Lipolytic Activity and Molecular Identification of *Pseudomonas aeruginosa* and *Lysinibacillus sphaericus* Isolated from Domestic Oil rich Wastewater

ABSTRACT

Microbial lipases have been heightened in bioremediation and various industries. Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, between September 2010 and August 2011.To identify the lipolytic enzyme producing microbial strains in domestic oil rich wastewater, the 16SrRNA was used for the amplification and the sequences identified by comparing with database using BLAST analysis. The enzyme activity was quantified by HPLC analysis. 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 genus *Pseudomonas* and one *Bacillus* sp. Comparing the weights on the first day to the twelfth day values when lipolytic organisms were grown in palm oil, some appreciable increases in weight difference were recorded in some isolates: *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. The two isolates; *Pseudomonas* sp. and *Bacillus* sp. showed high similar sequence (99%) to *Pseudomonas aeruginosa* and *Lysinibacillus sphaericus* respectively. When the lipolytic activity of these microbes were investigated in palm oil, *Lysinibacillus* sp. produced various forms of fatty acids in the medium; myristic acid (2.61%), palmitic acid (6.22%), stearic acid (5.18%) and arachidic (3.66%). These strains are versatile in utilizing the limited nutrient and had the ability to grow appreciably in the toxic condition (soap solution); suggesting these microbes may serve as a useful tool in treating dietary oil-rich wastewater.

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

1. INTRODUCTION

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 [1]. Many microorganisms such as bacteria, yeast and fungi are known to secret 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 [2]. 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 to monitor growth dynamics and identify the lipolytic organisms from domestic oil-rich wastewater disposed into the environment.

2. MATERIAL AND METHODS

2.1 Source of Bacteria

The bacteria isolates used in this study have been previously described [3]. Isolates were recovered from oil-rich wastewater samples from a restaurant in Ado-Ekiti, Nigeria. The isolates had previously been identified on the basis of standard physiological, biochemical, and serological assays. The isolates include one hundred and eleven bacteria belonging to the following genera; *Pseudomonas*, *Staphylococcus*, *Enterococcus, Escherichia*, *Bacillus*, *Streptococcus* and *Klebsiella*. These organisms were obtained from

47 wastewater after dish washing (with soap solution; 5g/100ml detergent in water), first and
48 second rinsing of the dishes, and run-off into open sewers along the drainage.

49 **2.2 Growth of bacteria isolates in wastewater and palm oil**

50 A pure colony of each isolate was inoculated on sterile Tributyrin agar (TBA) and
51 incubated at 37℃ for 24h. Lipolytic activity of each isolate was confirmed by the
52 development of zone (s) of clearance around each colony [4]. Subsequently, 0.1 ml and 10
53 ml of known concentration of strains was inoculated into 100 ml sterile oil-rich wastewater
54 sample and 90 ml of fresh palm oil respectively. The mixture was shaken and incubated at
55 ambient temperature and growth monitored for 12days. The chemical composition of the
56 wastewater and palm oil used as growth media is shown in Table 1. All the experiments
57 were carried out in triplicates and conducted twice.

58 **2.3 Characterization and enumeration of fatty acids in the methylated oils**

59 The AKTA basic 10/100 high performance liquid chromatography (Amersham
60 Pharmacia Biotech, Sweden) was used for the fatty acids determination. The analysis was
61 carried out using Supercosil LC-18 column, 25cm x 4.6mm ID, 5µm particles. The mobile
62 phase consisted of acetonitrile: acetone (59:41; v/v) with a flow rate of 1.0ml/min and fatty
63 acids were detected using a UV (UV-900) detector. Ten microlitre sample of the fresh and
64 biodegraded palm oil was injected for each run. The fatty acid components in the test
65 samples were identified by comparing retention times to those of the standards. Each fatty
66 acid in the test sample was expressed in percent of the total fatty acids present. The methyl
67 esters of lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids were
68 used as standards for identification [5].

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

70

71 **2.4 Molecular Characterization of Bacterial Isolates**

72 **2.4.1 Extraction of DNA**

73 One millilitre of fresh bacterial culture was dispensed into a labelled microcentrifuge
74 tube and centrifuged at 13,500 rpm for 3 mins. The supernatant was decanted and the pellet
75 resuspended in 200µl of STET buffer (0.1M NaCl, 10mM Tris-Cl (pH 8.0), 1mM EDTA (pH
76 8.0). and 5% (v/v) Tween 20). Subsequently, 50µl of lysozyme (10mg/ml, pH 8.0) was added
77 to the suspension, vortexed for 5 seconds and incubated at room temperature for 1min. To
78 the suspension, 600µl of Lysis buffer (68% guanidium thiocyanate) was added, vortexed for
79 1 min and incubated at room temperature for 15 min., afterwards, 800µl of isopropanol was
80 added to the solution, vortexed for 5 seconds and centrifuged at 13,500 rpm for 15 min. The
81 supernatant was discarded and 1ml of 70% ethanol was added to the pellet. The suspension
82 was vortexed for 5 seconds and centrifuged at 13,500rpm for 5 min. Finally the pellet was
83 resuspended in 200µl of 10mM Tris-Cl (pH 8.0), 1mM EDTA (TE) buffer and 5µl of the
84 extract was run on 1% Agarose gel to detect DNA if present.

85 **2.4.2 Polymerase Chain Reaction (PCR)**

86 About 2.5ng of bacterial genomic DNA was added to a 50 µl PCR mix which
87 contained 1 X Hot start reaction buffer, 0.25mM dNTPs, 0.01M (each) primers 27F and
88 1525R, and 2.5 U Hot start polymerase (Jenabioscience). Thermal cycling was done in a
89 veriti thermal cycler (Applied Biosystems, USA) and cycling conditions were 95°C for 3 min
90 followed 45°C cycles of 95°C for 30secs, by 45°C for 1 minute, 72°C for 1min 30secs with
91 ramp from 45°C to 72°C set at 40%. Subsequently, the reaction was held at 72°C for
92 10minutes after which it was held at 4°C till terminated. PCR products were resolved on 1%
93 agarose gel stained with ethidium bromide and viewed on a transilluminator.

94 **2.4.3 Sequencing of amplified 16S rRNA gene**

95 The PCR products were purified using Montage PCR Clean up kit (Millipore). The
96 purified PCR products of approximately 1,500 bp were sequenced using 4 primers (27F -
97 AGA GTT TGA TCM TGG CTC AG, 1492R - TAC GGY TAC CTT GTT ACG ACT T, 518F -
98 CCA GCA GCC GCG GTA ATA CG and 800R - TAC CAG GGT ATC TAA TCC).
99 Sequencing was performed using Big Dye terminator cycle sequencing kit v.3.1 (Applied

BioSystems, USA) and sequencing products were resolved on an Applied Biosystems model 3130XL automated DNA sequencing system (Applied BioSystems, USA) at the Macrogen, Inc., Seoul, Korea.

2.4.4 Bioinformatics analysis

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

2.4.5 Nucleotide sequence accession numbers

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

3. RESULTS AND DISCUSSION

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

3.1 Growth of lipolytic organisms in wastewater

The growth pattern of these lipolytic microbes in wastewater varied. However, all the lipolytic bacteria showed appreciable growth rates in the wastewater (between 0.67 and 1.67mg/day) within 5 days. The cells of *Bacillus* sp. (p) and *Pseudomonas* sp. (w) grew prominently in the wastewater. The percentage weight difference between the first and the twelfth day incubation indicated some substantial growth differences in *Pseudomonas* sp. (w) and *Bacillus* sp. (p) with each having a 100% weight increase. There was also an appreciable increase in the weight of *Pseudomonas* spp. (dd), 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 [6].

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 [7].

3.2 Growth of lipolytic organisms in palm oil

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

154 Generally, the optimum growth in all the lipolytic microbial isolates in the fresh oil
155 was between the fifth and eighth days. The growth rate of the organisms ranging between
156 0.1mg/day in *Pseudomonas* sp. (j) and 0.12mg/day in *Pseudomonas* sp. (n), *Klebsiella* sp.
157 (m), *Staphylococcus* sp. (r), and *Bacillus* sp. (p). The decomposition of dietary oil was
158 primarily dependent on the varied lipolytic ability of the different bacteria. The weight
159 increase of each of the microbial cells in palm oil culture was usually lesser than what was
160 obtained in the oil-rich wastewater culture. This might be as a result of phosphate introduced
161 by the detergent as a cleansing agent. This provided added environmental advantage more
162 than what dietary oil only could have provided for both biochemical and physiological
163 activities of the microbes. Most household phosphate inputs now come from human wastes
164 and automatic dishwasher detergent. Previous studies have shown that very low amount of
165 phosphate led to increased microbial growth of biofilm in drinking water distribution systems
166 (DWDS) [8].
167 Considering the chemical component of the wastewater and palm oil, the microbes grew
168 better in the wastewater in spite of some inhibitory substances associated with the detergent
169 used in the soap solution for dish washing. The presence of high moisture (90.7%), fibre
170 (4.7%) and protein (3.2%) might have encouraged more microbial growth in the wastewater
171 than in the palm oil, corroborating with the work of Shabtai, who reported that *P. aeruginosa*
172 was highly tolerant of high concentrations of the cationic detergent, cetyl trimethyl
173 ammonium bromide and of the fatty acid salts derived from bacterial hydrolysis of the oil [9].

174 **3.3 Molecular characterization of bacteria isolates**

175 Genomic DNA was extracted from all six lipolytic bacteria isolates. The entire 1.5kb
176 16S rRNA gene was amplified and sequenced (Fig 1). Electropherograms of four of the six
177 sequencing reactions revealed multiple peaks. Only those of *Pseudomonas* sp.(j) and
178 *Bacillus* sp.(p) had clean peaks and were consequently analysed further. A search of the
179 Genbank, using the BLAST tool showed *Pseudomonas* sp.(j) and *Bacillus* sp.(p) to be most

180 similar (99%) to *Pseudomonas aeruginosa* (CP006245.1) and *Lysinibacillus sphaericus*
181 (FR775037.1) respectively (Fig 2 and 3).

182 The electropherograms showed that only two PCR products were clean. The other
183 four had multiple peaks and this might be due to a number of factors; such as non-pure
184 isolates, non-specific binding of primers to template during amplification as a result of the
185 very low annealing temperature (45°C) and reduced ramp (40%) [10]. The PCR conditions
186 allowed for promiscous binding of primers to template which might be responsible for the
187 multiple peaks observed during sequencing. However, these conditions had to be used to
188 achieve amplification as samples could not be consistently amplified at higher temperatures.

189 Bioinfomatic analysis of the exploitable sequences confirmed the identity of the
190 *Pseudomonas* isolate but identified the *Bacillus* isolate as *Lysinibacillus sphaericus*.
191 Concordance between biochemical and molecular identification of bacteria as observed for
192 the *Pseudomonas* isolate has been documented in literature [11]. However, the discordance
193 in the identity of the second sequenced isolate validates the superiority of molecular
194 identification to biological typing [12].

195 The genus *Lysinibacillus* contains spore forming Gram positive rod shaped bacteria [13].
196 Lysinibacilli are very common in soil and have been documented to possess insecticidal
197 toxins [14]. To the best of our knowledge, this study documents the first isolation and
198 molecular characterization of *Lysinibacillus sphaericus* indigenous to Ekiti State, Nigeria and
199 probably Nigeria as a whole.

200 **3.4 Degradation of lipids by *Pseudomonas aeruginosa* and *Lysinibacillus***
201 ***sphaericus:* Production of fatty acids**

202 Following the molecular characterization of *Pseudomonas aeruginosa* and
203 *Lysinibacillus sphaericus*, the degradative activity of the organisms in palm oil was monitored
204 for 12 days. The palm oil used in this study was very rich in palmitic acid, oleic acid and
205 linoleic acid constituting about 38.9%, 34.7% and 13.1% of the total fatty acid content
206 respectively. The other major components of the oil are stearic (5.58%) and petroselinic

207 (5.59%) acids (Table 5a). The utilization of the dietary oil as substrate by the microbes may
208 be explained by fatty acids profiling of the palm oil over specific incubated periods (8th day)
209 where outstanding microbial growth was experienced and the end of experimentation (12th
210 day) (Table 4). *Pseudomonas aeruginosa* reduced the initial concentration of petroselinic
211 acid, oleic acid, and linoleic acid by 2.16%, 2.8%, 2.7% respectively after 8 days; indicating
212 that these FAs are the most utilized for the growth of the microbe. However, it was observed
213 that the strain synthesized more palmitic acid (5.05%) and stearic acid (2.09%) into the
214 medium during the same period while *Lysinibacillus sphaericus* produced greater amount of
215 palmitic acid (5.45%). When compared to *Pseudomonas aeruginosa,* it appeared *L.*
216 *sphaericus* preferred to utilize linoleic acid [3.65%] for growth better than it would with
217 petroselinic acid [1.63%].

218 Of the thirty two fatty acids analyzed, it was observed on day 12 that *Lysinibacillus*
219 *sphaericus* depended mostly on petroselinic acid, oleic acid and linoleic acid for growth;
220 metabolizing 3.66%, 4.55% and 5.61% of the initial amount of these acids (Table 5b). When
221 compared to the lipolytic activity of *Pseudomonas aeruginosa* in palm oil*, Lysinibacillus*
222 *sphaericus* produced more myristic acid, palmitic acid, stearic acid and arachidic acid
223 (2.61%, 6.22%, 5.18% and 3.66% respectively) that was absorbed into the medium during
224 this period.

225 Lipase from different microorganisms presents different chemical characteristics,
226 which may be useful for industries [1]. The lipases present different affinity for the substrate
227 {[15]; [16]; [17]}. The activity varies in accordance with the chain length of the fatty acids. So,
228 some bacteria can present affinity to p-nitrophenylacetate (C_2 small chains) or p-
229 nitrophenylcaprilate (C_8 medium chains). Other lipase isoforms present a better affinity for
230 esters with long carbon chain like p-nitrophenyl-laurate (C_{12}) or p-nitrophenylpalmitate (C_{16})
231 {[1]; [18]; [19]}. Following the characterization of *Pseudomonas aeruginosa* and
232 *Lysinibacillus* sp. the degradative activity of the organisms in palm oil was monitored for 12
233 days. The palm oil used in this study was very rich in palmitic acid, oleic acid and linoleic

234 acid constituting about 38.9%, 34.7% and 13.1% of the total fatty acid content respectively.
235 In this study, *Pseudomonas aeruginosa* reduced the initial concentration of petroselinic acid,
236 oleic acid, and linoleic acid in the palm oil by 2.16%, 2.8%, 2.7% respectively in 8 days;
237 indicating that these FAs are the most utilized for the growth of the microbe. These FAs are
238 easily metabolized based on their cis-form which is readily available for microbial attack
239 (degradation) than the trans-form of the fatty acid. On the other hand, when compared to
240 *Pseudomonas aeruginosa,* it appeared *L. sphaericus* preferred to utilize linoleic acid (3.65%)
241 for growth than it would with petroselinic acid (1.63%). This is because linoleic acid was
242 more unsaturated than petroselinic acid, thereby more prone to degradation. The lesser the
243 unsaturation the more difficult will be the biodegradation of the fatty acid; this might then be
244 responsible for the reason why the saturated fatty acids were not being degraded by the
245 microbes. It also depends on the quality and quantity of enzymes secreted by the individual
246 microbes. *Lysinibacillus sphaericus* might have secreted more specific and restrictive
247 enzymes (lipase and others) to degrade/metabolize readily available nutrients in oil than
248 *Pseudomonas aeruginosa*. A specific lipase activity of *P. fluorescens* S1K W1 was found in
249 a medium which contained emulsified olive oil as carbon source. The enzyme showed a high
250 lipolytic activity towards tricaproic (C_6) and tricaprylin (C_8) compared to the other
251 triacylglycerols examined; also, it preferentially hydrolyzed the ester bonds in 1 and 3 of
252 triolein [18]. It was observed that *Pseudomonas aeruginosa* left more palmitic acid
253 (5.05%) and stearic acid (2.09%) into the medium within 8 days while *Lysinibacillus*
254 *sphaericus* produced greater amount of palmitic acid (5.45%). This increase in saturated
255 fatty acids might be due to increase in their percentages in the medium with the decrease in
256 the percentages of the unsaturated fatty acids. High levels of lipase production were
257 reported from various thermophilic *Bacillus* sp. in the presence of olive oil as carbon source
258 in the culture medium {[20]; [21]}. These microbes have therefore shown evidence of ability
259 to biodegrade oil rich domestic wastewater, to concentrate some valuable fatty acids.
260

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

ND: Not detected

Table 2: List of identified lipolytic bacteria*

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

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

Table 3: Growth (mg) of lipolytic isolates[1] in wastewater

Isolates[1]	Incubation Period (day)												% weight* difference
	1	2	3	4	5	6	7	8	9	10	11	12	
Bacillus 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%**
Enterococcus 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%

Klebsiella 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%
Pseudomonas 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%**
Pseudomonas 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%
Pseudomonas 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%**
Pseudomonas 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%
Staphylococcus 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%
Staphylococcus 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%
Staphylococcus 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%
Staphylococcus 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%

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

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

Table 4: Growth (mg) of bacteria isolates[1] in Palm oil

Isolates[1]	Incubation Period (day)												% weight* difference
	1	2	3	4	5	6	7	8	9	10	11	12	
Bacillus 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%**
Klebsiella 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%**
Pseudomonas 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%**
Pseudomonas 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%**
Staphylococcus 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%**

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

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

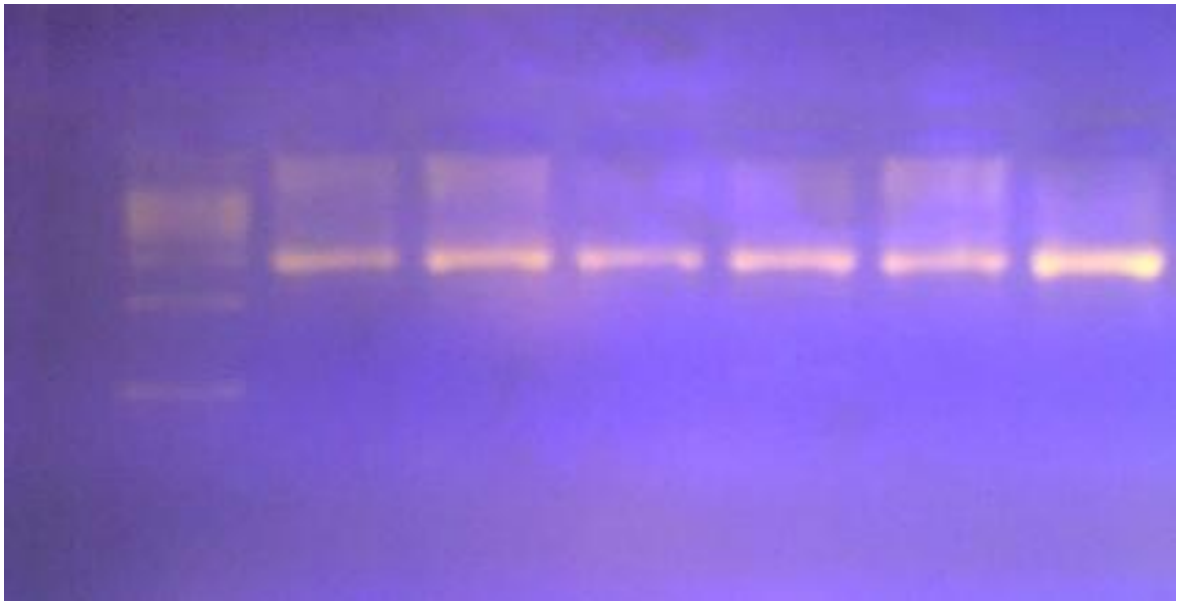


Fig 1: Gel electrophoresis of the PCR product showing the 1.5kb amplicon

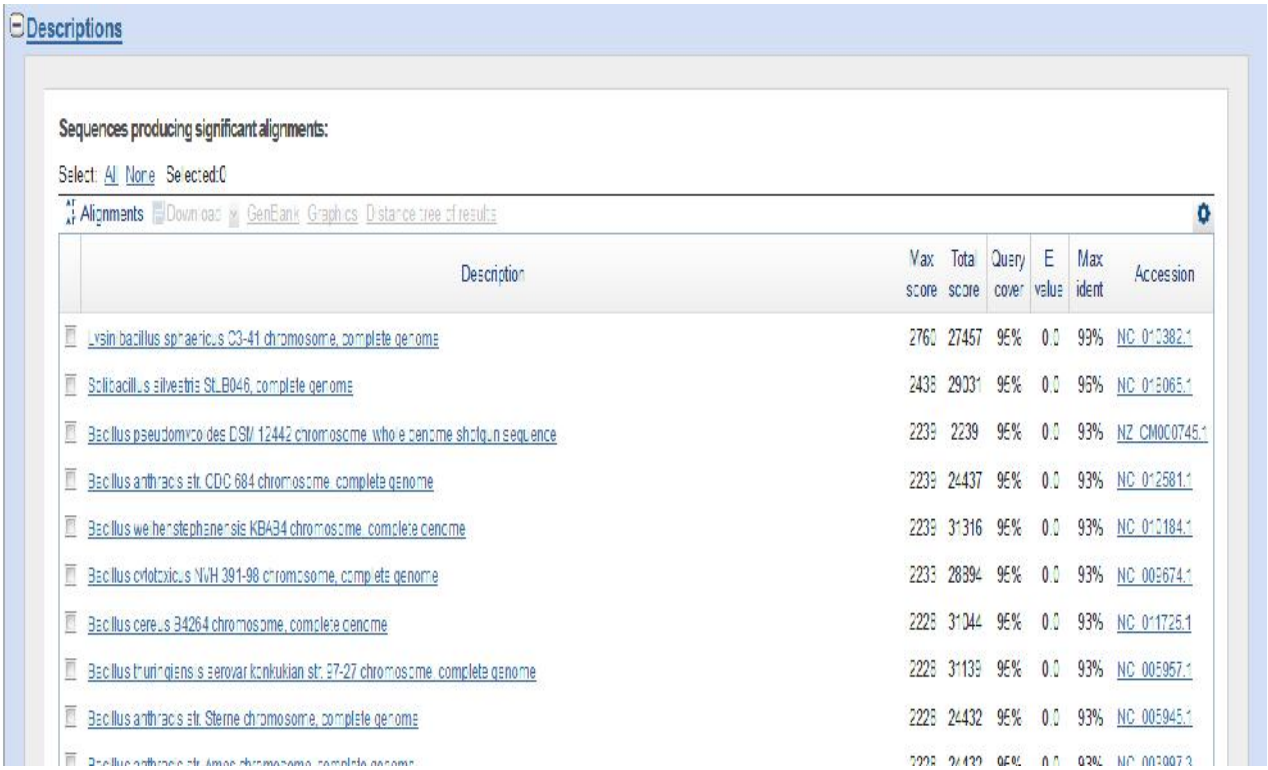
Key: Lane M - The marker is 0.5 - 5kb (from Jena bioscience)
L ane 1- *Pseudomonas* sp. (w) (positive); Lane 2 - *Pseudomonas* sp. (n) (positive)
Lane 3 - *Pseudomonas* sp. (j) (positive); Lane 4 - *Staphylococcus* sp. (r) (positive)
Lane 5 - *Klebsiella* sp. (m) (positive); Lane 6 - *Bacillus* sp. (p) (positive)

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Max ident	Accession
Pseudomonas aeruginosa B136-33, complete genome	2747	10960	95%	0.0	99%	CP004061.1
Pseudomonas aeruginosa PAO1 strain PAO1 16S ribosomal RNA, complete sequence	2747	2747	95%	0.0	99%	NR_074828.1
Pseudomonas aeruginosa partial 16S rRNA gene, type strain DSM 50071T	2747	2747	95%	0.0	99%	HE978271.1
Pseudomonas aeruginosa strain NC02 16S ribosomal RNA gene, partial sequence	2747	2747	95%	0.0	99%	JX035794.1
Pseudomonas aeruginosa DK2, complete genome	2747	10988	95%	0.0	99%	CP003149.1
Pseudomonas aeruginosa strain RI-1 16S ribosomal RNA gene, partial sequence	2747	2747	95%	0.0	99%	JQ773431.1
Pseudomonas aeruginosa NCGM2.S1 DNA, complete genome	2747	10988	95%	0.0	99%	AP012280.1
Pseudomonas aeruginosa M18, complete genome	2747	10988	95%	0.0	99%	CP002496.1
Pseudomonas aeruginosa strain BS8 16S ribosomal RNA gene, partial sequence	2747	2747	95%	0.0	99%	JN003525.1
Pseudomonas sp. NR2(2010) 16S ribosomal RNA gene, partial sequence	2747	2747	95%	0.0	99%	GU566322.1
Pseudomonas aeruginosa strain MW3A 16S ribosomal RNA gene, partial sequence >cb|GQ180118.1| Pseudomonas aeruginosa strain MW3AC 16S ribosoo	2747	2747	95%	0.0	99%	GQ180117.1
Pseudomonas aeruginosa strain WJ-1 16S ribosomal RNA gene, partial sequence	2747	2747	95%	0.0	99%	FJ948174.1
Pseudomonas aeruginosa LESB58 complete genome sequence	2747	10962	95%	0.0	99%	FM209186.1

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



Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Max ident	Accession
Lysinibacillus sphaericus C3-41 chromosome, complete genome	2762	27457	95%	0.0	99%	NC_010382.1
Solibacillus silvestris StLB046, complete genome	2438	29331	95%	0.0	95%	NC_015065.1
Bacillus pseudomycoides DSM 12442 chromosome, whole genome shotgun sequence	2239	2239	95%	0.0	93%	NZ_CM000745.1
Bacillus anthracis str. CDC 684 chromosome, complete genome	2239	24437	95%	0.0	93%	NC_012581.1
Bacillus weihenstephanensis KBAB4 chromosome, complete genome	2239	31316	95%	0.0	93%	NC_010184.1
Bacillus cytotoxicus NVH 391-98 chromosome, complete genome	2239	28394	95%	0.0	93%	NC_009674.1
Bacillus cereus B4264 chromosome, complete genome	2229	31044	95%	0.0	93%	NC_011725.1
Bacillus thuringiensis serovar konkukian str. 97-27 chromosome, complete genome	2229	31139	95%	0.0	93%	NC_005957.1
Bacillus anthracis str. Sterne chromosome, complete genome	2229	24432	95%	0.0	93%	NC_005945.1
Bacillus anthracis str. Ames chromosome, complete genome	2229	24432	95%	0.0	93%	NC_003997.3

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

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

Fatty Acids	Common Name [1,2,3,4]	*Pseudomonas aeruginosa*	*Lysinibacillus sphaericus*
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

[1] http://lipidlibrary.aocs.org/lipids/fa_mono/index.htm
[3] http://www.ncbi.nlm.nih.gov/pubmed/16964408
[2] http://che1.lf1.cuni.cz/html/Lipids02.pdf
[4] https://en.wikipedia.org/wiki/Polyunsaturated_fatty_acid

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

Fatty Acids	Common Name [1,2,3,4]	*Pseudomonas aeruginosa*	*Lysinibacillus sphaericus*
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)	Petroselaidic 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

[1] http://lipidlibrary.aocs.org/lipids/fa_mono/index.htm
[3] http://www.ncbi.nlm.nih.gov/pubmed/16964408
[2] http://che1.lf1.cuni.cz/html/Lipids02.pdf
[4] https://en.wikipedia.org/wiki/Polyunsaturated_fatty_acid

4. CONCLUSION

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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