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Characterisation Studies on Lipases of *Brevibacterium, Bacillus and Pseudomonas* Spp Produced Under Sub-Merged Fermentation of Different Carbon Sources

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ABSTRACT

Lipases are a class of hydrolytic enzymes that catalyze the hydrolysis of insoluble triacylglycerol to glycerol, acylglycerols, and free fatty acids. The present study is aimed at identifying Lipase producing bacteria isolated from soil determining the optimal conditions (temperatures, pH, and metal ion concentrations) of Lipases produced through submerged fermentation by bacteria of different three (3) genera. The different bacterial isolates with good hyper-producing potentials for Lipases were identified by Polymerase Chain Reactions (PCR). The effect of temperature on cellulase activity was determined by estimating the lipase activity at pH 9.0 within a temperature range of (30^0C-60^0C). In order to determine the behaviour of the enzymes within some metallic ions, the reaction of the enzyme and gum Arabica/ olive oil mixture was allowed to proceed at 50^0C with duplicate test tubes containing 50mM $CaCl_2$ (Ca^{2+}). $MgSO_4$ (Mg^{2+}), Nacl(Na^+), Kcl (K^+). The identifies of the lipase producing bacteria were identified as *Brevibacterium brevis* strains Hk 544, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* strain BS 01 for Isolate H, A, B and F., respectively. Optimum temperatures for the activities of *Brevibacterium brevis* strains Hk 544, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* strain were determined to be 50°C, 50°C , 45°C and 45°C, respectively. Optimum pH for the activities of *Brevibacterium brevis* strains Hk 544, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* strain were determined to be alkaline (8.0 to 9.0). The observation that sodium and potassium ions at 50mm concentration enhanced the activity of some of the lipases under this investigation showed that sodium and potassium are likely to be co-factors for the performance of these lipases.

INTRODUCTION

Lipases are ubiquitously produced by the plants (Belguith *et al.* 2009); animals (Carriere *et al.* 1994); and microorganisms (Ramesh *et al.*, 2013). Microbial lipases are the preferred potent source due to several industrial potentials (Hasan *et al.*, 2006). Lipases are becoming more and more popular in the field of biotechnology, as they have received great interest in industrial applications because of their properties (Almeida *et al.*, 2019) The uniqueness and its ability to benefit from a wide range of reaction materials and its high stability towards temperature and acidity function and organic solvents, as most industrial processes are carried out at a high temperature and therefore enzymes with high stability (Sahu & Martin, 2011). Lipases are considered to be the third biggest enzymes group following proteases and amylases, based on total sales volume. Because of its extensive range of applications lipase production is a billion dollar (Jaeger *et al.*, 1998). Lipases (Glycerol ester hydrolases E.C. 3.1.1.3) are much-demanded enzymes with significant commercial applications in industries. Lipases stimulate the hydrolysis of triacylglycerol to glycerol and free fatty acids. A real lipase will cleave emulsified esters of glycerin and lengthy chain fatty acids such as triolen and tripalmitin (Gayathri *et al.*, 2013). Many applications of lipases include specialty organic syntheses, hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing, resolution of racemic mixtures, and chemical analyses (Afaf *et al.*, 2020). Microbial lipases detoxify and degrade the oil effluents as one by innovative technologies (Shart & Elkhalil, 2020).

The present study is aimed at identifying Lipase producing bacteria isolated from soil, determining the optimal conditions (temperatures, pH, and metal ion concentrations) of Lipases produced through submerged fermentation by bacteria of different three (3) genera.

MATERIALS AND METHODS

Molecular identification of isolates

DNA extraction

DNA was extracted using the protocol stated by Fuguri *et al.*, (2015). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 °C. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were re-suspended in 520 µl of TE buffer (10) mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 °C, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and vortexed. The suspension was incubated for 10 min at 65 °C and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and

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isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50µl of TE buffer.

Polymerase chain reaction

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'-AAGGAGGTGATCCAGCC-3' primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins. And chill at 4°C. GEL (Hassan *et al*., 2016; Gunajit *et al*., 2017).

Integrity of DNA

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for mix 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then re-suspend with 20 µl of sterile distilled water and kept in -20°C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nano-drop of model 2000 from thermo scientific.

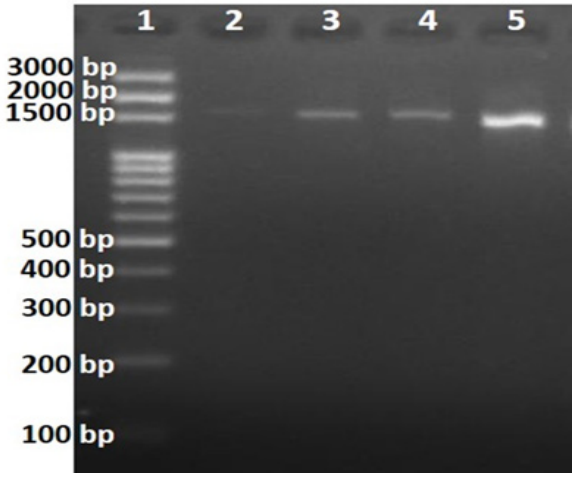

Plate 1: Agarose gel electrophoresis indicating the positive amplification of the bacteria isolate's samples using ITS universal primers (Band 1 is the marker, band 2, 3, 4 and 4 represents *Brevibacterium brevis* strains Hk 544, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* respectively.

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator V3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis.

Lipase production under different carbon source media

The determination of optimum carbon Lipase production medium hydrolytic activity of isolated bacteria lipase was done on composed of (g/L): peptone, 10; NaCl, 5; $CaCl_2.2H_2O$, 0.1; Trybutyrin selective agent, 10 mL (v/v). $(NH^{\neg}4)_2$ SO4(1.4g), K_2HPO_4 (2.0g), $CaCl_2$ (2.0g), $MgSO_4.7H_2O$ (0.3g), peptone (7.5g), $FeSO_4$ (5.0g), $MnSO_4$ (1.6g), $ZnSO_4$ (1.4g). In addition different Erlenmeyer flasks containing the above chemical compounds amended using different carbon sources such as Tobacco seed oil (5ml/100ml of production medium), Tobacco seed oil (10ml /100ml of production medium), Olive oil (5ml/100ml of production medium), Olive oil (10ml /100ml of production medium). The Erlenmeyer flasks were loaded at the shaker incubator at 150RPM for 5days. there after 5 days of incubation; the Lipase activities were determined using NaOH titration method as previously

described above.

Characterization of Enzyme Based on Stability

The purified fraction showing highest specific activity was characterized by varying the parameters that influence enzyme activity.

Effect of pH on Activity and Stability of Lipase

This was determined by emulsifying 25ml of olive oil with 75ml of 7% gum Arabic for 10mins. The reaction mixture containing 5ml of olive oil emulsion, 2ml of 0.1M phosphate buffer (pH 7.0) Gum Arabica 1% (w/v) as substrate suspended in various buffer systems: 0.1M sodium acetate buffer (pH 6.0 - 7.0): 0.1 M sodium phosphate buffer (6.0-7.0); tris-HCl buffer (8.0) and a glycerine- NaOH buffer (pH 9.0-). The pH stability studies was performed by pre-incubating the purified enzyme without substrate in pH values ranging from 4.0-10.0 at 50 0C for 2 h and subsequent analysis was determination under standard assay carried out (Okoli *et al*., 2017).

Effect of Temperature on Activity and Stability of Lipase

The effect of temperature on Lipase activity was determined by estimating the lipase activity at pH 9.0 within a temperature range of (30^0C-60^0C) for 2h using gum Arabica and olive oil mixture as substrate. The thermal stability of lipase was determined by pre-incubating the purified enzyme preparation (pH 9.0) at different temperatures (30^0C - 60^0C) for 2 h without substrate. The residual cellulase activity was determined under standard assay conditions (Sharma *et al*., 2018; Okoli *et al*., 2017).

Effect of Metal Ions on Enzyme Activity

The reaction of the enzyme and gum Arabica/olive oil mixture was allowed to proceed at 500C with duplicate test tubes containing 50mM $CaCl_2$ (Ca^{2+}). $MgSO_4$ (Mg^{2+}), Nacl(Na^+), Kcl (K^+). Test tubes were labeled according to the above mentioned salts. This was determined by emulsifying 25ml of olive oil with 75ml of 7% gum Arabic for 10mins. The reaction mixture containing 5ml of olive oil emulsion, 2ml of 0.1M phosphate buffer (pH 7.0), Gum Arabica 1% (w/v) as substrate suspended in 0.1M phosphate buffer, In addition. Into each of the tubes 50mm of the various metal ions (5ml) were added, incubated at 45^OC, and thereafter titrated against 0.1N NaOH using Phenolphthalein indicator.

Determination of Lipase activities

Lipase screening assay was carried out using olive oil emulsion prepared by emulsifying 25ml of olive oil with 75ml of 7% gum Arabic for 10mins.

Titration: 2-3 drops of phenolphthalein indicator were added to the reaction mixture and the liberated free fatty acids were titrated with 0.5N NaOH to the end point of pink color at pH 10.0 (Macedo *et al*., 1997; Lopes *et al*., 2011; Mendes *et al*., 2011; Bhavani, *et al*., 2012; Nagarajan *et al*., 2014; Ullah *et al*., 2015). Lipase activity was calculated as micromoles of free fatty acids formed from olive oil per ml of lipase enzyme as given by the equation:

$$\text{Activity} = \frac{(VS - VB).\ N.\ 1000}{S}$$

Where, VS is the volume of 0,05M NaOH solution consumed by the enzyme _substrate cocktail (ml); VB is the volume of 0.05M NaOH solution consumed in the titration by the substrate (control) cocktail; N is the molar strength of the NaOH solution used for titration (0.05M); S is the volume of substrate cocktail solution. One unit of lipase enzyme is defined as the amount of enzyme required to liberate 1µmol of fatty acids from triglycerides (Okoli *et al*., 2019).

RESULTS AND DISCUSIONS

The identifies of the lipase producing bacteria were identified as *Brevibacterium brevis* strains H k 544, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* strain BS 01 for Isolate H, A, B and F., respectively (Table 1). These bacterial isolates had their genomes blasted on the blast software of the NCCI, and similarities between 99-100% at different accession numbers were recorded (Table 1.)The bands of the nucleotide were also arranged in get as shown in plate.

Table 1: Summary of Molecular Identity of some selected lipase hyper-producing strains of Bacteria

S/N	Isolate codes	Identity	% Simi-larity	Accession Num-ber
1	H	*Brevibacillus brevis* strain HK 544	99.44	CPO42161.1
2	A	*Pseudomonas aeruginosa* WES2	99.93	MN960116.1
3	B	*Bacillus megaterium* strain WH13	100	Mn372086.1
4	F	*Bacillus subtilis* BS 01	100	MT372489.1

This current investigations that observed different strain of *Brevibacterium brevis*, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* strain BS 01 as hyper –producing bacteria is also tandem with other reports by other workers at different parts of the globe. Ertuğrul *et al*., (2007) reported the use of hyper – producing strain of *Bacillus* from lipase production. Kiran *et al*. (2008) also documented the use of *Pseudomonas* for sub nerved production of extra- cellular lipase.

This report on this study is also in tandem with periods study by Bradoo *et al*. (1999) which also documented lipase- hyper producing strain of *Bacillus* species resident in soil.

In independent related studies, Ambu *et al*., (2010) reported the presence of extra-cellular lipase producing *Acinetobacter Junii* in the soil of south Korea. Bompensieri *et al*. (1996) had also reported the isolation of Lipse-Producing bacterial such as *Acinetobacter* from different

environmental sources.

Thus, one concludes that the observation of *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Brevibacterium* strains in this study as Lipases hyper-Producing is within the pattern of previous observation in this subject matter. The physiology and genetics of enzyme expressions in microorganisms show that enzyme production potentials in bacteria, and fungi lie on genes and equally regulated by genes. This could be extended to mean that any microorganisms irrespective of genes or species can produce any enzyme provided it has the genes to express such enzymes. In a horizontal gene transfer within a wild environment, bacterial and fungal strains can transmit genes from one genus to closely related genus.

In addition, under a conventional condition, bacterial and fungal isolates that do not have to express lipase production, could along their co-existence with lipase-producing bacterial and fungal isolates acquire the genes for lipase production either on their genome/ chromosome of outside the chromosome (in this case, it is said that the microorganism has acquired plasmids for such enzyme productions).

The lipases isolated under different conduction from Isolate H (*Brevibacterium brevis*), Isolate A (*Pseudomonas aeruginosa*), Isolate B (*Bacillus megaterium*), and Isolate F (*Bacillus subtilis*) were subjected to temperature stability studies (Figure 1).

Brevibacterium brevis at temperatures of 25^0, 35^0, 40^0, 54^0, and 60^0 on a centigrade scale showed a lipase activity of 8.0, 9.0, 7.0, 10.0, 22, and Units of lipase respectively (Figure 1). This showed that lipase of *Brevibacterium brevis* has an optimum temperature of 50^0c. This implies that industrial application using this lipase of *Brevibacterium brevis* strain HK 544 must be maintained at 50°C to achieve the best desired hydrolysis.

Furthermore in a related study, Luz *et al.* (2021) reported *Pseudomonas fluorescens* isolated from water, bryophytes and soil showed different tolerance pattern to temperature. Luz *et al.* (2021) showed that S. marcescens lipase had an optimum temperature at 35°C while *Pseudomonas fluorescens* lipase had optional temperature at temperature of 55°C.

Pandey *et al.* (2016) working on lipase of Penicillum fungus discovered that temperature of 25^0C. Kojima *et al.* (1994) observed that similar strain of *Pseudomonas*

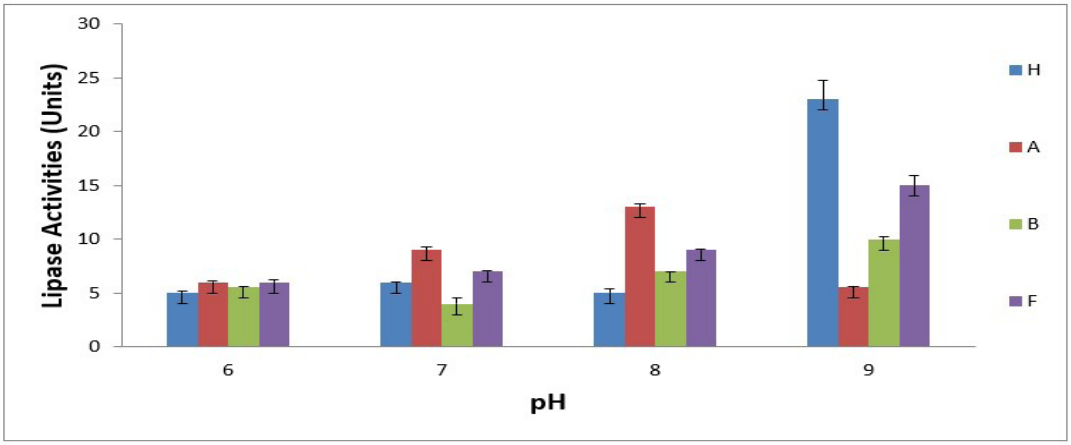

Figure 1: pH stability of the lipase enzymes from *Brevibacillus brevis* strain HK 544 (ISOLATE H) *Pseudomonas aeruginosa* WES2 (ISOLATE A), *Bacillus megaterium* strain WH13 (ISOLATE B) and *Bacillus subtilis* BS 01 (ISOLATE F)

fluorescens has an optimal temperature of 55^0C.

In addition, Lipase isolated from Isolate A (*Pseudomonas aeruginosa* WES 2) should lipase activities of 10.0 units 12.0 14.0, 19.0 30.0, and 13.0 units, at temperatures of 25^0C, 35^0C, 40^0C, 45^0C, and 60^0C respectively (Figure 1). This implies that 50°C remain the best /optimum temperature that can active the highest level of hydrolysis during the application of two other workers in Brazil (Chandra *et al.* 2020) in an extensive review reported that Lipases of *Chromobacterium viscosum*, *Aspergillus niger*, *Rhizopus* species and *Pseudomonas nitroaceducans* has an optimal activities at lower temperatures between 35°C – 40°C. This is in contrast with this current study as lipase of *Pseudomonas aeruginosa* has optimal activity a temperature of 50° on a centigrade scale.

This current work is in agreement with the report of Kiran *et al.* (2008) which reported lipase of marine strain of *Pseudomonas aeruginosa* having an optimal temperature of 40-50^0C.

Lipase of bacterial isolate B (molecularly identified as *Bacillus megaterium* strain WH 13) at temperature of (25°C, 35°C, 40°C, 45°C, 50°C and 60°C showed Lipase activities of 5.0, 6.0, 9.1, 20.0, 8.0, and 5.0 units, respectively (Figure 1). Statistical analyses using ANOVA (analyses of variance) showed that among the four (4) Lipases from four (4) different bacterial isolates in this study, Lipase of *Bacillus megaterium* had the least response in terms of Lipase activities. It is also recorded that the optimum temperature for Lipase isolated from *Bacillus megaterium* was observed at 45^0C. In another study on Lipase of *Bacillus* species isolated from the hot spring of had an optimal temperature of 60°C.

Thermal stability of Lipase of *Bacillus megaterium* strain WH 13 at 40°C could be also explained by the presence of polyamines in the general protein structure. The increased number of hydrogen bonds, salt bridges and

relatively high amount of thermo-tolerant amino acids may contribute to the thermal stability of this Lipase at 40°C. The result of this current study is in contrast with the work of Brune & Gotz (1992), which reported that Lipase of *Pseudomonas* sp KWA-56 showed an optimum temperature of 60°C and remain stable at 60°C.

In terms of ergonometric, it has become very important to develop novel enzymes that remain active and stable temperature ranges. This enzyme being most active at 40°C implies a lot of cost optimization implication as little heat energy is required to achieve enzyme- substrate catalysis during industrial applications (Brune & Gotz, 1992).

In terms of bio-energetics, Lipase of *Bacillus megaterium* at 40°C means that little/ relatively low amount of heat energy is required to achieve bund breaking in substrates in order to reduce the activation energy, create an alternative pathway and establish catalysis products (Fatty acids in this case). Studies showed that the Lipase of *Bacillus subtilis* (isolate F) at temperatures of 25°c, 35°c, 40°c, 45°c, 50°c, and 60°c showed Lipase activities of 6.0, 16.0, 8.0, 20.0, 4.0, and 4.0 units, respectively. In this case, it is deduced that the Lipase of *Bacillus subtilis* had two temperature optima (35°C and 45°C).

This implies that industrial applications of this *Bacillus subtilis* Lipase is best at 35°C and 45°C in order to achieve the best hydrolysis desired. Bakir & Metin (2017) identified a thermophilic *Bacillus* species from the hot springs of Ayidin, Turkey and reported in a similar fashion to this work that the thermophilic *Bacillus* sp strain remained most active at temperature of 45°C – 50°C.

Other similar independent studies also had previously confirmed that *Bacillus coagulans* Lipases have optimum temperature ranges of 45°C – 55°C (Fojan, 2000; Lima *et al*., 2004; Sulong *et al*., 2006). The pH responses of the Lipase invested from *Pseudomonas aeruginosa* (Isolate A) showed that pH values of 6.0, 7.0, 8.0, and 9.0, showed Lipase activities of 6.0, 9.0, 13.0 and 5.6 units statistical analysis using ANOVA showed that there are significant differences in the units of activities from pH 6.0 to pH 9.0. Inductively, the Lipase of this *Pseudomonas aeruginosa* strain WES 2 (Isolate A) has alkaline pH optima and applications must be maintained within alkaline ranges to ensure optimal hydrolysis by the enzyme. This observation is in tandem with similar studies in Japan where Yoshitaka *et al.* (1982) reported the presence of alkaline Lipase from different strain of Gram-negative *Alcaligenes* species.

Relatively recently, Liew *et al.* (2015) reported the isolation of alkaline Lipase from *Burkholderia cepacia* after optimization using submerged fermentation technology. The result obtained in this study is further agreement with the work of Gupta *et al.* (2009) which reported isolation of alkaline Lipase from *Burkholderia* sp C20.

Furthermore, Liu *et al.* (2006) and Sharma *et al.* (2002) independently observed at different location that strain of Arthrobacter sp had Lipases with optimum activity at pH of HP8.0 – 9.0.

In addition, the work of Gupta *et al.* (2004) contradicts this current study. Gupta *et al.* (2009) reported that some bacterial Lipase showed optimum activities at neutral pH (7.0). The pH responses of the three (3) Lipase investigations were studies and documented in Figure 2.

Lipase harvested from isolate H (*Brevibacillus brevis* strain Hk 544) at pH values of 6.0, 7.0, 8.0, and 9.0 showed Lipase activities of 5.0, 6.0, 5.0, and 23.0 units, respectively. This implies that the Lipase of *Brevibacillus brevis* strain HK 544 is an alkaline Lipase and all application of the enzyme must be carried out using pH 8.0. (Figure 2) Similarly in Pradesh, India Bora & Bora (2012) reported the isolation of alkaline Lipase from thermophilic *Bacillus* species resident in soil. The study in strong agreement with the report of Rathi *et al* (2001) which observed strains of *Bacillus* species with Lipase having optimal activities beyond 17.5 units of activity at pH of 8.5 (alkaline).

The Lipase harvested from *Bacillus megaterium* WH 13 (isolate B) at pH values of 6.0, 7.0, 8.0, and 9.0 had activities of 5.6, 4.0, 7.0 at 10.0 units, respectively. This showed that the Lipase from WH 13 strain of *Bacillus*

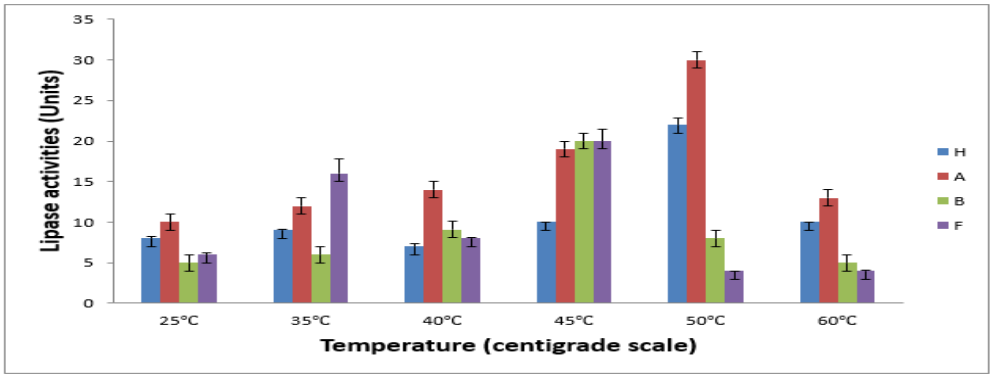

Figure 2: pH stability of the lipase enzymes from *Brevibacillus brevis* strain HK 544 (ISOLATE H) *Pseudomonas aeruginosa* WES2 (ISOLATE A), *Bacillus megaterium* strain WH13 (ISOLATE B) and *Bacillus subtilis* BS 01 (ISOLATE F).

megaterium is an alkaline type of Lipase with pH 9.0 as optimum pH for optimal activity or hydrolysis.

Rasmey *et al.* (2017) took a different position and reported that *Pseudomonas monteili* 2403 showed optimum Lipase activities at pH 6.0 which is moderately acidic. Other scholars such as Qamsari *et al.* (2011), Sooch and Kaulder (2013) had independently reported that different species and strains of *Bacillus* and *Pseudomonas* spp expressed

Lipases that had optimum activities at pH ranges of 6.0 – 6.5.

In addition, Lipase harvested from *Bacillus subtilis* (isolate F), at pH values of 6.0, 7.0, 8.0, 9.0 showed a lipolytic activity of 6.0, 7.0 9.0 and 15.0, respectively (Figure 2). This equally showed alkaline optimum pH, and could be used at pH 9.0 for the best hydrolytic performance.

This observation in the current investigation, is in agreement with previous study by Prasasty *et al.* (2016) which documented that *Bacillus* species and *Pseudomonas alcaligenes* had Lipases with optimum activities of 1.0 x 104 U/mg and 0.8 x 104 U/mg, respectively (Prasaty *et al.* (2016.).

However, Tang and Xia (2005). showed that *Bacillus coagulans* ZJU strain works optimally at 7.0 – 10.0 pH values and activity remained depressed at acidic pH ranges. In further characterization based on reaction with heavy metals from alkalis and alkaline earth metals in the periodic group, the performance as regards activities of the three (3) Lipases have been documented as in figure 3.

Lipase of *Brevibacterium brevis* HK 544 got higher lipolytic activity of 20 units at 50mM concentration of sodium and at 50MmM, concentrations of Potassium (K), Calcium (Ca), and magnesium (Mg) the lipase harvested from *Brevibacterium brevis* stain HK 544 got repressed to 6.0, 8.0 and 8.0 units, respectively (Figure 3).

The observation that sodium ion at 50mm concentration enhanced the activity of lipase of *Brevibacterium* showed that sodium is likely to be a co-factor for the performance of this lipase from B. brevis.

The lipase of *Pseudomonas aeruginosa* (Isolate A), showed a repressed enzyme activity of 6.0/12.0 and 9.0 units at

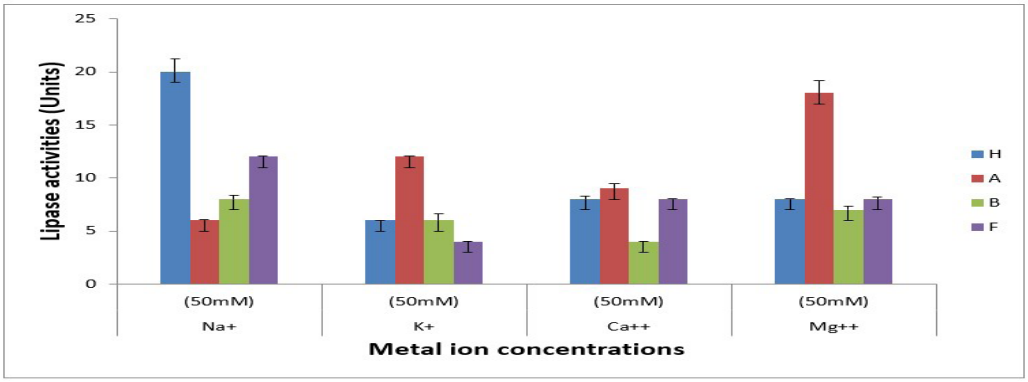

Figure 3: Metallic ion stability of the lipase enzymes from *Brevibacillus brevis* strain HK 544 (ISOLATE H) *Pseudomonas aeruginosa* WES2 (ISOLATE A), *Bacillus megaterium* strain WH13 (ISOLATE B) and *Bacillus subtilis* BS 01 (ISOLATE F)

50mM concentration of Sodium (Na^+) Potassium (K^+), and Calcium (Ca^{2+}) ions, while at 50mM concentration of magnesium (Mg^{2+}) had an enhanced activity of 18.0 units of lipase activity. The essence of this characterization has identified magnesium as possible co-factor for the activity of *Pseudomonas aeruginosa* Wes2 strain.

In a related pattern, the lipase of *Bacillus subtilis* BS 01 (isolate f) had a strong increase in activity on exposure of substrate and enzyme under 50mM concentration of sodium ion (12.0 units) while the activities of the *Bacillus subtilis* enzyme /lipase at 50mm concentrations of K^+, Ca^{2+} and Mg^{2+} had repressed enzyme units of 4.0, 8.0, and 8.0 units (figure 3).

The Lipase of *Bacillus megaterium* strain WH13 (Isolate B,) showed complete repression on exposure with 50mm concentrations of sodium (Na^+), potassium (K^+), Calcium(Ca^{2+}), and Magnesium (Mg^{2+}) as 8.0, 6.0, 4.0, and 7.0 Units of Lipase activities lose recorded respectively (Figure 3).

CONCLUSION

The Properties of Lipases from *Brevibacterium brevis* strains Hk 544, *Pseudomonas aeruginosa* strain WES, *Bacillus megaterium* strain WH13, and *Bacillus subtilis* strain BS 01have been tracked and industrial application of any of the Lipases can now be achieved under the scientifically proven-conditions as observed in this study. The study is expected to utilize the produced Lipases for production of Biodiesel. However, at this point, the aspect of Biodiesel production using the Lipases cannot be established as a result of limitation in funds.

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