

Lipolytic Activity and Molecular Characterization of *Pseudomonas aeruginosa* and *Lysinibacillus sphaericus* from domestic oil rich wastewater

ABSTRACT

The role of lipases in bioremediation and other industrial applications has heightened the importance of lipolytic microbes. In this study, the growth dynamics of microbes in lipid rich environments were monitored and highly lipolytic microbes were characterised by PCR amplification and sequencing of the 16S rRNA gene.

Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, between September 2010 and August 2011. The sequences were analyzed using the BLAST (Basic Local Alignment Search Tool) bioinformatics program on the NCBI (National Center for Biotechnology Information) website. Also, fatty acids in methylated oils were characterized and quantified using High Performance Liquid Chromatography (HPLC). All the lipolytic bacteria showed appreciable growth rates in the wastewater (between 0.67 and 1.67mg/day) within 5 days. The most effective lipolytic bacteria isolates in the oil-rich wastewater were two species of the genera *Pseudomonas* and one *Bacillus* sp. Comparing the weights on the first day to the twelfth day values in palm oil, some appreciable increases in weight difference were recorded for some isolates of the genera *Pseudomonas*, *Staphylococcus*, *Bacillus* and *Klebsiella*; 28.3%, 7.84%, 4.44% and 6.98% respectively. The weight increase of each of the microbial cells in palm oil culture was usually lesser than what was obtained in the oil-rich wastewater culture. Only one isolate of *Pseudomonas* sp. and *Bacillus* sp. had informative electropherograms, and consequent BLAST revealed that the *Pseudomonas* sp. and *Bacillus* sp. were most similar (99%) to *Pseudomonas aeruginosa* (CP006245.1) and *Lysinibacillus* sp. (FR775037.1) respectively. When compared to the lipolytic activity of *Pseudomonas aeruginosa* (CP006245.1) in palm oil, *Lysinibacillus sphaericus* (FR775037.1) synthesized more myristic acid, palmitic acid, stearic acid and arachidic acid (2.61%, 6.22%, 5.18% and 3.66% respectively) into the medium.

Keywords: Lipolytic, oil-rich wastewater, PCR, *Pseudomonas aeruginosa*, *Lysinibacillus sphaericus*, fatty acids

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19 **1. INTRODUCTION**

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The study of product authenticity based on genomic profiling has been rapidly developed and improved with the progress of molecular biology, from the development of polymerase chain reaction (PCR) to direct gene sequencing. Invented in 1983 PCR offers a simple *in vitro* process to amplify a targeted fragment of DNA [1] by a repeated cycling process of defined stages [2]. Also, DNA sequencing is the most informative and precise technique for species identification. DNA sequencing is a modification of DNA replication, which differs from normal replication in the inclusion of dideoxynucleotides (ddNTPs) instead of dNTPs [3]. PCR products are used as sequencing template. This technique, called direct sequencing, eliminates the need for cloning and thereby significantly saves time and materials.

Microbial lipases are highly sourced due to their specificity of reaction, stereo specificity and less energy consumption than conventional methods. Lipase-producing microorganisms have been found in different habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, and decaying food [4]. Many microorganisms such as bacteria, yeast and fungi are known to secrete lipases. The oily environment (oil mill effluent) may provide a good environment for isolation of lipase producing microorganisms.

Several lipase-producing bacteria were isolated from wastewater of an oil processing plant [5]. The strain possessing the highest lipase activity was identified both biochemically and by sequencing of 16S rRNA gene as a *Pseudomonas aeruginosa* strain KM110. An overall 3-fold enhanced lipase production (0.76 U mL^{-1}) was achieved after improving conditions of production medium. The olive oil and peptone was found to be the most suitable substrate for maximum enzyme production. Also the enzyme exhibited maximum lipolytic activity at 45°C where it was also stably maintained. The present study is

45 to monitor growth dynamics and characterize the lipolytic organisms from domestic oil-rich
46 wastewater disposed into the environment.

47 48 **2. MATERIAL AND METHODS**

49 50 **2.1 Source of Bacteria**

51 The bacteria isolates used in this study have been previously described [6]. Isolates
52 were recovered from oil-rich wastewater samples from a restaurant in Ado-Ekiti, Nigeria.
53 The isolates had previously been identified on the basis of standard physiological,
54 biochemical, and serological assays. The isolates include one hundred and eleven bacteria
55 belonging to the following genera; *Pseudomonas*, *Staphylococcus*, *Enterococcus*,
56 *Escherichia*, *Bacillus*, *Streptococcus* and *Klebsiella*. These organisms were obtained from
57 wastewater after dish washing (with soap solution; 5g/100ml detergent in water), first and
58 second rinsing of the dishes, and run-off into open sewers along the drainage.

59 **2.2 Growth of bacteria isolates in wastewater and palm oil**

60 A pure colony of each isolate was inoculated on sterile Tributyrin agar (TBA) and
61 incubated at 37°C for 24h. Lipolytic activity of each isolate was confirmed by the
62 development of zone (s) of clearance around each colony [7]. Subsequently, 0.1 ml and 10
63 ml of known concentration of organisms was inoculated into 100 ml sterile oil-rich
64 wastewater sample and 90 ml of fresh palm oil respectively. The mixture was incubated at
65 ambient temperature and growth monitored for 12days. The chemical composition of the
66 wastewater and palm oil used as growth media is shown in Table 1. All the experiments
67 were carried out in triplicates and conducted twice.

68 **2.3 Characterization and enumeration of fatty acids in the methylated oils**

69 The AKTA basic 10/100 high performance liquid chromatography (Amersham
70 Pharmacia Biotech, Sweden) was used for the fatty acids determination. The analysis was
71 carried out using Supercosil LC-18 column, 25cm x 4.6mm ID, 5µm particles. The mobile
72 phase consisted of acetonitrile: acetone (59:41; v/v) with a flow rate of 1.0ml/min and fatty

acids were detected using a UV (UV-900) detector. Ten microlitre sample of the fresh and biodegraded palm oil was injected for each run. The fatty acid components in the test samples were identified by comparing retention times to those of the standards. Each fatty acid in the test sample was expressed in percent of the total fatty acids present. The methyl esters of lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids were used as standards for identification [8].

Calculation: % Fatty acid = $\frac{\text{Peak area of the sample} \times \text{conc. of fatty acid} \times \text{diluting factor}}{\text{Peak area of the standard}}$

2.4 Molecular Characterization of Bacterial Isolates

2.4.1 Extraction of DNA

One millilitre of fresh bacterial culture was dispensed into a labelled microcentrifuge tube and centrifuged at 13,500 rpm for 3 mins. The supernatant was decanted and the pellet resuspended in 200µl of STET buffer (0.1M NaCl, 10mM Tris-Cl (pH 8.0), 1mM EDTA (pH 8.0). and 5% (v/v) Tween 20). Subsequently, 50µl of lysozyme (10mg/ml, pH 8.0) was added to the suspension, vortexed for 5 seconds and incubated at room temperature for 1min. To the suspension, 600µl of Lysis buffer (68% guanidium thiocyanate) was added, vortexed for 1 min and incubated at room temperature for 15 min., afterwards, 800µl of isopropanol was added to the solution, vortexed for 5 seconds and centrifuged at 13,500 rpm for 15 min. The supernatant was discarded and 1ml of 70% ethanol was added to the pellet. The suspension was vortexed for 5 seconds and centrifuged at 13,500rpm for 5 min. Finally the pellet was resuspended in 200µl of 10mM Tris-Cl (pH 8.0), 1mM EDTA (TE) buffer and 5µl of the extract was run on 1% Agarose gel to detect DNA if present.

2.4.2 Polymerase Chain Reaction (PCR)

About 2.5ng of bacterial genomic DNA was added to a 50 µl PCR mix which contained 1 X Hot start reaction buffer, 0.25mM dNTPs, 0.01M (each) primers 27F and 1525R, and 2.5 U Hot start polymerase (Jenabioscience). Thermal cycling was done in a veriti thermal cycler (Applied Biosystems, USA) and cycling conditions were 95°C for 3 min

100 followed 45°C cycles of 95°C for 30secs, by 45°C for 1 minute, 72°C for 1min 30secs with
101 ramp from 45°C to 72°C set at 40%. Subsequently, the reaction was held at 72°C for
102 10minutes after which it was held at 4°C till terminated. PCR products were resolved on 1%
103 agarose gel stained with ethidium bromide and viewed on a transilluminator.

104 **2.4.3 Sequencing of amplified 16S rRNA gene**

105 The PCR products were purified using Montage PCR Clean up kit (Millipore). The
106 purified PCR products of approximately 1,500 bp were sequenced using 4 primers (27F -
107 AGA GTT TGA TCM TGG CTC AG, 1492R - TAC GGY TAC CTT GTT ACG ACT T, 518F -
108 CCA GCA GCC GCG GTA ATA CG and 800R - TAC CAG GGT ATC TAA TCC).
109 Sequencing was performed using Big Dye terminator cycle sequencing kit v.3.1 (Applied
110 BioSystems, USA) and sequencing products were resolved on an Applied Biosystems model
111 3130XL automated DNA sequencing system (Applied BioSystems, USA) at the Macrogen,
112 Inc., Seoul, Korea.

113 **2.4.4 Bioinformatics analysis**

114 Sequences were analyzed using the BLAST (Basic Local Alignment Search Tool)
115 bioinformatics program on the NCBI (National Center for Biotechnology Information) website.
116 BLAST was done to identify 16S rRNA sequences in Genbank most similar to the query
117 sequence.

118 **2.4.5 Nucleotide sequence accession numbers**

119 Sequences reported here have been submitted to the Genbank Nucleotide
120 Sequence Database under the accession numbers KF420486 and KF420487

121 **3. RESULTS AND DISCUSSION**

122
123 Twenty one (18.9%) of the one hundred and eleven isolates showed lipolytic activity
124 with zones of clearance on TBA above 0.25mm (Table 2). Of these only nine organisms
125 exhibited very high lipolytic activity (between 0.35 and 0.41mm, clearance zone): one
126 *Bacillus* sp. (p), one *Klebsiella* sp. (m), five *Pseudomonas* spp. (g, j, s, w and y) and two
127 *Staphylococcus* spp. (c and r).

128 **3.1 Growth of lipolytic organisms in wastewater**

129 The growth pattern of the lipolytic microbes in wastewater varied (Table 3).
 130 Following 24h incubation, no microbial mass was recorded in the wastewater inoculated with
 131 *Bacillus* sp. (p) and *Pseudomonas* sp. (w). However, *Pseudomonas* sp. (aa) grew well
 132 (2.0mg) within this period. About eighty two (81.8%) percent of the isolates (9/11) recorded
 133 maximum growth (5.0mg and above) after six (6) days of incubation in the wastewater.
 134 *Klebsiella* sp. (m) and *Pseudomonas* sp. (j) grew very well (weight > 9.0mg) after 6 days. On
 135 the other hand, *Pseudomonas* sp. (w) attained maximum growth on the fifth day. Meanwhile
 136 on day 12, only *Bacillus* sp. (p) and *Pseudomonas* sp. (w) still had twice the initial cell mass
 137 recorded after 24h incubation. *Pseudomonas* sp. (dd) exhibited about sixty seven percent
 138 weight increase (Table 3).

139 All the lipolytic bacteria showed appreciable growth rates in the wastewater
 140 (between 0.67 and 1.67mg/day) within 5 days. The percentage weight difference between
 141 the first and the twelfth day incubation indicated some substantial growth differences in
 142 *Pseudomonas* sp. (w) and *Bacillus* sp. (p) with each having a 100% weight increase. There
 143 was also an appreciable increase in the weight of *Pseudomonas* spp. (dd), with 66.7%
 144 weight increase. Hence, the most effective lipolytic bacteria isolates in the oil-rich
 145 wastewater were *Pseudomonas* sp. (dd), *Pseudomonas* sp. (w) and *Bacillus* sp. (p).

146 **3.2 Growth of lipolytic organisms in palm oil**

147 The weight of the isolates after 24h incubation in palm oil ranged between 0.33mg
 148 and 0.48mg, and the final weight of these organisms on the 12th day was between 0.43mg
 149 and 0.51mg. Generally, the optimum growth in all the lipolytic microbial isolates in the fresh
 150 oil was between the fifth and eighth days (Table 4). The growth rate of the organisms within
 151 5 days ranged between 0.12mg/day in *Pseudomonas* sp. (n), *Klebsiella* sp. (m),
 152 *Staphylococcus* sp. (r), and *Bacillus* sp. (p) and 0.1mg/day in *Pseudomonas* sp. (j).
 153 Comparing the weights on the first day and the twelfth day, there were some appreciable
 154 increases in weight difference noticed for isolates *Pseudomonas* sp. (j), *Staphylococcus* sp.

(r), *Bacillus* (p), and *Klebsiella* sp. (m) with values: 28.3%, 7.84%, 4.44% and 6.98% respectively. The weight increase of each of the microbial cells in palm oil culture was usually lesser than what was obtained in the oil-rich wastewater culture.

In selecting bacteria isolates for further studies, the weight of each selected bacteria isolate on the twelfth day was compared to the weight after the first day of incubation. The percentage weight difference was between 2.04% and 28.3%, showing significant difference at $p \leq 0.05$. The decomposition of dietary oil was primarily dependent on the varied lipolytic ability of the various bacteria as was observed by the varied cell weight differences.

3.3 Molecular characterization of bacteria isolates

Genomic DNA was extracted from all six lipolytic bacteria isolates. The entire 1.5kb 16S rRNA gene was amplified and sequenced. Electropherograms of four of the six sequencing reactions revealed multiple peaks. Only those of *Pseudomonas* (j) and *Bacillus* (p) had clean peaks and were consequently analysed further. A search of the Genbank, using the BLAST tool showed *Pseudomonas* (j) and *Bacillus* (p) to be most similar (99%) to *Pseudomonas aeruginosa* (CP006245.1) and *Lysinibacillus sphaericus* (FR775037.1) respectively.

3.4 Degradation of lipids by *Pseudomonas aeruginosa* and *Lysinibacillus sphaericus*: Production of fatty acids

Following the molecular characterization of *Pseudomonas aeruginosa* and *Lysinibacillus sphaericus*, the degradative activity of the organisms in palm oil was monitored for 12 days. The palm oil used in this study was very rich in palmitic acid, oleic acid and linoleic acid constituting about 38.9%, 34.7% and 13.1% of the total fatty acid content respectively. The other major components of the oil are stearic (5.58%) and petroselinic (5.59%) acids (Table 5a). The utilization of the dietary oil as substrate by the microbes may be explained by fatty acids profiling of the palm oil over specific incubated periods (8th day) where outstanding microbial growth was experienced and the end of experimentation (12th day) (Table 4). *Pseudomonas aeruginosa* reduced the initial concentration of petroselinic

182 acid, oleic acid, and linoleic acid by 2.16%, 2.8%, 2.7% respectively after 8 days; indicating
183 that these FAs are the most utilized for the growth of the microbe. However, it was observed
184 that the organism synthesized more palmitic acid (5.05%) and stearic acid (2.09%) into the
185 medium during the same period while *Lysinibacillus sphaericus* produced greater amount of
186 palmitic acid (5.45%). When compared to *Pseudomonas aeruginosa*, it appeared *L.*
187 *sphaericus* preferred to utilize linoleic acid [3.65%] for growth better than it would with
188 petroselinic acid [1.63%].

189 Of the thirty two fatty acids analyzed, it was observed on day 12 that *Lysinibacillus*
190 *sphaericus* depended mostly on petroselinic acid, oleic acid and linoleic acid for growth;
191 metabolizing 3.66%, 4.55% and 5.61% of the initial amount of these acids (Table 5b). When
192 compared to the lipolytic activity of *Pseudomonas aeruginosa* in palm oil, *Lysinibacillus*
193 *sphaericus* produced more myristic acid, palmitic acid, stearic acid and arachidic acid
194 (2.61%, 6.22%, 5.18% and 3.66% respectively) that was absorbed into the medium during
195 this period.

196 **3.5 Discussion**

197 Twenty one (18.9%) of the one hundred and eleven isolates screened in this work
198 showed lipolytic activity with zones of clearance on TBA above 0.25mm. Of these only nine
199 organisms exhibited very high lipolytic activity (between 0.35 and 0.41mm, clearance zone),
200 one *Bacillus* sp. (p), one *Klebsiella* sp. (m), five *Pseudomonas* spp. (g, j, s, w and y) and two
201 *Staphylococcus* spp. (c and r). The growth pattern of these lipolytic microbes in wastewater
202 varied. However, all the lipolytic bacteria showed appreciable growth rates in the wastewater
203 (between 0.67 and 1.67mg/day) within 5 days. The cells of *Bacillus* sp. (p) and
204 *Pseudomonas* sp. (w) grew prominently in the wastewater. The percentage weight difference
205 between the first and the twelfth day incubation indicated some substantial growth
206 differences in *Pseudomonas* sp. (w) and *Bacillus* sp. (p) with each having a 100% weight
207 increase. There was also an appreciable increase in the weight of *Pseudomonas* spp. (dd),
208 with 66.7% weight increase. However, the most effective lipolytic bacteria isolates in the oil-

rich wastewater were *Pseudomonas* sp. (dd), *Pseudomonas* sp. (w) and *Bacillus* sp. (p). These findings are in accordance with Mishra Ankit and others, who obtained four strains of highly lipolytic isolates identified as *Bacillus* sp. and *Pseudomonas* sp. with the former producing the highest lipase activity [9].

The *Bacillus* sp. produced 45.2 ± 0.4 U/ml of lipase within 48 hours of incubation time, at room temperature, in a basal mineral medium consisting of 2% olive oil. Among all the screened microbes from the samples of oil-contaminated soil and waste water, the potential lipolytic bacterium, *Staphylococcus warneri*, unicellular yeast, *Candida rugosa* and filamentous fungus, *Fusarium solani* were selected because of their high specific activities [10].

Generally, the optimum growth in all the lipolytic microbial isolates in the fresh oil was between the fifth and eighth days. The growth rate of the organisms ranging between 0.1mg/day in *Pseudomonas* sp. (j) and 0.12mg/day in *Pseudomonas* sp. (n), *Klebsiella* sp. (m), *Staphylococcus* sp. (r), and *Bacillus* sp. (p). The decomposition of dietary oil was primarily dependent on the varied lipolytic ability of the different bacteria. The weight increase of each of the microbial cells in palm oil culture was usually lesser than what was obtained in the oil-rich wastewater culture. This might be as a result of phosphate introduced by the detergent as a cleansing agent. This provided added environmental advantage more than what dietary oil only could have provided for both biochemical and physiological activities of the microbes. Most household phosphate inputs now come from human wastes and automatic dishwasher detergent. Previous studies have shown that very low amount of phosphate led to increased microbial growth of biofilm in drinking water distribution systems (DWDS) [11].

Considering the chemical component of the wastewater and palm oil, the microbes grew better in the wastewater in spite of some inhibitory substances associated with the detergent used in the soap solution for dish washing. The presence of high moisture (90.7%), fibre (4.7%) and protein (3.2%) might have encouraged more microbial growth in

the wastewater than in the palm oil, corroborating with the work of Shabtai, who reported that *P. aeruginosa* was highly tolerant of high concentrations of the cationic detergent, cetyl trimethyl ammonium bromide and of the fatty acid salts derived from bacterial hydrolysis of the oil [12].

The electropherograms showed that only two PCR products were clean. The other four had multiple peaks and this might be due to a number of factors; such as non-pure isolates, non-specific binding of primers to template during amplification as a result of the very low annealing temperature (45°C) and reduced ramp (40%) [13]. The PCR conditions allowed for promiscuous binding of primers to template which might be responsible for the multiple peaks observed during sequencing. However, these conditions had to be used to achieve amplification as samples could not be consistently amplified at higher temperatures.

Bioinformatic analysis of the exploitable sequences confirmed the identity of the *Pseudomonas* isolate but identified the *Bacillus* isolate as *Lysinibacillus sphaericus*. Concordance between biochemical and molecular identification of bacteria as observed for the *Pseudomonas* isolate has been documented in literature [14]. However, the discordance in the identity of the second sequenced isolate validates the superiority of molecular identification to biological typing [15].

The genus *Lysinibacillus* contains spore forming Gram positive rod shaped bacteria [16]. Lysinibacilli are very common in soil and have been documented to possess insecticidal toxins [17]. To the best of our knowledge, this study documents the first isolation and molecular characterization of *Lysinibacillus sphaericus* indigenous to Ekiti State, Nigeria and probably Nigeria as a whole.

Lipase from different microorganisms presents different chemical characteristics, which may be useful for industries [4]. The lipases present different affinity for the substrate { [18]; [19]; [20]}. The activity varies in accordance with the chain length of the fatty acids. So, some bacteria can present affinity to p-nitrophenylacetate (C₂ small chains) or p-nitrophenylcaprilate (C₈ medium chains). Other lipase isoforms present a better affinity for

esters with long carbon chain like p-nitrophenyl-laurate (C₁₂) or p-nitrophenylpalmitate (C₁₆)
 {[4]; [21]; [22]}. Following the characterization of *Pseudomonas aeruginosa* and
Lysinibacillus sp. the degradative activity of the organisms in palm oil was monitored for 12
 days. The palm oil used in this study was very rich in palmitic acid, oleic acid and linoleic
 acid constituting about 38.9%, 34.7% and 13.1% of the total fatty acid content respectively.
 In this study, *Pseudomonas aeruginosa* reduced the initial concentration of petroselinic acid,
 oleic acid, and linoleic acid in the palm oil by 2.16%, 2.8%, 2.7% respectively in 8 days;
 indicating that these FAs are the most utilized for the growth of the microbe. These FAs are
 easily metabolized based on their cis-form which is readily available for microbial attack
 (degradation) than the trans-form of the fatty acid. On the other hand, when compared to
Pseudomonas aeruginosa, it appeared *L. sphaericus* preferred to utilize linoleic acid (3.65%)
 for growth than it would with petroselinic acid (1.63%). This is because linoleic acid was
 more unsaturated than petroselinic acid, thereby more prone to degradation. The lesser the
 unsaturation the more difficult will be the biodegradation of the fatty acid; this might then be
 responsible for the reason why the saturated fatty acids were not being degraded by the
 microbes. It also depends on the quality and quantity of enzymes secreted by the individual
 microbes. *Lysinibacillus sphaericus* might have secreted more specific and restrictive
 enzymes (lipase and others) to degrade/metabolize readily available nutrients in oil than
Pseudomonas aeruginosa. A specific lipase activity of *P. fluorescens* S1K W1 was found in
 a medium which contained emulsified olive oil as carbon source. The enzyme showed a high
 lipolytic activity towards tricaproic (C₆) and tricapyrin (C₈) compared to the other
 triacylglycerols examined; also, it preferentially hydrolyzed the ester bonds in 1 and 3 of
 triolein [21]. It was observed that *Pseudomonas aeruginosa* left more palmitic acid
 (5.05%) and stearic acid (2.09%) into the medium within 8 days while *Lysinibacillus*
sphaericus produced greater amount of palmitic acid (5.45%). This increase in saturated
 fatty acids might be due to increase in their percentages in the medium with the decrease in
 the percentages of the unsaturated fatty acids. High levels of lipase production were

290 reported from various thermophilic *Bacillus* sp. in the presence of olive oil as carbon source
 291 in the culture medium {[23]; [24]}. These microbes have therefore shown evidence of ability
 292 to biodegrade oil rich domestic wastewater, to concentrate some valuable fatty acids.

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294 **Table 1: Chemical composition of wastewater and palm oil**

Substrate	Ash (mg/ml)	Moisture (%)	Fibre (%)	Protein (%)	Fat (mg/ml)	Oil (mg/ml)	CHO (%)	Silica (%)	Soda-Ash (mg/ml)	Surfactant (mg/ml)	PO ₄ (mg/ml)
Waste water	0.5	90.7	4.7	3.2	1.2	2.4	3.1	0.2	0.4	0.01	0.3
Palm Oil	0.4	0.4	ND	1.9	85.6	5.8	6.1	-	-	-	ND

295 ND: Not detected

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298 **Table 2: List of identified lipolytic bacteria***

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Bacteria species	Zone of clearance (mm)	
	0.25-0.34	0.35 and above
<i>Bacillus</i> sp. (p)	-	0.36
<i>Enterococcus</i> sp. (a)	0.30	-
<i>Escherichia</i> sp. (f)	0.27	-
<i>Escherichia</i> sp. (t)	0.29	-
<i>Klebsiella</i> sp. (m)	-	0.40
<i>Pseudomonas</i> sp. (e)	0.34	-
<i>Pseudomonas</i> sp. (g)	-	0.37
<i>Pseudomonas</i> sp. (j)	-	0.39
<i>Pseudomonas</i> sp. (s)	-	0.37
<i>Pseudomonas</i> sp. (w)	-	0.36
<i>Pseudomonas</i> sp. (y)	-	0.35
<i>Pseudomonas</i> sp. (aa)	0.33	-
<i>Pseudomonas</i> sp. (dd)	0.31	-
<i>Pseudomonas</i> sp. (ee)	0.29	-
<i>Staphylococcus</i> sp. (c)	-	0.41
<i>Staphylococcus</i> sp. (i)	0.31	-
<i>Staphylococcus</i> sp. (l)	0.34	-
<i>Staphylococcus</i> sp. (r)	-	0.38
<i>Staphylococcus</i> sp. (z)	0.30	-
<i>Staphylococcus</i> sp. (bb)	-	0.33

*From wash basin, through the drainage to the receiving stream

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308 **Table 3: Growth (mg) of lipolytic isolates¹ in wastewater**
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Isolates ¹	Incubation Period (day)												% weight* difference
	1	2	3	4	5	6	7	8	9	10	11	12	
<i>Bacillus</i> sp. (p)	0.0	3.0	5.0	5.0	7.0	5.0	5.0	1.0	3.0	2.0	2.0	1.0	+100%**
<i>Enterococcus</i> sp. (b)	1.0	3.0	6.0	7.0	7.0	8.0	8.0	4.0	2.0	2.0	2.0	2.0	+50.0%
<i>Klebsiella</i> sp. (m)	1.0	5.0	5.0	7.0	9.0	9.0	9.0	5.0	4.0	4.0	3.0	2.0	+50.0%
<i>Pseudomonas</i> sp. (w)	0.0	6.0	8.0	7.0	9.0	7.0	8.0	5.0	4.0	3.0	2.0	2.0	+100%**
<i>Pseudomonas</i> sp. (aa)	2.0	1.0	3.0	3.0	5.0	6.0	6.0	2.0	2.0	3.0	2.0	3.0	+33.3%
<i>Pseudomonas</i> sp. (dd)	1.0	2.0	6.0	6.0	7.0	7.0	6.0	4.0	2.0	4.0	2.0	3.0	+66.7%**
<i>Pseudomonas</i> sp. (j)	1.0	2.0	2.0	8.0	9.0	11.0	10.0	6.0	6.0	3.0	2.0	2.0	+50%
<i>Staphylococcus</i> sp. (c)	1.0	3.0	3.0	5.0	7.0	7.0	8.0	5.0	3.0	3.0	2.0	2.0	+50.0%
<i>Staphylococcus</i> sp. (i)	1.0	1.0	3.0	3.0	5.0	6.0	6.0	3.0	3.0	4.0	3.0	2.0	+50.0%
<i>Staphylococcus</i> sp. (r)	1.0	3.0	5.0	5.0	5.0	4.0	3.0	2.0	2.0	1.0	1.0	2.0	+50.0%
<i>Staphylococcus</i> sp. (bb)	1.0	2.0	3.0	4.0	5.0	5.0	4.0	3.0	3.0	2.0	2.0	2.0	+50.0%

310 *Difference in percentage weight after 24h and 12days incubation **Bacteria isolates selected for
311 further analyses

312 ¹Zero point one (0.1) ml of known concentration of organism in 100ml wastewater

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316 **Table 4: Growth (mg) of bacteria isolates¹ in Palm oil**
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Isolates ¹	Incubation Period (day)												% weight* difference
	1	2	3	4	5	6	7	8	9	10	11	12	
<i>Bacillus</i> sp. (p)	0.43	0.46	0.49	0.60	0.61	0.59	0.52	0.53	0.52	0.50	0.46	0.45	+4.44%**
<i>Klebsiella</i> sp. (m)	0.40	0.44	0.47	0.55	0.58	0.57	0.59	0.60	0.54	0.46	0.44	0.43	+6.98%**
<i>Pseudomonas</i> sp. (j)	0.33	0.37	0.39	0.46	0.50	0.50	0.51	0.58	0.64	0.58	0.46	0.46	+28.3%**
<i>Pseudomonas</i> sp. (n)	0.48	0.51	0.53	0.57	0.59	0.57	0.60	0.64	0.59	0.54	0.50	0.49	+2.04%**
<i>Staphylococcus</i> sp. (r)	0.47	0.54	0.57	0.58	0.59	0.57	0.68	0.61	0.57	0.55	0.53	0.51	+7.84%**

318 *Difference in percentage weight after 24h and 12days **Isolates selected for further analyses

319 ¹Ten milliliter of known concentration of inoculum in 90 ml of palm oil

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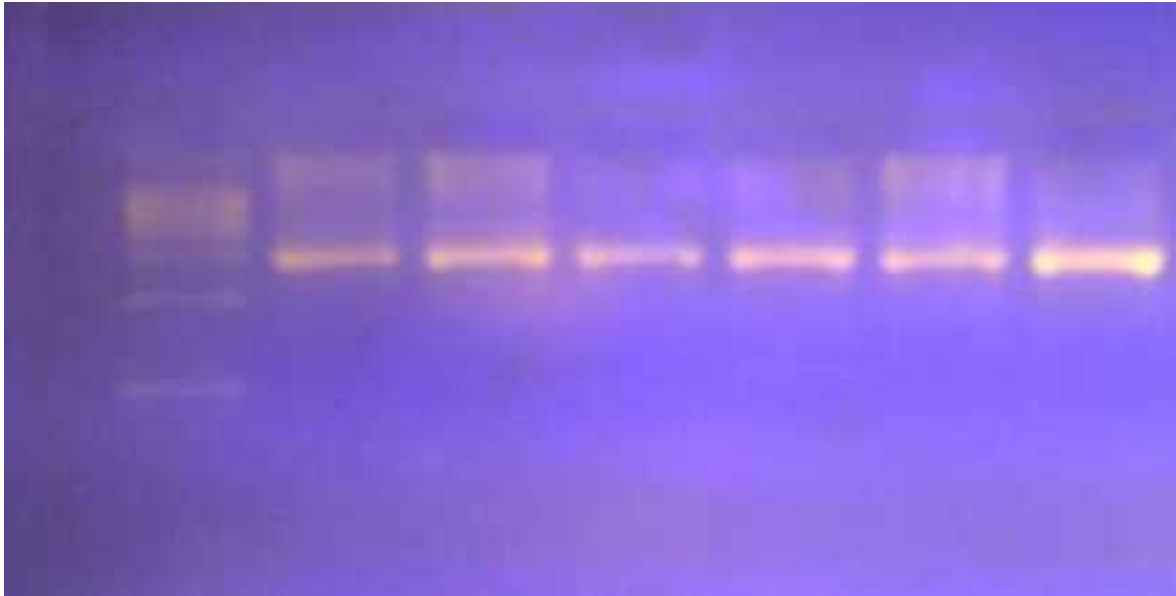
329

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332 LINES M 1 2 3 4 5 6
334



335
336 Fig 1: Gel electrophoresis of the PCR product showing the 1.5kb amplicon
337

338 Key: Lane M - The marker is 0.5 - 5kb (from Jena bioscience)
339 Lane 1 - *Pseudomonas* sp. (w) (positive); Lane 2 - *Pseudomonas* sp. (n) (positive)
340 Lane 3 - *Pseudomonas* sp. (j) (positive); Lane 4 - *Staphylococcus* sp. (r) (positive)
341 Lane 5 - *Klebsiella* sp. (m) (positive); Lane 6 - *Bacillus* sp. (p) (positive)
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Select: All None Selected:0

Alignments Download GenBank Graphics Display all results

Description	Max score	Total score	Query cover	E value	Max ident	Accession
Pseudomonas aeruginosa B136.33 complete genome	2747	10360	96%	0.0	99%	CP004551.1
Pseudomonas aeruginosa PAO1 strain PAO1 16S ribosomal RNA, complete sequence	2747	2747	96%	0.0	99%	FJ074829.1
Pseudomonas aeruginosa partial 16S rRNA gene, type strain DSM 5027T	2747	2747	96%	0.0	99%	HE978271.1
Pseudomonas aeruginosa strain NC02 16S ribosomal RNA gene, partial sequence	2747	2747	96%	0.0	99%	JX035794.1
Pseudomonas aeruginosa PAO2 complete genome	2747	10388	96%	0.0	99%	CP003149.1
Pseudomonas aeruginosa strain R-1 16S ribosomal RNA gene, partial sequence	2747	2747	96%	0.0	99%	J2773431.1
Pseudomonas aeruginosa VCGM2.01 DNA complete genome	2747	10388	96%	0.0	99%	AF112260.1
Pseudomonas aeruginosa T18 complete genome	2747	10300	96%	0.0	99%	CP002436.1
Pseudomonas aeruginosa strain 558 16S ribosomal RNA gene, partial sequence	2747	2747	96%	0.0	99%	JN103625.1
Pseudomonas sp. NR020101 16S ribosomal RNA gene, partial sequence	2747	2747	96%	0.0	99%	GI557392.1
Pseudomonas aeruginosa strain MW34 16S ribosomal RNA gene, partial sequence + cp GQ137112.1 Pseudomonas aeruginosa strain MW34C 16S riboso	2747	2747	96%	0.0	99%	GQ137112.1
Pseudomonas aeruginosa strain YJ-1 16S ribosomal RNA gene, partial sequence	2747	2747	96%	0.0	99%	FJ043174.1
Pseudomonas aeruginosa JE3550 complete genome sequence	2747	10302	96%	0.0	99%	FM211182.1

Fig. 2: Bioinformatic analysis of isolate 3 sequence data producing probable significant alignments as *Pseudomonas aeruginosa*.

Descriptions

Sequences producing significant alignments:

Sort: [All](#) [Name](#) [Selected](#) [All](#)

[Alignments](#) [Download](#) [GenBank](#) [Descriptions](#) [Distance tree of results](#)

Description	Max score	Total score	Query cover	E value	Max ident	Accession
Lysinibacillus sphaericus C0-41 chromosome, complete genome	2750	27457	95%	0.0	95%	NC_013322.1
Solibacillus silvestris SLE645, complete genome	2438	24031	95%	0.0	95%	NC_013025.1
Bacillus pasteurianus DSM 12442 chromosome, whole genome shotgun sequence	2239	2239	95%	0.0	95%	NC_04303745.1
Bacillus anthracis str. C02644 chromosome, complete genome	2239	22437	96%	0.0	95%	NC_012551.1
Bacillus weihenstephanensis K5654 chromosome, complete genome	2239	21016	95%	0.0	95%	NC_012154.1
Bacillus cereus H41391-46 chromosome, complete genome	2238	22894	96%	0.0	95%	NC_012614.1
Bacillus cereus F4754 chromosome, complete genome	2220	21044	95%	0.0	95%	NC_011725.1
Bacillus thuringiensis serovar kurstaki strain 97-27 chromosome, complete genome	2228	21139	96%	0.0	95%	NC_005957.1
Bacillus anthracis str. Sterne chromosome, complete genome	2228	22479	96%	0.0	95%	NC_005945.1
Bacillus anthracis str. Ames chromosome, complete genome	2228	22479	96%	0.0	95%	NC_005946.1

Fig. 3: Bioinformatic analysis of isolate 6 sequence data producing probable significant alignments of *Lysinibacillus sphaericus*.

397 **Table 5a: Fatty acids content (%) of microbiological degradation products of**
 398 **palm oil (Day 8)**

Fatty Acids	Common Name ^{1,2,3,4}	<i>Pseudomonas aeruginosa</i>	<i>Lysinibacillus sphaericus</i>
C5:0	Pentanoic acid	ND – 0.0000 = ND	ND – 0.0000 = ND
C6:0	Caproic acid	0.0000 – ND = ND	0.0000 – ND = ND
C8:0	Caprylic acid	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
C10:0	Capric acid	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
C12:0	Lauric acid	0.0111 – 0.0079 = 0.0032	0.0111 – 0.0092 = 0.0019
C14:0	Myristic acid	0.8346 – 1.7267 = - 0.8921	0.8346 – 1.2390 = 0.4014
C14:1 (Cis-9)	Myristoleic acid	0.0338 – 0.0239 = 0.0099	0.0338 – 0.0278 = 0.0060
C16:0	Palmitic acid	38.9032 – 43.9487 = - 5.0455	38.9032 – 44.3489 = -5.4457
C16:1 (Cis-9)	Palmitoleic acid	0.1207 – 0.0853 = 0.0354	0.1207 – 0.0993 = 0.0214
C18:0	Stearic acid	5.5831 – 7.6778 = - 2.0947	5.5831 – 5.8024 = 0.2193
C18:1 (Trans-6)	Petroselaidic acid	0.0518 – 0.0366 = 0.0152	0.0518 – 0.0426 = 0.0092
C18:1 (Cis-6)	Petroselinic acid	5.5851 – 3.4299 = 2.1552	5.5851 – 3.9503 = 1.6348
C18:1 (Trans-9)	Elaidic acid	0.0034 – 0.0024 = 0.0010	0.0034 – 0.0028 = 0.0006
C18:1 (Cis-9)	Oleic acid	34.6824 – 31.8857 = 2.7967	34.6824 – 34.0752 = 0.6072
C18:1 (Trans-11)	Vaccenic acid	0.0590 – 0.0417 = 0.0173	0.0590 – 0.0485 = 0.0105
C18:2 (Cis-9, 12)	Linoleic acid	13.1259 – 10.4223 = 2.7036	13.1259 – 9.4789 = 3.6470
C18:2 (Trans-9, 11)	Linoelaidic acid	0.0434 – 0.0307 = 0.0127	0.0434 – 0.0357 = 0.0077
C20:0	Arachidic acid	0.1030 – 0.0728 = 0.0302	0.1030 – 0.1336 = 0.0306
C18:3	γ-Linolenic acid	0.1728 – 0.1221 = 0.0507	0.1728 – 0.1420 = 0.0308
C20:1 (Cis-11)	Gondoic acid	0.1055 – 0.0746 = 0.0309	0.1055 – 0.0867 = 0.0188
C18:3 (Cis-9, 12, 15)	α-Linolenic acid	0.1865 – 0.1318 = 0.0547	0.1865 – 0.1532 = 0.0333
C20:2 (Cis-11, 14)	Eicosadienoic acid	0.0146 – 0.0103 = 0.0043	0.0146 – 0.0120 = 0.0026
C22:0	Behenic acid	0.0950 – 0.0672 = 0.0278	0.0950 – 0.0781 = 0.0169
C20:3 (Cis-8, 11, 14)	Dihomo-γ-linolenic	0.1202 – 0.0850 = 0.0352	0.1202 – 0.0988 = 0.0214
C22:1 (Cis-13)	Erucic acid	0.0327-- 0.0231 = 0.0096	0.0327-- 0.0269 = 0.0058
C20:3 (Cis-11, 14, 17)	Eicosatrienoic acid	0.0630 – 0.0445 = 0.0185	0.0630 – 0.0518 = 0.0112
C20:4 (Cis-5, 8, 11, 14)	Arachidonic acid	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
C22:2 (Cis-13, 16)	Docosadienoic acid	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0096 = 0.0021
C24:0	Lignoceric acid	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0096 = 0.0021
C20:5 (Cis-5, 8, 11, 14, 17)	EPA	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0096 = 0.0021
C24:1 (Cis-15)	Nervonic acid	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0096 = 0.0021
C24:6 (Cis-4, 7, 10, 13, 16, 19)	Tetracosahexaenoic	0.0221 – 0.0156 = 0.0065	0.0221 – 0.0182 = 0.0096

399 ¹http://lipidlibrary.aocs.org/lipids/fa_mono/index.htm
 400 ³<http://www.ncbi.nlm.nih.gov/pubmed/16964408>
 401 ²<http://che1.lf1.cuni.cz/html/Lipids02.pdf>
 402 ⁴https://en.wikipedia.org/wiki/Polyunsaturated_fatty_acid
 403

404 **Table 5b: Fatty acids content (%) of microbiological degradation products of**
 405 **palm oil (Day 12)**

Fatty Acids	Common Name ^{1,2,3,4}	<i>Pseudomonas aeruginosa</i>	<i>Lysinibacillus sphaericus</i>
C5:0	Pentanoic acid	ND – 0.0000 = ND	ND – 0.0000 = ND
C6:0	Caproic acid	0.0000 – ND = ND	0.0000 – ND = ND
C8:0	Caprylic acid	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
C10:0	Capric acid	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
C12:0	Lauric acid	0.0111 – 0.0103 = 0.0008	0.0111 – 0.0088 = 0.0023
C14:0	Myristic acid	0.8346 – 2.2883 = -1.4537	0.8346 – 3.4486 = -2.614
C14:1 (Cis-9)	Myristoleic acid	0.0338 – 0.0312 = 0.0026	0.0338 – 0.0266 = 0.0072
C16:0	Palmitic acid	38.9032 – 43.6616 = -4.7584	38.9032 – 45.1265 = -6.2233
C16:1 (Cis-9)	Palmitoleic acid	0.1207 – 0.1114 = 0.0093	0.1207 – 0.0951 = 0.0256
C18:0	Stearic acid	5.5831 – 8.8155 = -3.2324	5.5831 – 10.7671 = -5.1840
C18:1 (Trans-6)	Petroselaic acid	0.0518 – 0.0478 = 0.0004	0.0518 – 0.04080 = 0.0038
C18:1 (Cis-6)	Petroselinic acid	5.5851 – 3.1066 = 2.4785	5.5851 – 1.9243 = 3.6608
C18:1 (Trans-9)	Elaidic acid	0.0034 – 0.0031 = 0.0003	0.0034 – 0.0026 = 0.0008
C18:1 (Cis-9)	Oleic acid	34.6824 – 31.8335 = 2.8488	34.6824 – 30.1320 = 4.5504
C18:1 (Trans-11)	Vaccenic acid	0.0590 – 0.0544 = 0.0046	0.0590 – 0.0464 = 0.0126
C18:2 (Cis-9, 12)	Linoleic acid	13.1259 – 9.1088 = 4.0171	13.1259 – 7.5119 = 5.6142
C18:2 (Trans-9, 11)	Linoelaidic acid	0.0434 – 0.0401 = 0.0033	0.0434 – 0.0342 = 0.0092
C20:0	Arachidic acid	0.1030 – 0.0950 = 0.0080	0.1030 – 0.1593 = -0.0563
C18:3	γ-Linolenic acid	0.1728 – 0.1593 = 0.0135	0.1728 – 0.1359 = 0.0369
C20:1 (Cis-11)	Gondoic acid	0.1055 – 0.0973 = 0.0082	0.1055 – 0.0830 = 0.0225
C18:3 (Cis-9, 12, 15)	α-Linolenic acid	0.1865 -- 0.1719 = 0.0146	0.1865 – 0.1467 = 0.0398
C20:2 (Cis-11, 14)	Eicosadienoic acid	0.0146 – 0.0135 = 0.0011	0.0146 -- 0.0115 = 0.0031
C22:0	Behenic acid	0.0950 – 0.0876 = 0.0074	0.0950 – 0.0748 = 0.0202
C20:3 (Cis-8, 11, 14)	Dihomo-γ-linolenic	0.1202 -- 0.1108 = 0.0094	0.1202 – 0.0946 = 0.0256
C22:1 (Cis-13)	Erucic acid	0.0327-- 0.0301 = 0.0026	0.0327-- 0.0257 = 0.0070
C20:3 (Cis-11, 14, 17)	Eicosatrienoic acid	0.0630 – 0.0581 = 0.0049	0.0630 – 0.0496 = 0.0134
C20:4 (Cis-5, 8, 11, 14)	Arachidonic acid	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
C22:2 (Cis-13, 16)	Docosadienoic acid	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0092 = 0.0025
C24:0	Lignoceric acid	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0092 = 0.0025
C20:5 (Cis-5, 8, 11, 14, 17)	EPA	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0092 = 0.0025
C24:1 (Cis-15)	Nervonic acid	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0092 = 0.0025
C24:6 (Cis-4, 7, 10, 13, 16, 19)	Tetracosahexaenoic	0.0221 – 0.0204 = 0.0017	0.0221 – 0.0175 = 0.0047

406 ¹http://lipidlibrary.aocs.org/lipids/fa_mono/index.htm

407 ³<http://www.ncbi.nlm.nih.gov/pubmed/16964408>

408 ²<http://che1.lf1.cuni.cz/html/Lipids02.pdf>

409 ⁴https://en.wikipedia.org/wiki/Polyunsaturated_fatty_acid

411 4. CONCLUSION

412
413 This study has shown that wastewater needs proper and adequate treatment before
414 dislodging into the environment. The ability of some lipolytic bacteria such as *Pseudomonas*
415 *aeruginosa* and *Lysinibacillus sphaericus* to degrade/utilize dietary oil has been reported in
416 this work. These organisms are versatile in utilizing the limited nutrients and had the ability to
417 grow appreciably in the wastewater in spite of the toxic component (soap solution; 5g/100ml
418 of water). This suggests that these bacteria may serve as useful tool in treating dietary oil-
419 rich wastewater in Nigeria.

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425 COMPETING INTERESTS

426
427
428 Authors have declared that no competing interests exist.

429 REFERENCES

- 430
431
432
433 1. Watson JD, Berry A. DNA: The Secret of Life. New York: Alfred A Knopf, Ward RD,
434 Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species.
435 *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*,
436 2003; 360: 1847–1857.
- 437 2. Rapley R, Harbron S. Molecular Analysis and Genome Discovery. Chichester John Wiley
438 and Sons Ltd. 2004; 388pp.
- 439 3. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors,
440 *Proc. Natl. Acad. Sci. U.S.A.*, 1997; 74: 5463-5467.
- 441 4. Sharma R, Chisti Y, Banerjee UC. "Production, purification, characterization, and
442 applications of lipases," *Biotechnology Advances*, 2001; 19 (8): 627–662.

- 443 5. Mobarak-Qamsari E, Kasra-Kermanshahi R, Moosavi-nejad, Z. Isolation and
 444 identification of a novel, lipase-producing bacterium, *Pseudomonas aeruginosa* KM110.
 445 *Iranian Journal of Microbiology*, 2011; 3 (2): 92-98.

- 446 6. Odeyemi AT. Proficiency of lipolytic organisms in dietary oil rich wastewater treatment.
 447 Ph. D. thesis. Ekiti State University, Ado-Ekiti. 2013; pp. 174pp.

- 448 7. Merck KG. Darmstadt, Germany. 2002. [http://service.merck.de/micro](http://service.merck.de/microbiology/tedisdata/prods/4973-1_01957_0500.html)
 449 [biology/tedisdata/prods/4973-1_01957_0500.html](http://service.merck.de/microbiology/tedisdata/prods/4973-1_01957_0500.html)

- 450 8. APHA. Standard Methods for the Examination of Water and Wastewater. 17th edition,
 451 American Public Health Association, Washington D.C., 1989; 1,268.

- 452 9. Mishra Ankit SK Yaginik MP, Yadav SK. Screening and Temperature Optimization for
 453 Lipase-Producing Bacteria from Waste Contaminated Water. *Asian Journal of Biochemical*
 454 *and Pharmaceutical Research Issue*, 2011; 1 (1): 63-69. ISSN: 2231-2560.

- 455 10. Pakorn W, Chutima K, Kingkaew P, Warawut C, Tikamporn Y, Jisnuson S. Immobilized
 456 lipase from potential lipolytic microbes for catalyzing biodiesel production using palm oil as
 457 feedstock. *African Journal of Biotechnology*, 2011; 10 (9): 1666-1673.

- 458 11. Hyun-Jung J, Yung-June C, Hee-Myong R, Jong-Ok K. Effect of phosphate addition on
 459 biofilm bacterial communities and water quality in annular reactors equipped with stainless
 460 steel and ductile cast iron pipes. *The Journal of Microbiology*. 2012; 50(1): 17-28.

- 461 12. Shabtai Y. Isolation and characterization of a lipolytic bacterium capable of growing in a
 462 low-water-content oil-water emulsion. *Appl. Environ. Microbiol.*, 1991; 57 (6): 1740–1745.

- 463 13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. "Basic local alignment search
 464 tool." *J. Mol. Biol.* 1990; 215:403-410.

- 465 14. Janda JM, Sharon LA. 16S rRNA Gene Sequencing for Bacterial Identification in the
466 Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *Journal of Clinical Microbiology*, 2007; 45
467 (9): 2761–2764.
- 468 15. Kaouther BA, Elaine EV, Willem M de Vos. Advanced Molecular Tools for the
469 Identification of Lactic Acid Bacteria. *The Journal of Nutrition*. 2007; 137 (3): 741S-747S.
- 470 16. Young-Do N, Myung-Ji S, Seong-Il L, So-Young L. Genome Sequence of *Lysinibacillus*
471 *boronitolerans* F1182, isolated from a traditional Korean Fermented Soybean Product. *J.*
472 *Bacteriol.* 2012; 194 (21): 5988pp.
- 473 17. Renaud B, Olivie RS, Maud D, Yannick L, JöRg S, Carola S, Pablo P, Rolf D, Moreno G.
474 Novel organic solvent-tolerant esterase isolated by metagenomics: insights into the
475 lipase/esterase classification. *Revista argentina de microbiología*. 2013; 45: 3-12.
- 476 18. George E, Tamerler C, Martinez A, Martinez MJ, Keshavarz T. Influence of growth
477 medium composition on the lipolytic enzyme activity of *Ophiostoma piliferum* (Cartapip).
478 *Journal of Chemical Technology and Biotechnology*, 1999; 74 (2): 137–140.
- 479 19. Gupta R, Rathi P, Gupta N, Bradoo S. Lipase assays for conventional and molecular
480 screening: an overview. *Biotechnology and Applied Biochemistry*, 2003; 37 (1): 63–71.
- 481 20. Haba E, Bresco O, Ferrer C, Marqués A, Busquets M, and Manresa A. Isolation of
482 lipase-secreting bacteria by deploying used frying oil as selective substrate. *Enzyme and*
483 *Microbial Technology*, 2000; 26 (1): 40–44.
- 484 21. Silva Filho GN, Vidor C. Solubilização de fosfatos por microrganismos na presença de
485 fontes de carbono” *Revista Brasileira de Ciência do Solo*, 2000; 24: 311–319.

486 22. Dong H, Gao S, Han S, Cao S. "Purification and characterization of a *Pseudomonas* sp.
487 lipase and its properties in non-aqueous media," *Biotechnology and Applied Biochemistry*,
488 1999; 30 (3): 251–256.

489 23. Lee DW, Koh YS, Kim KJ, Kim BC, Choi HJ, Kim DS. Isolation and characterization of a
490 thermophilic lipase from *Bacillus thermoleovorans* ID-1. *FEMS Microbiol. Lett.*, 1999; 179:
491 393-400.

492 24. Eltaweel MA, Rahman RNZRA, Salleh AB, Basri, M. An organic solvent-stable lipase
493 from *Bacillus* sp. strain 42. *Ann. Microbiol.*, 2005; 55: 187-192.