The study of the influence of some physico-chemical parameters on the isolate showed an optimal growth temperature at 38°C, an optimum pH at 7, the best carbon source is palm kernel oil at 1% (v/v), the best nitrogen source is ammonium chloride at 2% (m/v) and the best salt source is magnesium sulphate at 3% (m/v). Macroscopic and microscopic analyses reveal that the DI_1A isolate is a Gram+ bacillus, a positive catalase capable of sporulating.

Introduction

Enzymes have been used since ancient civilizations. Nowadays, more than 4000 enzymes are known and about 200 are used for commercial purposes, most of them fungal and bacterial (Sharma *et al.*, 2001). Until the 1960s, the enzyme market was worth only a few million dollars a year, but since then the market has evolved dramatically (Wilke, 1999). The market for industrial enzymes is growing rapidly, because of the emergence of

new fields of application. In 2007, the global market for industrial enzymes was estimated at $2.3 Billions while lipases accounted for only 5% of the total market (Jyoti and Avneet 2006). This rate can increase significantly through a range of application areas. Thus, the growing demand for enzymes of particularly bacterial origin owes its applications to several production fields: Food processing industries, pharmaceutical industries, chemical industries and textile industries (Patil *et al.,* 2011). In addition to lipolytic properties, lipases have esterification properties (Jaeger and Ritz, 1998).

The growing importance of lipases in biotechnological perspectives can easily be seen in the number of journals and articles that cover the variable aspects of this highly versatile enzyme: Biochemistry, Molecular Biology, Purification Approach and Biotechnology Applications (Gupta *et al.,* 2004). Lipases are enzymes produced by many plants, animals and microorganisms. The most exploited bacteria lipases are: *Archomobacter, Alicagens, Arthrobacter, Baccillusburkhoderia, Chromobacterium and Pseudomonas sp.* (Gupta *et al.,* 2004).

Despite the considerable progress made in recent years in the production of bacterial lipase, the isolation of this type of microorganism remains a major challenge. For this reason, scientists must move to isolate microorganisms capable of producing lipases in order to increase the number of bacteria available. The objective of this work is to isolate a thermostable lipase producing bacterium.

Materials and Methods

Isolation

The liquid fermentation medium consists of: 0.2% (m/v) yeast extract; 0.5% (m/v) peptone; 0.02% (m/v) $MgSO_4$; 0.3% NaCl; 0.1% (m/v) KH_2PO_4; 0.5% (v/v) olive oil; 0.05% (v/v) tween 80 as emulsifier. The whole was dissolved in distilled water and the pH of the medium adjusted to 8 by adding 0.3% (m/v) Na_2CO_3 and then distributed to the Erlenmeyer. Each Erlenmeyer contains 1/10 of its volume in a liquid medium. This medium was autoclaved at 121°C for 20 minutes. After cooling, 5 grams of soil sample taken from palm oil waste dumps were introduced and incubated under agitation in a water bath at 37 °C for 24 hours.

The solid isolation medium has the same composition as the liquid fermentation medium in the presence of Rhodamine B and agar. This enrichment medium was diluted decimal, then 5 microlitres of each fraction were spread on the surface on the Agar-Rhodamine B medium contained in the petri dishes according to the method used by Bhavani *et al., in* 2012, then incubated in an oven at 37°C for 24 hours.

The lipolytic capacity of a colony has been materialized by the presence of a fluorescent halo around it. The isolate with a larger diameter halo and a small colony was retained and maintained on inclined agar.

Production of the crude enzyme

It was carried out using fermentation in a liquid medium (Hupé, 2008). Indeed, 10ml of a fermentation medium previously autoclaved at 121°C for 20 minutes are distributed in 50 ml Erlenmeyer.

After cooling, a suspension of bacterial colonies was introduced. The Erlenmeyer are incubated under oscillation in a water bath at 30°C for 24 hours. After fermentation, the various media are centrifuged. The recovered supernatant is considered to be the crude enzyme. It is stored at +4°C for further work.

Screening of the best isolate

Evaluation of the relative activity

Halodiameters measuring method

Relative activity is the distance of diffusion of the enzyme through the gel. It consisted in measuring the diameters of the colonies (Dc) and those of the haloes (Dh) using a graduated ruler and making the ratio Da/Dh. Hydrolytic activity was based on this ratio. The colonies with the highest ratios were selected for the evaluation of enzyme activity.

Well method

60 µL of the crude enzyme obtained is introduced into the wells and incubated at 37°C for 48 hours. Opaque haloes around the wells are observed, demonstrating that these enzymes are capable of hydrolyzing lipids. The diameter of each halo is based on the lipase activity. The larger it is, the higher the activity.

Evaluation of enzymatic activity

The enzymatic activity of the pre-selected isolates was determined by the titration method described by Sharma et *al* (2012) with slight modifications in the incubation time. Lipolytic activity is determined in an emulsified system using olive oil as substrate and tween 80 as emulsifier. The substrate must be properly emulsified because the lipolytic activity varies directly with the surface of the substrate available for the enzyme. Vigorous agitation of the emulsion is acceptable (Sharma *et al.*, 2012).

Study of the influence of some physicochemical parameters

Factors affecting the growth of microorganisms such as fermentation time, pH, temperature, sources and quantities of substrate (lipids), as well as sources and quantities of nitrogen and mineral salts affecting lipase production are partially studied by varying experimental conditions. The experiments are carried out in 50 ml Erlenmeyers flask containing 10 ml of liquid medium by varying the parameters to be studied. The pH is maintained at 7.5 and the temperature at 37°C.

Results and Discussion

Isolation

Lipolytic strains are those with haloes (light areas around the colony) (photograph 1).

There is a predominance of isolates in Pool No. 2 and an absence in Pool No. 3 and the YATO site. In Pool No. 1 and the Souza site, only a few bacteria are observed. Yato site, only 2 years old, its microorganisms would not yet be suitable for the production of fatty acid hydrolases (lipases). Pool No. 3, devoid of any form of fat, cannot also serve as a biotope for these microorganisms. The remaining sites, which are older and content requirements of fat, are the ideal biotopes for these microorganisms (Table 1).

Screening of the best isolate

Evaluation of the relative activity and partial identification of the best isolate

Halodiameters measuring method

In order to determine the hydrolytic activity (R=Da/Dh) of lipases excreted by the colonies, the diameters of the colonies (Dc) and halos (Dh) were measured after 48 hours of incubation at 37°C. The screening of lipolytic strains was made possible by the value of this activity (R=Dc/Dh). The screening technique highlights the microorganisms that produce lipolytic

enzymes. It can be seen that the microorganisms in Pool N°2 have the highest activities while the other sites have microorganisms with relatively lower activities (Table 2). This could be explained by the saturation of the Souza site and the Pool No. 1 with fat, as the Pool No. 2 has a very low oil content

Well method

Knowing that microorganisms have the lipolytic capacity, this manipulation aims to evaluate the activity of the crude enzyme produced.

All the isolates obtained showed lipase activity through the appearance of haloes around the wells (Photograph 2). Statistical analyses show that relative activity varies significantly between isolates (H = 21.206; p' 0.0001).

Figure 1 below shows that some isolates have a better relative activity compared to others. These are mainly isolates from the lagoon and mainly from pools N° 1 and N° 2. This may be due to their age and the presence of fat in the environment.

Determination of the activity of the best pre-selected isolates

Titrimetry was performed as described by sharma *et al.,* in 2012. The purpose of this activity is to select the best isolate.

Although these isolates showed good activity compared to some strains already studied, statistical analyses of the data showed a significant variation in activity with each isolate (H = 20,500; p = 0.0046). Figure 2 shows that the enzyme produced by DI1A has the highest activity at 5.63±0.31. This activity is significantly higher than that obtained by Aspergillus niger 1.5 (IU/ml) by Sharma et al, (2001) and *Bacillus safensis* (0.248 IU/ml); *Bacillus megaterum* (0.203 IU/ml) by Khataminezhad *et al.,* (2014) who used the titrimetric method.

Partial identification of DI_1A

Macroscopic, microscopic examinations and biochemical tests are performed for the partial identification of DI_1A (Table 3).

The DI1A isolate is whitish, producing a green pigmentation. It is capable of growing at 55°C, the edge of its colonies are regular, semi-bomled with a shiny and creamy surface, positive catalase. Under a microscope, DI_1A is a Gram+ bacillus capable of sporulating (Table 3). Khataminezhad *et al.,* (2014) showed through studies on *Bacillus safensis* and *Bacillus megaterum*that several bacteria producing lipolytic enzymes were generally Gram-positive, catalase-positive, sporulated, aerobic bacilli; characteristics common to the majority of *Bacillus* species (Seeley and VanDemark, 1981; Fergus, 2008).

Study of the influence of some physico-chemical parameters on the production of DI_1Alipases.

Influence of temperature on enzymatic production

Microorganisms are deeply affected by the temperature of their environment since it significantly influences the growth of these microorganisms and therefore the production of enzymes (Figure 3).

The influence of temperature variations (25, 30, 37, 40, 45, 55 and 60°C) is studied at pH 8, at 107 rpm and in the presence of olive oil (0.5%) as a carbon source. Statistical analyses of the data show thatenzymatic activity varies

significantly with temperature (H=18.130; p=0.0059).

Figure 3 shows a peak at 38°C (5.30 ± 0.39 IU/ml): This is the optimal temperature. This activity drops lightly between 40 and 45°C and then drops sharply above 55°C. Each microorganism has an optimal temperature at which maximum enzyme production occurs. In ourstudy, the optimal temperature for enzyme production is 38°C. Meenakshi and Hindumathy (2014) reported maximum enzyme production at 37°C by *odoratiminusmyroids.* The optimum production at 38°C would have originated from the opening and increase in the permeability of the bacterial membrane, which would have triggered the proper functioning of the body's enzymatic synthesis machinery. The decrease in production above 45°C would come from the decrease in cell growth due to the increase in temperature.

Influence of initial pH on enzymatic production

The initial pH of the medium playsa key rule in bacterial growth. The influence of the initial pH (5, 6, 7, 7, 8, 9) is studied at 30°C, at 107 rpm and in the presence of olive oil (0.5%) as a carbon source. The variation in activity according to pH is significant (H= 11.525; p=0.0213).

Figure 4 shows a gradual increase in activity from pH 5 onwards, with a maximum at pH 7 for an activity of 3.89 ± 0.33 IU/ml. Abovethis pH, there is a gradual decrease in activity between pH 8 and pH 9. These results are similar to those reported by Huda (2013) in *Actinobacter baumannii*. However, maximum production at pH 9 has been reported in *Bacillus safensis* and *Bacillus megaterum* by Khataminezhad *et al.,* (2014). This suggests that DI_1A isalkaline.

Influence of shaking speed on enzymatic production

The study of the influence of the shaking speed (90, 107, 124, 124, 140 and 155 rpm) was carried out at 30°C, pH 8, and in the presence of olive oil (0.5%). Production does not vary significantly with agitation rate (H=8.775; p=0.0670)**.**

At 140 rpm, an enzymatic activity of 4.22 ± 0.48 IU/ml is obtained, which is the optimal shaking speed. Above 140 rpm, this activity decreases considerably when reaching 155 rpm (3.96 ± 0.64 IU/ml) (Figure 5).

The literature reports that bacterial cultures in agitated environments cause morphological changes, including changes in cell permeability (Darah *et al.*, 2013). Shaking is required for aerobicbacteria to produce lipase since there is virtually no production of lipase in the stationary state (without shaking) (Veerapagouet*al*., 2013). From the results obtained, it is observed that shaking at 90 rpm increases lipase production. The optimal shaking speed for the production of lipase by DI_1A is 140 rpm. Beyond this speed, production falls (Figure 5). Lipase production could be increased by increasing the oxygen transfer rate, contact area and good dispersion of the substrate (oil) in the culture medium during fermentation under agitation However, at high shaking speeds, enzyme production decreases. Veerapagou *et al.,* (2013) also reported that lipase production is optimal at 160 rpm and decreases when the agitation rate is increased. This could be due to the fact that high agitation makes the substrate less available. Darah *et al.,* (2011) report that high agitation rates contribute to low enzyme production leading to shear forces, resulting in high cell destruction rates and consequently lower enzyme production. The damage created by these shear forces cannot be

repaired by excess oxygen in the environment.

Influence of fermentation time on enzymatic production

The fermentation time is decisive for the enzymatic production. The influence of fermentation time on enzyme production is studied at different times (1; 6; 12; 24; 36; 48, 72, 90 and 120 hours), at pH 8, 30°C, 107 rpm and with olive oil (0.5%) as substrate. This production varies significantly over time (H=16.251; p=0.0125) according to statistical analyses.

After 1 hour of fermentation, there is an increase in enzymatic activity (1.56 ± 022 IU/ml). This activity varies little between 6 and 12 hours and then increases sharply to 3.41 ± 0.56 IU/ml (maximum activity) after 48 hours of fermentation. It tends to decrease after 72 hours (3.22 ± 0.51 IU/ml). At 120 hours, there is a considerable decrease of the activity (Figure 6). These results are similar to those reported by Leonov (2010) who worked on *Pseudomonas fluorescens* and obtained an optimal production time of 48 hours of fermentation, Meenakshiand Hindumathy (2014) who worked on *Myroïdesodoratiminus* and Abdollahi *et al.,* (2014) on *Pseuddomonas sp.* An increase in fermentation time leads to a reduction in enzymatic production, this could be due to the exhaustion of nutrients in the medium (Sourav *et al,* 2011) or probably to the production of secondary metabolites, inhibitors of protein synthesis (Chibuogwu *et al.,* 2009).

Influence of the substrate (carbon source) on enzymatic production

Peanut, olive, refined (R) and crude palm (R), palm kernel and cocoaoils are used as a substrate (carbon source) at 0.5%, pH 8, 30°C and 107 rpm to determine their influence on enzyme production. This enzymatic production varies significantly depending on the carbon source used (H = 15.971; p = 0.0069) (Figure 7).

The activity of the enzyme produced from palm kernel oil as the only carbon source (8.22 ± 1.79 IU/ml) is higher than that of the enzyme produced from crude palm oil (6,67±1.47 IU/ml), olive (5.07±1.70 IU/ml), groundnut (4.15±0.96 IU/ml), refined palm (R) (2.52±0.34 IU/ml) and cocoa (2.26±0.93 IU/ml). (Figure 7). (Savitha *et al.,* 2007) and Meenakshi and Hindumathy (2014).

Influence of substrate concentration (carbon source) on enzyme production

The influence of palm kerneloil concentration on production was studied (0.5; 1; 1; 2; 2; 3; 4 and 5%) at 30°C, pH 8 and 107 rpm.

The activity of the enzyme produced at 1% is 3.19 ± 0.34UI/ml (maximum activity) significantly higher than that of enzymes produced at other concentrations. It ranges from 2.26 ± 0.28 IU/ml, at 1%, a gradual decrease in the curve reaches the maximum at 1%, decreases and remains around 1.26 ± 0.74 IU/ml at 5% (Figure 8). The amount of substrate is a determining factor in enzyme production because the amount of substrate available conditions the growth of microorganisms. These results can be extrapolated to those of Savitha *et al.,* (2007) and Meenakshi and Hindumathy (2014) who all reported that coconut oil with a composition very close to that of palm kernel oil was the best substrate at 1% for some fungal isolates and *Odoratiminus myroids* respectively.

Based on these results, it can be said that the production of enzymes is inductive because the enzyme activity is conditioned by the substrate in presence.

Influence of the nitrogen source on enzymatic production

Soybean meal (*Glycine hispida*), beanmeal (*Phaseolus vulgaris L.*), ammonium sulphate ($(NH_4)_2SO_4$), ammonium chloride (NH_4Cl), peptone, yeast extract and urea are used as sources of nitrogen at 2%, pH 8, 30°C and 107 rpm to determine their influence on enzymatic activity. The variation was not significant (H = 9.887; p =0.1295). The activity of the enzyme produced from yeast extract as a source of nitrogen (8.15±1.95 IU/ml) is higher than that of enzymes produced from ammonium chloride (NH4Cl) (7.96±3.06 IU/ml), urea (4.63±1.16IU/ml), ammonium sulphate ((NH 3)2SO4) (3.52±1.40 IU/ml), peptone (3.33±1.92 IU/ml), soybean meal (*Glycine hispida*) (2.04±0.85 IU/ml) and beans flour (*Phaseolus vulgarisL.*) (1.48±1.16 IU/ml) (Figure 9). This would be due to the fact that ammonium chloride has nitrogen directly available for the bacteria and the yeast extract would be very rich in available amino acids which can be quickly used for metabolic needs while the others require additional efforts. Ammonium chloride is chosen because yeast extract is more expensive on the market. These results are similar to those obtained by Veerapagou *et al.*, in 2013, where he demonstrated in a study on lipase-producing bacteria that yeast extract was the best organic source while ammonium chloride was the best inorganic source for lipase-producing bacteria

Influence of ammonium chloride concentration (nitrogen source) on enzymatic production

The influence of ammonium chloride concentration (0.5; 1; 1; 2; 3; 4 and 5%) at 30°C, pH 8 and 107 rpm was investigated.

The activity of the crude enzyme produced increases between 0.5 (0.3 ± 0.23IU/ml) and 1% (2.07 ± 0.28IU/ml), until it reaches its maximum at 2% (2.70 ± 0.28IU/ml). Above 2%, it gradually decreases to 0.89 ± 0.22 IU/ml at 5%. (Figure 10). The low activity between 0.5 and 1% is due to the lack of sufficient elements for metabolism. The decrease at a certain concentration is due to the saturation of the medium with organic elements, including bacterial poisoning**.**

Influence of mineral salts on enzymatic production

Mineral salts are essential in enzymatic activity. Some inhibit it while others increase it. At the cellular level, they facilitate the diffusion of nutrients through the membrane. We studied the influence of some salts, namely NaCl; $(NH_4)2SO_4$; KH_2PO_4; NaH_2PO_4; $CaCl_3$; $FeSO_4$ and$MgSO_4$

These mineral salts all appear to be suitable for enzyme production but statistical analyses show a significant degree of difference (H=15.602; p=0.0081) between the salts tested. However, iron sulphate ($FeSO_4$) appears to be the least suitable while magnesium sulphate ($MgSO_4$) shows better activity (Figure 11). Magnesium ions are cofactors that are sometimes essential for the functioning of certain lipases. Janssen *et al.*, 1994 also report that lipase production by a thermophilic *Bacillus* was optimal when magnesium, iron, and calcium ions were added to the production medium. Similarly, Pokorny *et al.*, reported in 1994 that *A. Niger*'s lipase production was increased in the presence of magnesium.

Influence of $MgSO_4$ concentration on enzyme production

The influence of magnesium sulphate salt concentration (0.5; 1; 1; 2; 2; 3; 4 and 5%) was studied at 30°C, pH 8 and 107 rpm. Enzyme production varies significantly with

salt concentration (H = 13.094; p =0.0225). The concentration of salts is very important for enzymatic production. It ranges from 0.70±0.23 IU/ml to 0.5%, reaches 2.04±0.39 IU/ml at 3% (optimal concentration) and drops sharply to 0.56±0.22 IU/ml at 5% due to saturation effects. (Figure 12). The production of extracellular lipase by *Acinobacter calcoaceticus* BD 413 was increased when the medium was supplemented with magnesium, (Kok *et al.,* 1995). Sharon *et al.,* (1998) report that the maximum lipase production by *P. pseudoalcaligenes*Kka-5 occurred at 0.8 M magnesium; however, the exclusion of magnesium ions from the medium caused an approximately 50% reduction in lipase production, but supplementing the medium with calcium ions did not affect lipase production. This implies that magnesium ions would be essential for enzymatic production in some microorganisms.

Influence of temperature and pH on enzymatic activity of the crude enzyme

Influence of temperature on enzymatic activity

The influence of temperature on enzymatic activity is significant (H=13,429; p= 0,0367). It was determined at different temperatures (20, 30, 37, 40, 45, 55,60, 70 and 80°C). The enzymatic activity is equal to 0.81 ± 0.42UI/ml at 20°C. This value gradually increases to a peak corresponding to 3.37 ± 0.45 IU/ml at 55°C and then drops slightly to 3.22 ± 0.62 IU/ml at 60°C. (Figure 13). These results are similar to those reported by Abdollahi *et al.*, (2014) who recorded maximum enzymatic activity at 60°C and a sharp decrease from 65°C in lipases isolated from *Pseuddomonas sp.* and those obtained by Qing *et al.,* (2014) who observed maximum enzymatic activity at 60°C in lipase isolated from a metagenomic strain.

Influence of pH on enzymatic activity of the crude enzyme

The influence of pH variations (5, 6, 7, 7, 8, 9) is studied on the crude enzyme at 30°C. Analyses show that pH significantly influences enzymatic activity (H=12.308; p = 0.0152). Figure shows 14 an increase in activity from pH 5 (0.85 ± 0.50 IU/ml) to its maximum at pH 7 (3.89 ± 0.33 IU/ml) which is maintained at pH 8, above which there is a decrease in activity. At acidic pH, enzyme activity gradually decreased. The lipase produced hydrolyzed the substrate over a relatively short pH range of 7 to 8 giving an activity of 4.81 ± 0.36 IU/ml (Figure 14). these results are similar to those obtained by Shakila *et al.*, (2012) where a study on *Serratiamarcescens* MBB05 lipase showed maximum activity at pH7. The enzyme of DI_1A is therefore alkaline.

Table.1 Distribution of isolates obtained according to sampling sites

Sampling site		Number of samples	Number of isolates
YAT		12	**0**
SOU		22	**5**
DIZANGUE	Pool N°1	12	**5**
	Pool N°2	15	**25**
	Pool N°3	5	**0**
Totals		**66**	**35**

Table.2 Hydrolytic activity of isolates

Sites	Summer camps	Diameter of the halo (48 hours): Da (mm)	Diameter of the Colony (48 hours): Dc (mm)	Report R=Da /Dh
SOUZA	F_1	35	10	**3,5**
	F_2	20	10	**2**
	F_3	20	10	**2**
	F_4	20	9	**2,22**
	F_5	17	8	**2,12**
	DB_1	20	10	**2**
	DB_1	21	12	**1,75**
(Pool No.	DI_1	40	10	**4**
	DI_1	26	12	**2,16**
	DI_1	30	7	**3,66**
	DF_2C	50	6	**8,33**
	DI_2C	75	7	**10,7**
	DI_2D	65	5	**13**
	DD_2D	23	8	**2,87**
	DD_2C	18	5	**3,6**
	DF_2A	29	7	**4 ,14**
	DI_2B	45	6	**7,5**
(Pool No. 2)	DH_2E	30	3	**10**
	$DH_2B($	26	3	**8,66**
	DC_2A	50	10	**5**
	DE_2A	56	7	**8**
	DD_2B	60	9	**6,66**
	DG_2A	38	5	**7,6**
	DG_2E	15	5	**3**
	DC_2C	28	3	**9,33**
	DI_2E	25	5	**5**
	DD_2A	45	4	**11,2**
	DF_2B	35	5	**7**
	DC_2B	35	7	**5**
	DE_2B	45	18	**2,5**
	DH_2B	28	4	**7**
	DH_2B	32	5	**6,4**
	DF_2A	54	12	**4,5**
	DH_2C	17	12	**1,42**
	DG_2	**22**	**6**	**3,67**

Table.3 Characteristics of DI1A

CHARACTERISTIC	RESULTS
Macroscopic observations of colonies	
colonies	**Regular**
Edge	**Regular**
Elevation	**Semi-bombed**
Surface area	**Bright and**
Color	**Whiteish**
Pigmentation	**Green**
Breathing mode	**Aerobics**
Mobility	**Positive**
Growth at 55°C	**Positive**
Microscopic observations	
Shape	**Stick**
Formation of	**Yes**
Mobility	**Positive**
Gram staining	**Positive**
Catalase test	**Positive**
Oxydase	**Positive**

Photo.1 Lipolytic strains presenting the halo on Olive oil Rhodamine B Agar medium

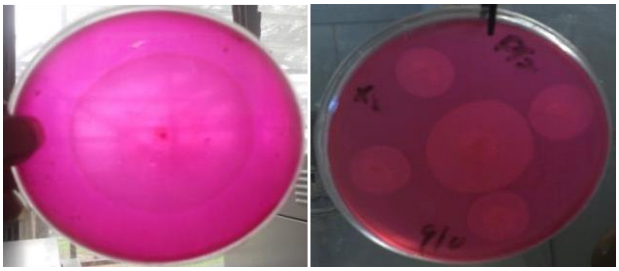


Photo.2 Relative activity of the crude enzyme on Rhodamine B agar medium

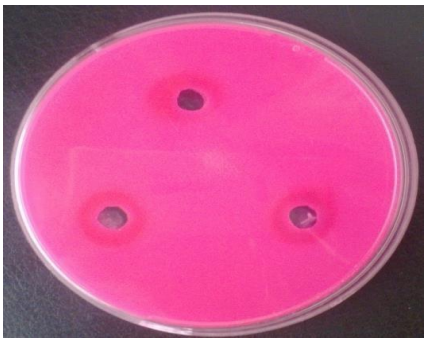


Fig.1 Relative activity of isolates

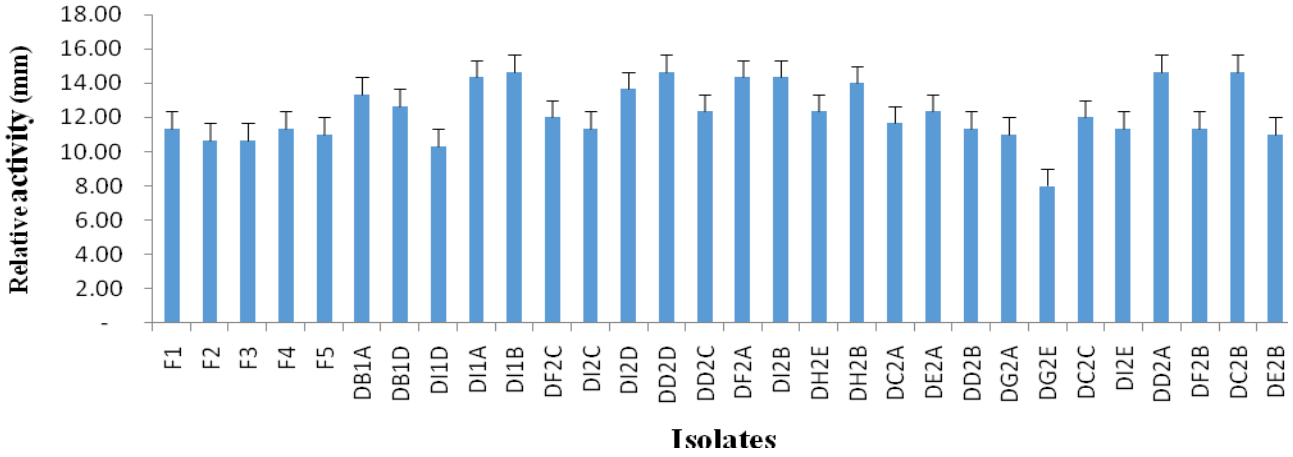


Fig.2 Enzymatic activity of the best pre-selected isolates

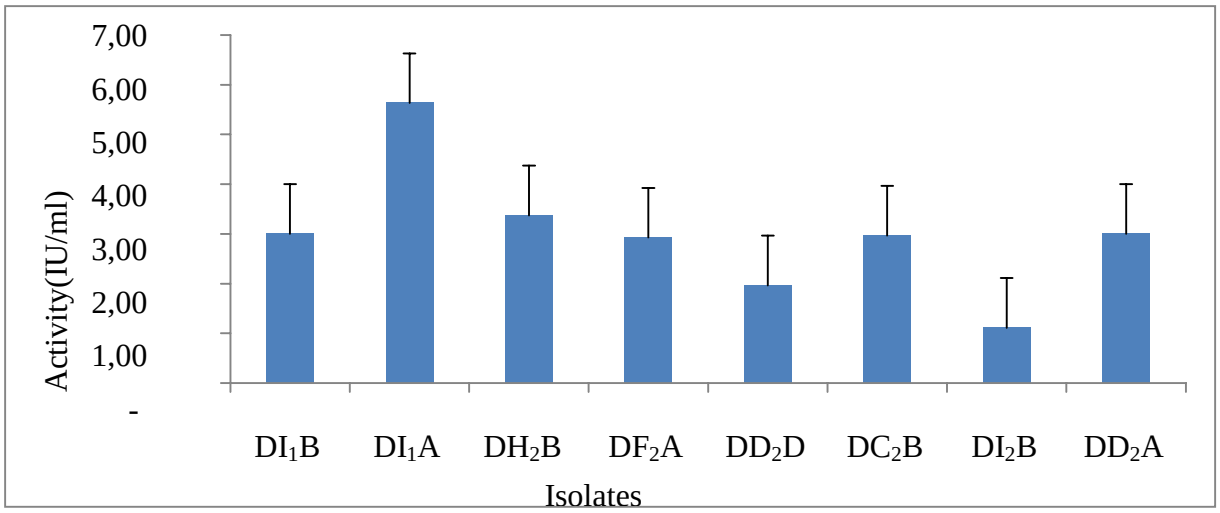


Fig.3 Influence of temperature on enzymatic production

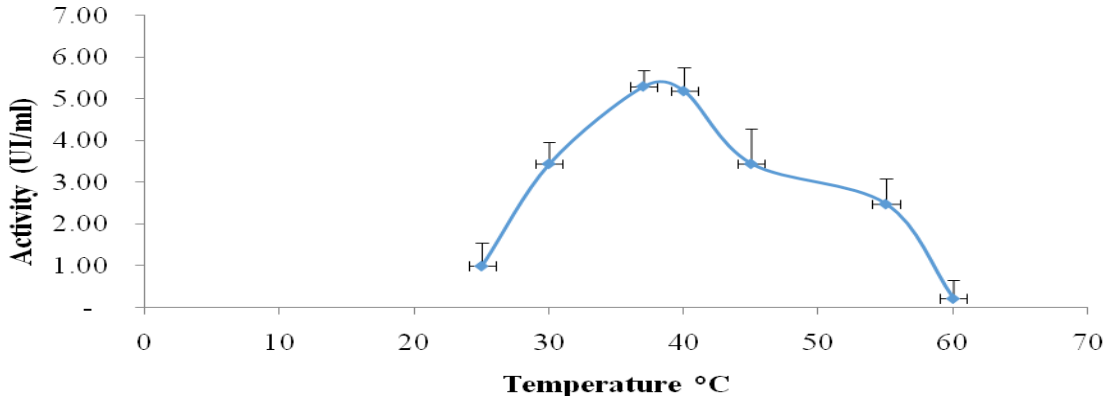


Fig.4 Influence of pH on enzymatic production

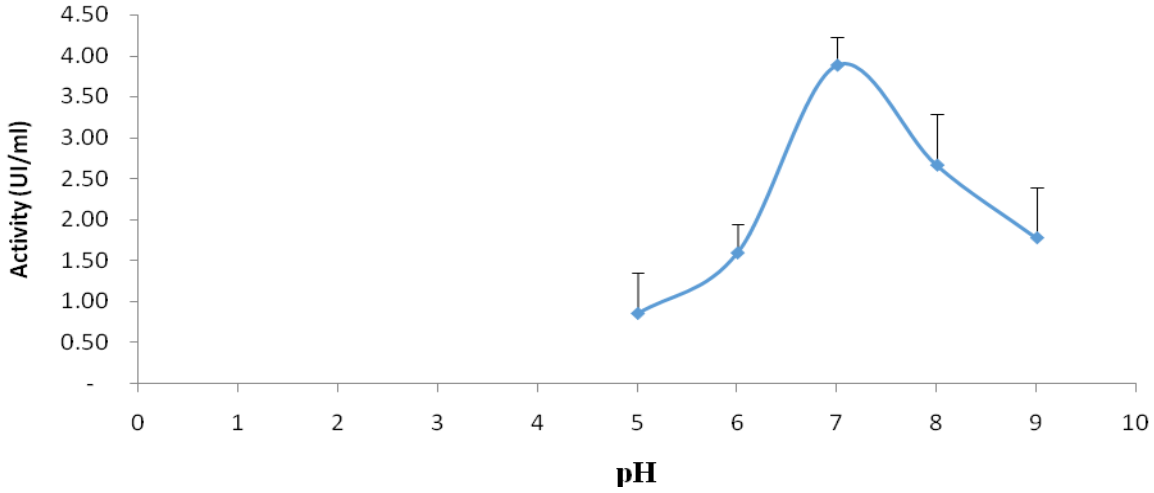


Fig.5 Influence of the shaking speed

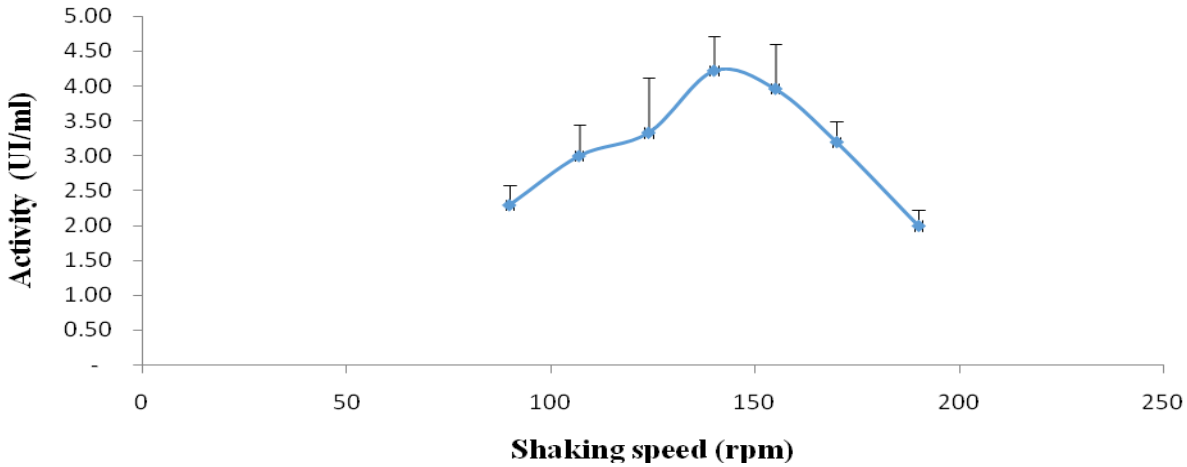


Fig.6 Influence of fermentation time

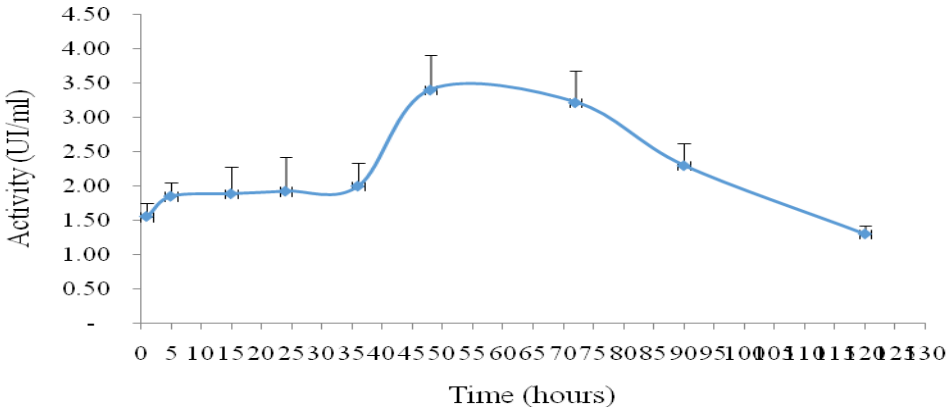


Fig.7 Influence of the carbon source

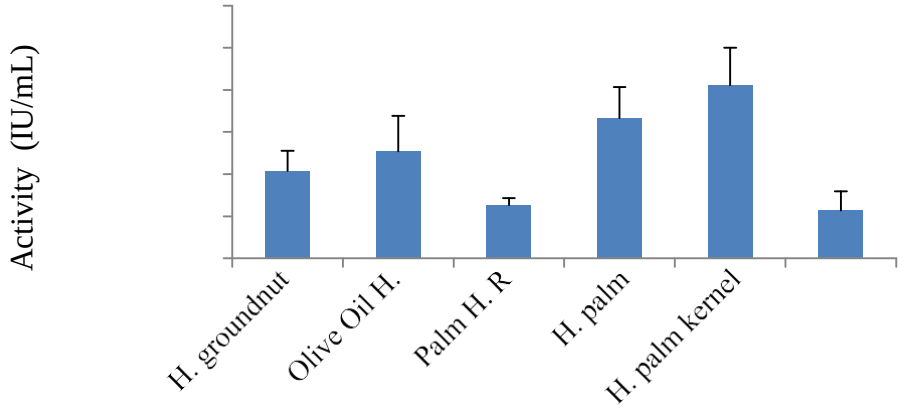


Fig.8 Influence of substrate concentration

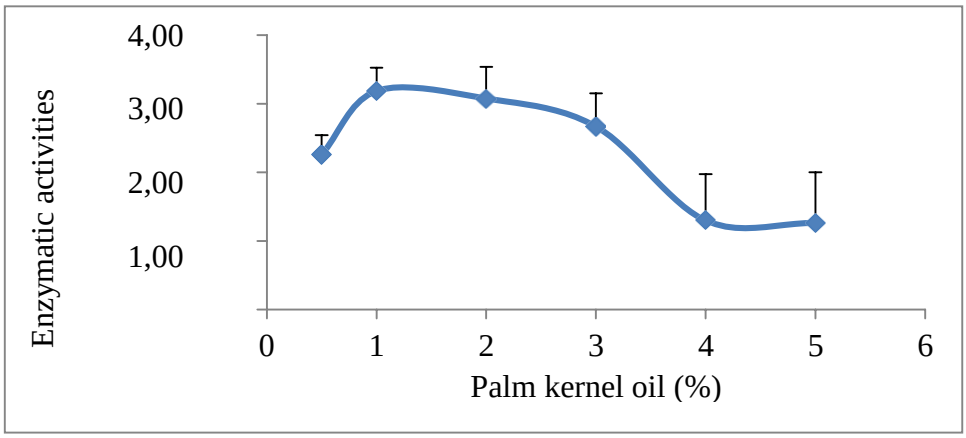


Fig.9 Influence of nitrogen source

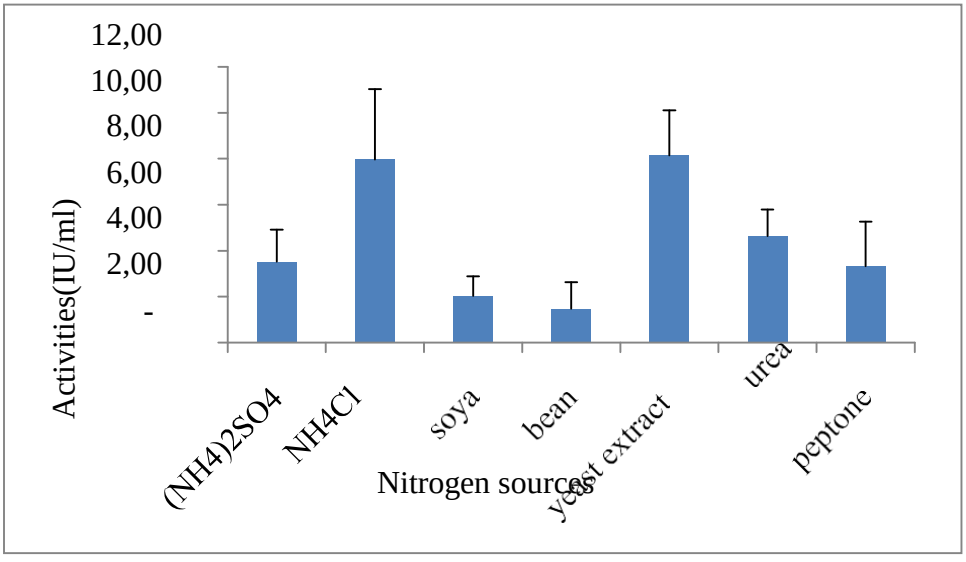


Fig.10 Influence of ammonium chloride concentration (nitrogen source)

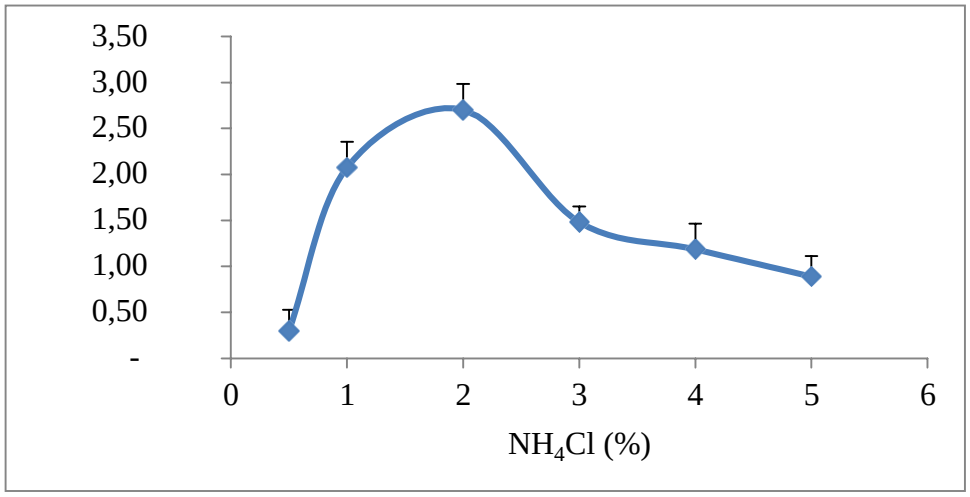


Fig.11 Influence of mineral salts

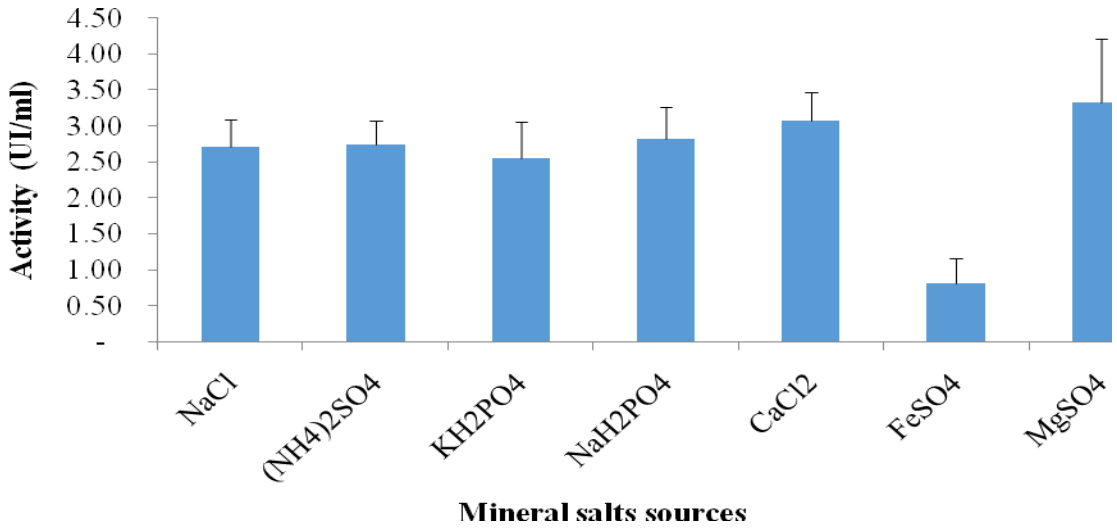


Fig.12 Influence of $MgSO_4$ concentration

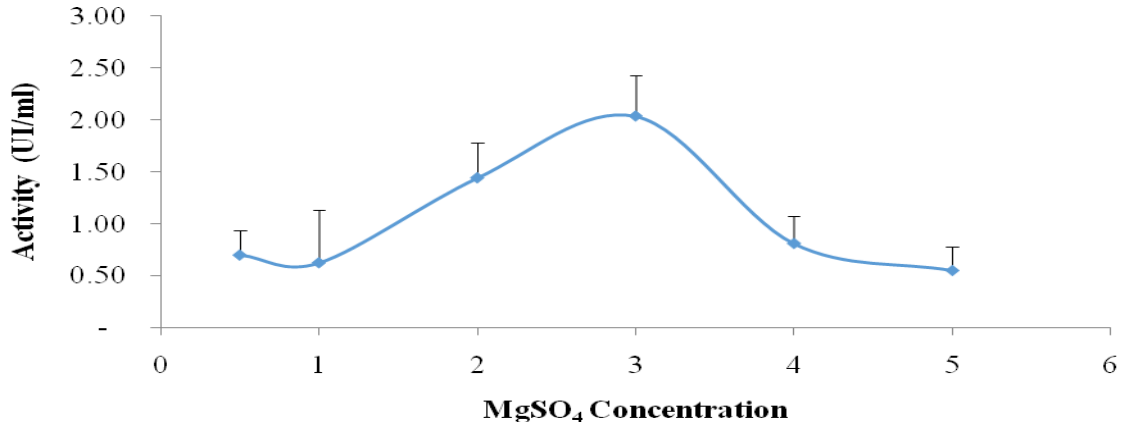


Fig.13 Influence of temperature on enzymatic activity

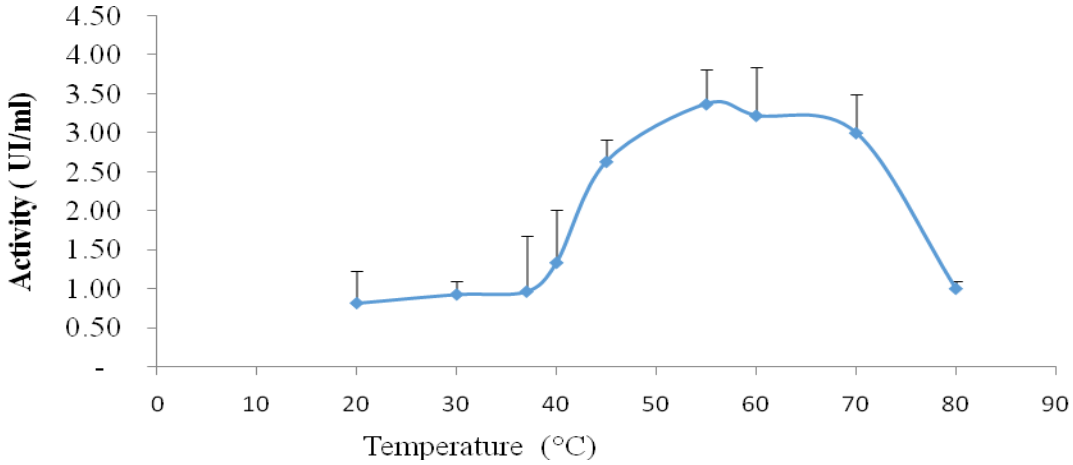
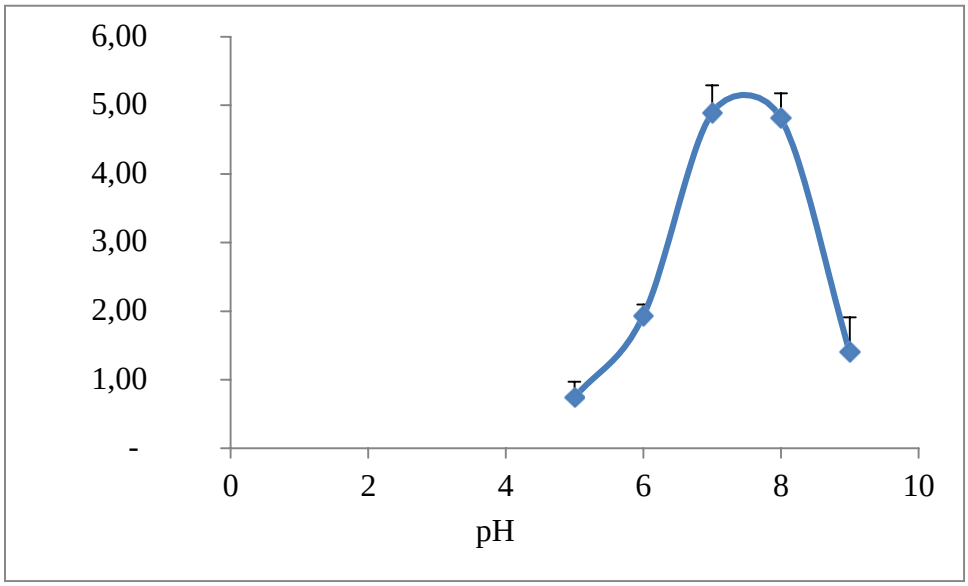


Fig.14 Influence of pH on enzymatic activity



In conclusion, soil samples from palm oil production areas are colonized by bacteria. The latter have high lipolytic capacities. The study of these bacteria shows that this property can be improved by varying the physicochemical parameters. The enzymes produced are of good thermostability and can therefore be used in industry

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