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Screening of Microorganisms Isolated from Different Environmental Samples for Extracellular Lipase Production

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Abstract

Lipases are hydrolytic enzymes that hydrolyse triglycerides to free fatty acids and glycerol and those from microbes have occupied a prominent position as industrial biocatalysts. Microorganisms isolated from oil polluted environmental samples were screened for their lipase producing ability. A total of thirty seven bacteria and seventeen fungal strains belonging to the genera Trichoderma (16 spp.) and Aspergillus (1 sp.) were obtained from the samples. Acinetobacter sp. had the highest frequency of occurrence (37.8%). The lipolytic activity of the bacteria when screened on solid agar ranged within 3.0^{m} - 15.5^{a}mm, 3.0^{n} - 15.5^{a}mm and 3.0 - 16^{a} mm at 24, 48 and 72 hours of incubation, respectively. Acinetobacter sp. (OG3) had the highest activity at different hours of incubation. Eight isolates did not show any activity throughout the incubation period. On submerged fermentation, the growth of the lipolytic bacteria ranged within 0.093 - 1.003 in which Acinetobacter sp. had the highest growth while Bifidobacterium sp. had the lowest. Lipase production ranged within 1.25 U/ml - 8.65 U/ml in which Acinetobacter sp. had the highest production. During submerged fermentation, the growth of the selected fungal isolate ranged within 0.00 - 0.200^{a}, 0.00 - 0.25^{a} and 0.00 - 0.3190^{a} in which T. FISO1 had the highest at 3 and 7 days of incubation while T. virens FSU/AW3 had the highest at 14 days of incubation. There was a significant difference in lipase production by the fungi, it ranged within 0.70^{n} - 12.35^{a} U/ml, 1.8^{k} - 19.15^{a} U/ml and 2.50^{n} - 19.8^{a} U/ml in which Hypocrea patella FAD1, T. stromaticum FSUAW1 and T. virens FSU/AW3 had the highest at day 3, 7 and 14, respectively. The study has shown that the bacteria and Trichoderma strains isolated are potential lipase producers.

Keywords: *Lipase, Screening, Bacteria, Trichoderma spp.*

Introduction

Lipases are the enzymes capable of catalysing the hydrolysis and synthesis of esters formed from glycerol and long-chain fatty acids (Sharma *et al*. 2001; and Svendsen 2000). Lipases are produced by many microorganisms (Kamimura *et al*. 2001; Elibol and Ozer 2000) and higher eukaryotes. The ease with which enzymes could be isolated from microbes has made both bacteria and fungi predominant sources of lipase. However, fungi are certainly the best lipase sources and are preferably used for industrial applications (Gupta and Soni 2000; Mahadik *et al.* 2002).

Lipases are able to catalyze hydrolysis, esterification, transesterification (Gupta *et al*. 2011) and lactonization (intramolecular esterification) (Jaeger and Eggert 2002).

Lipase producers have been isolated mainly from soil, or spoiled food material that contains vegetable oil. Lipase production from a variety of bacteria, fungi and actinomycetes has been reported in several works (Sztajer *et al*. 1988; Kulkarni and Gadre 2002).

Lipase producing fungi are present on a wide range of substrates in the ambient environment and these results could also provide basic data for further investigations on fungal extracellular enzymes (Griebeler *et al.* 2011). Among the available lipase

producing microorganisms, filamentous fungi belonging to various species of genera *Aspergillus, Rhizopus Penicillium* and *Trichoderma* are described as the most prospective lipase producers (Karanam and Medicherla 2008; Lima *et al*. 2003; and Kashmiri *et al*. 2006). Lipases find promising applications in organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleochemical industry, the dairy industry, the agrochemical industry, paper manufacture, nutrition, cosmetics, pharmaceutical processing and the development of lipase-based technologies for the synthesis of novel compounds (Gupta and Soni 2000). However, one limiting factor is a shortage of lipases having the specific required processing characteristics. Because of the numerous potential uses of lipase enzyme and the need to discover new lipase with specific processing characteristics, this study was undertaken to isolate, characterize and screen lipase producing microorganisms from vegetable oil polluted environmental samples.

Materials and Methods

Sample Collection

Samples were collected from different sites: soils exposed to vegetable oil, palm oil and engine oils for long periods; spoiled food substances and waste materials; and soil from Jatropha-based biodiesel processing plant in Bode-Sa'adu, Moro local government area of Kwara State, Nigeria.

Isolation and identification of lipolytic bacterial and fungal strains

Serial dilution of the collected samples was carried out (Olutiola *et al*. 2000) and 1 ml of the diluents was pour plated on nutrient agar (NA) and Potato Dextrose agar (PDA) supplemented with streptomycin. NA plates were incubated at 37^{o}C for 24 hours while PDA plates were incubated at 28^{o}C for 3 days. Morphological appearances of the inoculated plates (at room temperature) were observed and distinct colonies were sub-cultured to obtain pure isolates which were then maintained on NA and PDA slants and stored at 4^{o}C for further study. The pure bacterial isolates were further identified by microscopic and biochemical examination, Bergey's Manual of Determinative Bacteriology (Holt *et al*. 1994) was used as a reference for identification based on the result of various biochemical tests. The fungi were identified using information from Barnett and Hunter (1972), the Compendium of Soil Fungi (Domsch *et al*. 1980) and other relevant electronic documents on the identification of the genus *Trichoderma*.

Screening of the bacterial strains for lipase production on solid agar

A plate detection method containing a chromogenic substrate (Congo red) was used to screen the strains for lipase producing ability. The medium used for screening has the following composition in (g/l): peptone 10; NaCl 5; Calcium chloride; 0.1; castor oil, 1ml agar, 50; Congo red, 0.5; and distilled water, 1,000 ml. The sterile medium was pour plated and allows solidifying. The agar plates were spot inoculated with the bacterial and fungal isolates and the plates were incubated at 30^{o}C for 24-48 hours for bacterial isolates and 3-5 days for fungal isolates. Lipolysis was indicated by the appearance of clear zone of inhibition around the spot of inoculation. The diameters of the colonies and clearance zones were measured after 24, 48 and 72 hours for bacteria isolates and 3, 7 and 14 days for fungal isolates.

Screening of the selected isolates for lipase production using submerged fermentation

All the isolates were screened for lipase production in submerged fermentation medium. This was carried out using the modified method of Gupta *et al*. (2004). The sterile basal medium was inoculated with seed cultures of the bacterial and fungal isolates, respectively. Fermentation was carried out at room temperature (27±2°C) for 24, 48 and 72 hours for bacterial strains and 3, 7 and 14 days for fungal strains, respectively. Lipase production was determined by assaying lipase activity in crude culture filtrate at standard assay conditions (Harrigan and McCance 1966; Gupta *et al*. 2003; Shukla and Gupta

2007). One unit (U) of lipase activity was defined as the amount of enzyme capable of releasing one milligram of oleic acid per minute.

Dry Cell Weight Determination

The mycelium from each flask was filtered and then washed. The washed mycelium was dried in British-made Gallenkamp oven at 110^{o}C to a constant mass and the mass was determined using an automatic electronic balance.

Statistical Analysis

Experiments were performed in triplicate and the results were analysed statistically. The treatment effects were compared and the significant difference among replicates has been presented as Duncan's multiple range tests in the form of probability values.

Results and Discussion

Microorganisms isolated form environmental samples such as oil contaminated soils, spoilt food substances and waste materials were screened for their lipase producing ability on solid agar and submerged fermentation. A total of 37 bacteria (*Acinetobacter* sp., *Yersinia* sp., *Bifidobacterium* sp., *Arthrobacter* sp., *Brevibacterium* sp., *Staphylococcus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Citrobacter* sp., *Serratia marcescens*, *Bacillus* sp., *Acetobacterium* sp., *Acidomonas* sp. and *Aeromonas hydrophila*) and 17 fungi (*Trichoderma minutisporum* FAP_2S, *Trichoderma harziarium* FISO6, *Trichoderma* sp. (1, 2, 3 and 4), *Trichoderma longibrachiatum* FSU/AW2, *Hypocrea neorufa* FSU/AW4, *Trichoderma* polysporum FIS03, *Trichoderma* ressei FISO14, *Trichoderma* stromaticum FSU/AW1, *Trichoderma* virens FSU/AW3, *Hypocrea stilbohypoxyli* FISO10, *Trichoderma pleuroticola* FISO12, *Hypocrea patella* FAD1, *Trichoderma fertile* FOG1 *and Aspergillus* sp. FISO11) were isolated from different environmental samples and screened on solid agar as well as submerged fermentation for lipase production.

Figure 1 shows the percentage frequency of occurrence of the different bacteria isolated from the samples. *Acinetobacter* sp. had the highest frequency of occurrence (37.8%), followed in order by *Yersinia* sp. (10.8%), *Bifidobacterium* sp (8.1%), *Arthrobacter* sp (8.1%), *Brevibacterium* sp (5.4%), *Staphylococcus* sp (5.4%) and *Aeromonas hydrophila* (5.4%), *Acetobacterium* sp., *Serratia marcescens*, *Acidomonas* sp., *Lactobacillus* sp., *Bacillus* sp., *Streptococcus* sp and *Citrobacter* sp (2.7%). Bacteria were the predominant organisms isolated from the samples. The existence of lipase producing microorganisms in diverse environment such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, and decaying food, compost heaps, coal tips, and hot springs has been studied (Sztajer *et al*. (1988; Wang *et al*. 1995).

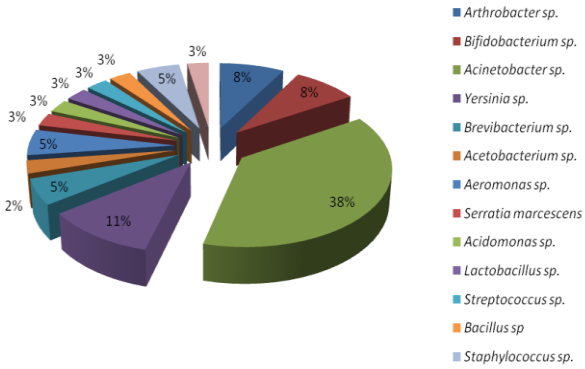

Fig. 1. Percentage frequency of occurrence of the bacterial isolates.

Similar bacteria isolates have been reported by Riaz *et al.* (2010), Barbaro *et al.* (2001), and Pandey *et al.* (1999). Gupta *et al.* (2004) also referenced 38 distinct bacterial sources from which common lipases are derived. Some of the commercially important lipase-producing fungi have been reported by Yadav *et al*. (1998) and Gulati *et al*. (1999). Abdel-Fattah and Hammad (2002) reported the use of tributyrin clearing zone techniques in the production of lipase by certain soil fungi. Table 1 shows the cultural, morpho-logical and microscopic characteristics of the fungal isolates. The most frequently isolated

genera were *Trichoderma* with a few being *Aspergillus*.

The screening of bacterial isolates for lipase production on solid agar is shown in Table 2. The lipolytic activity ranged within 3.0^{m} - 15.5^{a} mm, 3.0^{n} - 15.5^{a} mm and 3.0 - 16^{a} mm at 24, 48 and 72 hours of incubation, respectively. *Acinetobacter* sp. (OG3) had the highest activity at different hours of incubation. Eight isolates did not show any activity throughout the incubation period.

Table 3 shows lipase production and growth of the bacteria isolates using submerged fermentation. The growth of the bacterial isolates in submerged fermentation ranged within 0.093 - 1.003 in which *Bifidobacterium* sp. had the lowest while *Acinetobacter* sp. had the highest, respectively. Lipase production ranged within 1.25 U/ml - 8.65 U/ml in which *Acinetobacter* sp. had the highest production.

Table 4 shows the screening of the fungal isolates for growth and lipase production using submerged fermentation. The growth of the selected fungal isolate ranged within 0.00 - 0.200a, 0.00 - 0.25a and 0.00 - 0.3190a in which *Trichoderma* sp.1. FISO1 had the highest at 3 and 7 days of incubation while *T. virens* FSU/AW3 had the highest at 14 days of incubation.

It could be inferred from the obtained results that maximum biomass production was attained at the late period of (14^{th} day) of fermentation. This, however, is in contrast with the report of Rashimiri *et al.* (2006) who reported that maximum biomass production by *T. viride* was observed during the early hours of fermentation. There was a significant difference in lipase production by the fungi.

Table 1. Cultural, morphological and microscopic characteristics of fungal isolates.

Isolate code	Appearance on Agar	Microscopic spore shape	Examination of Hyphae	Probable Identity
FAP25	Presenting a whitish green granular colony	Smooth green ellipsoidal conidia	Phialides typically crowded arising from broad cells	*Trichoderma nutisporum*
FISO6	Yellow green conidia formed densely over the center and in undulating concentric rings	Conidia subglobose to ovoidal	Globose, intercalary hyphae and Terminal phialides	*Trichoderma harzianum*
FSU/AW2	Dark green, mottled with white flecks	Chlamydospores terminal, globose to subglobose. Smooth green conidia	Phialides mainly arising singly, in divergent whorls and typically cylindrical	*Trichoderma longibrachiatum*
FSU/AW4	Dark grown colonies	Conidia ellipsoidal, thick-walled, smooth and grayish green	Phialides flask shaped, widest below	*Hypocrea neorufa*
FISO3	White dense colony	White, smooth and oblong conidial chlamydospores not formed.	Phialides in whorls at the tip of fertile branches	*Trichoderma polysporum*
FISO14	Deep green with appressed mycelium with conspicuous radial lines	Conidia green, smooth and oblong or ellipsoidal	Long straight phialides, typically flask-shaped and enlarged in the middle	*Trichoderma ressei*
FSU/AW1	Dense mycelium, appressed off-white	White conidia, smooth, oblong chlamydospores forming in abundance	Phialides typically terminating cells of branches in pairs	*Trichoderma stromaticum*
FSU/AW3	Diffusing yellow pigment conidiation	Conidia broadly ellipsoidal to ovoid	Phialides mainly arising in closely appressed whorls. Less frequently in pairs	*Trichoderma virens*
FISO10	Conidia typically forming moderately well in concentric rings	Smooth and ellipsoidal conidia	Phialides produced singly	*Hypocrea stilbohypoxyli*
FISO12	Dark green, dense wooly colony	Green conidia, smooth and sub-globose	Phialides conspicuously swollen in the middle and with a sharply constricted, straight long neck	*Trichoderma pleuroticola*
FAD1	Yellowish green, uniformly dispersed colonies	Thin-walled, smooth, green, ellipsoidal conidia hlamydospore not formed	Straight phialides widest at the middle	*Hypocrea patella*
FOG1	Conidia formed densely in a central disk and concentric rings of conidial production. No pigment in the agar	Smooth green conidia and ellipsoidal in shape	Basal phialides tending to be held in more or less divergent whorl while terminal phialides slightly hooked	*Trichoderma fertile*
FISO11	Deep brown powdery colony with evanescent greenish tone in very old cultures	Conidia globose more or less pear shaped and coarsely roughened	Non-septate hyphae vessels thin-walled. Metullae and phiallides present	*Aspergillus* sp.

Table 2. Screening of the bacterial strains for lipase production on solid agar.

Isolate Code	Bacterial Isolates	Lipolytic Activity Diameter (mm)		
		Incubation Time		
		24	48	72
ISO1	*Artrobacter* sp.	0.00	0.00	0.00
ISO2A	*Artrobacter* sp.	5.0^{l}	8.0^{h}	9^{f}
ISO2B	*Bifidobacterium* sp.	7.0^{j}	8.0^{h}	8^{h}
ISO3	*Acinetobacter calcoaceticus*	0.00	8.5^{g}	8.5^{g}
ISO4	*Arthrobacter* sp.	0.00	6.0^{j}	6.0^{j}
ISO5	*Yersinia* sp.	0.00	0.00	0.00
ISO7	*Acinetobacter* sp.	5.0^{l}	5.0^{l}	5.0^{l}
ISO8	*Brevibacterium* sp.	11.0^{c}	11.0^{c}	11.0^{c}
ISO9	*Acetobacterium* sp.	8.0^{h}	8.0^{h}	8.0^{h}
ISO10	*Bifidobacterium* sp.	0.00	0.00	0.00
ISO11	*Aeromonas hydrophilla*	4.0^{m}	7.0^{i}	7.5^{h}
ISOI2	*Acinetobacter* sp.	7.5^{i}	8.0^{h}	8.0^{h}
ISO13	*Acinetobacter* sp.	9.0^{f}	9.0^{f}	9.0^{f}
ISO14	*Brevibacterium* sp.	4.0^{m}	4.0^{m}	4.0^{m}
ISO15	*Yersinia* sp.	8.0^{h}	8^{h}	8.0^{h}
ISO16	*Acinetobacter* sp.	0.00	0.00	3.0^{n}
ISO17	*Serratia marcescens*	0.00	0.00	0.00
ISO18	*Acidomonas* sp.	7.0^{j}	7.0^{i}	7.0^{i}
SU/AW1	*Lactobacillus* sp.	0.00	0.00	0.00
SU/AW2	*Bifidobacterium* sp.	4.0^{m}	4.0^{m}	4^{m}
SU/AW3	*Streptococcus* sp.	0.00	0.00	0.00
SU/AW4	*Acinetobacter* sp.	5.0^{l}	5.0^{l}	5^{l}
SU/AW5	*Acinetobacter* sp.	5.0^{l}	5.5^{k}	5.5^{k}
SU/AW6	*Yersinia* sp.	0.00	0.00	0.00
ID1	*Acinetobacter* sp.	10.5^{d}	10.5^{d}	10.5^{d}
AP11	*Bacillus* sp.	0.00	0.00	0.00
AP12	*Aeromonas* sp.	0.00	0.00	4.0^{m}
AP21	*Acinetobacter* sp.	8^{n}	8.0^{h}	8.5^{g}
AP22	*Yersinia* sp.	7.5^{i}	8.0^{h}	8.0^{h}
AP23	*Acinetobacter* sp.	7.5^{i}	8.0^{h}	8.0^{h}
OG1	*Staphylococcus* sp.	0.00	0.00	0.00
OG2	*Acinetobacter* sp.	11.5^{b}	11.5^{b}	12.5^{b}
OG3	*Citrobacter* sp.	15.5^{a}	15.5^{a}	16.0^{a}
AD1	*Acinetobacter* sp.	3.0^{n}	3.0^{n}	3.0^{n}
AD2	*Acinetobacter* sp.	9.5^{e}	10.0^{e}	10^{e}
AD3	*Staphylococcus* sp.	8.5^{g}	9.0^{f}	9^{f}
AD4	*Acinetobacter* sp.	5.5^{k}	5.5^{k}	5.5^{k}

Lipase production ranged within 0.70^{n} - 12.35^{a} U/ml, 1.8^{k} - 19.15^{a} U/ml and 2.50^{n} - 19.8^{a} U/ml in which *Hypocrea patella* FAD1, *T. stromaticum* FSUAW1 and *T. virens* FSU/AW3 had the highest at day 3, 7 and 14, respectively. *Trichoderma viriens* FSU/AW3 had the highest production at day 14 followed in order by *Trichoderma stromaticum* FSUAW1 (19.15 U/ml) while *Trichoderma minutisporum* FAP2S had the least production at day 7, respectively. Lipases are ubiquitous in nature and can be derived from various sources such as plants, animals and microorganisms. Microbial lipases represent the most widely used class of enzymes in biotechnological applications and organic chemistry (Saxena *et al*. 1999; Jaeger *et al*. 1994). It could thus be inferred that there was an increase in lipase production by some of the isolates (*T. Polysporum, T. harzianum and T. pleuroticola*) as the incubation period increases. However, in most of the isolates the lipase production decreased with the increase of the incubation period. This is in agreement with work of Rajesh *et al*. (2010) who stated that the lipase production increases as the incubation time increases but later falls with the increase in incubation time. Reduction in lipase production could be due to proteolytic degradation of the enzyme system. Pera *et al*. (2006) have reported 51% increase in extracellular lipase production by *Aspergillus niger* after 4 days of incubation.

Table 3. Screening of bacterial isolates for lipase production and growth determination using submerged fermentation.

Isolate Code	Bacterial Isolates	Lipase Production (U/ml)	Growth (Absorbance@600nm)	Growth (Dry Weight (g))
ISO1	*Artrobacter* sp.	2.9	0.568	0.188
ISO2A	*Artrobacter* sp.	2.7	0.278	0.137
ISO2B	*Bifidobacterium* sp.	7.2	0.093	0.122
ISO3	*Acinetobacter calcoaceticus*	4.7	0.462	0.166
ISO4	*Arthrobacter* sp.	3.15	0.129	0.033
ISO5	*Yersinia* sp.	5.7	0.744	0.204
ISO7	*Acinetobacter* sp.	1.8	0.376	0.212
ISO8	*Brevibacterium* sp.	1.95	0.493	0.054
ISO9	*Acetobacterium* sp.	1.9	0.825	0.074
ISO10	*Bifidobacterium* sp.	2.9	0.295	0.063
ISO11	*Aeromonas hydrophilla*	5.6	0.365	0.069
ISOI2	*Acinetobacter* sp.	5.8	0.535	0.052
ISO13	*Acinetobacter* sp.	5.7	0.347	0.065
ISO14	*Brevibacterium* sp.	3.3	0.435	0.233
ISO15	*Yersinia* sp.	1.25	0.527	0.245
ISO16	*Acinetobacter* sp.	4.2	0.119	0.222
ISO17	*Serratia marcescens*	8.0	0.397	0.091
ISO18	*Acidomonas* sp.	1.95	0.852	0.062
SU/AW1	*Lactobacillus* sp.	3.0	0.792	0.000
SU/AW2	*Bifidobacterium* sp.	3.9	0.914	0.000
SU/AW3	*Streptococcus* sp.	3.4	0.543	0.000
SU/AW4	*Acinetobacter* sp.	1.5	0.441	0.000
SU/AW5	*Acinetobacter* sp.	8.65	0.527	0.000
SU/AW6	*Yersinia* sp.	3.3	0.691	0.006
ID1	*Acinetobacter* sp.	4.2	1.003	0.006
AP11	*Bacillus* sp.	3.8	0.415	0.000
AP12	*Aeromonas* sp.	4.5	0.277	0.000
AP21	*Acinetobacter* sp.	7.65	0.6	0.000
AP22	*Yersinia* sp.	7.05	0.7	0.000
AP23	*Acinetobacter* sp.	3.6	0.412	0.000
OG1	*Staphylococcus* sp.	3.2	0.839	0.021
OG2	*Acinetobacter* sp.	1.8	0.65	0.000
OG3	*Citrobacter* sp.	7.25	0.633	0.012
AD1	*Acinetobacter* sp.	1.75	0.801	0.216
AD2	*Acinetobacter* sp.	6.45	0.626	0.014
AD3	*Staphylococcus* sp.	2.3	0.645	0.005
AD4	*Acinetobacter* sp.	2.0	1.003	0.106

Table 4. Screening of fungal isolates for growth and lipase production using submerged fermentation.

Isolate Code	Fungal Isolates	Growth			Lipase Production (U/ml)		
		Incubation Time (Days)			Incubation Time (Days)		
		3	7	14	3	7	14
FAP25	*Trichoderma minutisporum*	0.0130^{g}	0.028^{h}	0.1490^{f}	4.90^{h}	1.00^{l}	2.60^{m}
FISO12	*Trichoderma pleuroticola*	0.1500^{b}	0.064^{g}	0.2690^{b}	1.50^{l}	5.45^{e}	9.55^{c}
FISO1	*Trichoderma*	0.2000^{a}	0.2440^{a}	0.2590^{c}	2.80^{k}	4.80^{g}	6.50^{e}
FISO7	*Trichoderma*	0.0000^{l}	0.1970^{d}	0.0490^{k}	3.2^{l}	2.40^{l}	2.40^{o}
FISO3	*Trichoderma polysporum*	0.0000^{l}	0.2170^{c}	0.0890^{h}	2.95^{j}	2.00^{j}	3.40^{k}
FISO15	*Trichoderma*	0.1200^{c}	0.0400^{h}	0.1990^{f}	6.50^{f}	3.50^{h}	2.50^{n}
FSU/AW3	*Trichoderma virens*	0.0070^{ih}	0.2500^{a}	0.3190^{a}	8.31^{d}	2.35^{l}	19.8^{a}
FSUAW2	*Trichoderma longibrachiatum*	0.0000^{l}	0.0760^{f}	0.0720^{i}	4.90^{h}	17.10^{b}	2.05^{p}
FISO6	*Trichoderma harzianum*	0.0000^{l}	0.0000^{m}	0.0050^{n}	1.20^{m}	4.60^{g}	4.47^{i}
FISO11	*Aspergillus* sp.	0.055^{e}	0.0280^{h}	0.2090^{e}	7.99^{e}	1.80^{k}	16.75^{b}
FAD1	*Hypocrea patella*	0.136^{c}	0.1010^{e}	0.1190^{g}	12.35^{a}	7.60^{d}	7.65^{d}
FISO9	*Trichoderma*	0.119^{d}	0.2240^{b}	0.0590^{j}	10.00^{c}	1.00^{l}	2.80^{l}
FISO14	*Trichoderma ressei*	0.012^{gh}	0.2150^{c}	0.2190^{d}	10.60^{b}	4.90^{f}	3.65^{j}
FSUAW1	*Trichoderma stromaticum*	0.023^{f}	0.0250^{ih}	0.0000^{n}	5.70^{g}	19.15^{a}	5.60^{f}
FOGI	*Trichoderma fertile*	0.0120^{gh}	0.0220^{j}	$0.015l^{m}$	2.95^{j}	4.60^{g}	2.58^{mn}
FISO10	*Hypocrea stilbohypoxyli*	0.0000^{l}	$.00000^{l}$	0.0000^{n}	0.70^{n}	11.05^{c}	5.35^{h}
FSU/AW4	*Hypocrea neorufa*	0.0060^{i}	0.004^{k}	0.0000^{n}	10.00^{c}	5.35^{f}	5.45^{g}

Means with different superscripts within the column are significantly different. $P \geq 0.005$.

Conclusion

The present study revealed that *Acinetobacter* sp. And *Trichoderma virens* gave the best lipase production (8.65 U/ml and 19.80 U/ml) amongst the 37 and 17 bacterial and fungal isolates screened for lipase production using submerged fermentation. Other isolates such as: *T. stromaticum*, *T. longibrachiatum*, *Aspergillus* sp., *Hypocrea patella*, *H. stilbohypoxyli*, *T. ressei*, and *H. neorufa* with 19.15 U/ml, 17.10 U/ml, 16.75 U/ml, 12.35 U/ml, 11.05 U/ml, 10.60 U/ml, and 10.00 U/ml enzyme activity, respectively, are also high potential lipase producers. Research on production, characterization and purification of their enzyme through optimization of process parameters such as pH, temperature and various substrate utilizations would reveal those strains with higher lipase production potential.

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