Utilization of nutrients for growth and lipase production by some selected lipolytic fungi

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Abstract: Nutrient utilization for growth and lipase production by some lipolytic fungi isolated from oil polluted environmental samples was investigated. Olive oil was found to be the best utilizable carbon by the lipolytic strains. Sucrose and xylose was best utilized for growth by *Trichoderma virens* and *Hypocrea patella*. 90% of the strains utilized coconut oil maximally for lipase production. Tween80 was best utilized by *T. viriens* (2.80[a]U/ml) for lipase production. Ammonium dihydrogen phosphate was the best utilizable nitrogen source for growth of all the strains. The growth ranged from 0.000[e] – 0.035[a], 0.000[f] – 0.051[a] and 0.00[f] – 0.031[a] for *Hypocrea patella, Aspergillus* sp. and *Trichoderma virens* respectively. Urea was the best utilizable nitrogen source for lipase production (2.35U/ml) followed in order by ammonium chloride (2.05U/ml) and ammonium sulfate (2.00U/ml). Lipase production ranged from 0.90[e] – 2.05[a], U/ml 1.25[f] – 2.35[a]U/ml and 0.30[e] – 1.60[a]U/ml for *H. patella*, *Aspergillus* sp. and *T. virens* respectively. The combination of $MgSO_4$. $7H_2O+CaCl_2$ was found to stimulate the highest growth of *Hypocrea patella* on the 3rd day of incubation while $MgSO_4$. $7H_2O$ stimulated the best growth on the 7th day of incubation. Maximum lipase production was observed when $MgSO_4.7H_2O+CaCl_2$ (1.70[a]U/ml), $MgSO_4.7H_2O+ CaCl_2 + NaCl$ (1.15[a]U/ml) and $MgSO_4.7H_2O+CaCl_2$ (1.10aU/m) was utilized at different days of fermentation. Zn^{2+} was the best utilizable metal ions for growth (0.168[a]) and lipase production (1.25 U/ml) by *Hypocrea patella* on the 7th day of incubation. There was no significant difference in the utilization of Cu^{2+} and Mn^{2+} on the growth of *Hypocrea patella* while significant difference was not observed in lipase production with all the metal ions on the 14th day of incubation.

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1. Introduction

Lipases are defined as a carboxylesterases involved in catalytic hydrolysis and synthesis of long-chain acylglycerols. Lipases have ability to develop hydrolysis, esterification, perhydrolisis, alcoholysis, intersterification and aminolysis reactions. In the past years interest in microbial lipase production has increased as a result of the versatility of the molecular structure and catalytic properties. Lipases have high potential applications in different industrial sectors such as detergent, flavor development, food, paper recycle, chemical systems, racemic mixtures, waste water treatment,cosmetics, oleochemical, pharmaceutics (Jaeger, 1999; Serano and Alicia, 2001; Castro *et al.,* 2004; Sharma *et al.,* 2001). In the fuel sector, lipase are used as catalyst for synthesis of esters and transesterification of the oil for the production of biodiesel (Ranganathan *et al.,* 2008; Thamalampudi *et al.,* 2008). In plastic and textile industries lipases are also used for biodegradation of plastics and for the treatment of PET (Polyethylene terapthelate) fabric surface to enhance wettability, absorbency, stain resistance dyeability properties and also to improve the pilling properties (Gombert *et al.,*1999; Li and Hardin,1999; Anderson *et al.,* 1999). These unique properties is due to their broad specificity for a wide spectrum of substrates, stability in organic solvents and enantioselectivity (Snellman and Colwell, 2004; Fadnavis and Deshpande, 2002).

Lipases from microorganisms have drawn much attention for their potential use in biotechnology, mainly due to their availability and stability (Ghosh *et al*., 1996; Wang *et al*., 1995). Microbial lipases represent the most widely used class of enzymes in biotechnological applications and organic chemistry (Jaeger *et al*., 1994). Fungi has been reported as the best lipase sources and are usually used for industrial applications (Mahadik *et al.,* 2002). Many lipases of microbiological origin with different properties and substrates specificity has been isolated and characterized by some researcher.

Lipase producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, and decaying food, compost heaps, coal tips, and hot springs (Sztajer *et al.,* 1988; Wang *et al.*,1995).

Microbial lipases are more stable as compared to plant and animal lipases and they can be obtained cheaply. Some of the commercially important lipase-producing fungi have been isolated and characterized (Yadav *et al*., 1998a, b; Gulati *et al*., 1999). Studied on investigation of lipase production by *Trichoderma Reesei* and optimization of production parameters has been done by Rajesh *et al*. (2010). Lipases are very useful biocatalysts as a result of its wide-ranging versatility in industrial applications. Due to their importance improvement of both the producing strains and the biochemical and physicochemical properties of lipolytic enzyme (Prim *et al.,* 2003) is of great concerned.

This research aimed at investigating nutrient utilization for growth and lipase production by lipolytic strains of fungi previously isolated from oil contaminated environmental samples.

2. Materials and methods

2.1 Culture collection and Inoculum preparation

Three strains of lipolytic fungi (*Hypocrea patella* FAD1, *Aspergillus* sp. FISO11 and *Trichoderma* virens FSU/AW3) were obtained from a culture collection of our previous work in the Department of Microbiology University of Ibadan, Ibadan Nigeria. The stock cultures were maintained on Potato Dextrose Agar (PDA) and stored at 4^oC. The seed culture was grown in a 250ml flask containing 50ml of sterile seed medium (containing yeast extract, 5; peptone, 1.5; $MgSO_4.7H_2O$ 1; KH_2PO_4, 3; distilled water, 1000ml, pH 5.5) inoculated with a known volume of the stock culture and incubated for 5 days.

2.2 Lipase production by the selected lipase producing fungal strains

Lipase production by the lipolytic fungi strain was carried out using the modified method of Gupta *et al*. (2005). The sterile basal medium (containing yeast extract, 5; peptone, 1.5; NaCl, 1; $MgSO_4.7H_2O$ 1; KH_2PO_4, 3; Castor oil, 10ml; distilled water, 1000ml, pH 5.5) was inoculated with seed cultures of fungal strains respectively. Fermentation was carried out at room temperature (27±2°C) for 3, 7 and 14 days. Lipase production was determined by assaying lipase activity in crude culture filtrate at standard assay conditions (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.

2.3 Utilization of carbon and nitrogen for growth and lipase production by the lipolytic fungi

Utilization of carbon and nitrogen sources for growth and lipase production by the isolates was investigated. Eleven carbon sources both lipids and carbohydrates (Coconut oil, palm kernel oil, olive oil, glucose, sucrose, fructose, lactose, soluble starch, xylose, and Tween80 and Groundnut oil) and nitrogen sources (Ammonium dihydrogen phosphate, ammonium chloride, sodium nitrate, corn steep liquor, ammonium sulfate, potassium nitrate and urea) was used. Lipase production was carried out by supplementing the different carbon and nitrogen sources respectively. Lipase production was determined by assaying lipase activity in crude culture filtrate at standard assay conditions (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.

2.4 Utilization of mineral salts and metal ions for growth and lipase production by Hypocrea patella FAD1

Utilization of mineral salts for growth and lipase production by *Hypocrea patella* FAD1 was studied by using different types of mineral salts singly as well as in combination ($CaCl_2$, $MgSO_47H_2O$ + $CaCl_2$, $MgSO_4.7H_2O$+$CaCl_2$+NaCl, $CaCl_2$+NaCl and $MgSO_4.7H_2O$) and metal ions in the form of salts (KCl, $MnCl_2$, $CuSO_4$, $FeSO_4$ and $ZnSO_4$) was investigated. Lipase production was determined by assaying lipase activity in crude culture filtrate at standard assay conditions (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.

2.5 Dry Cell Weight Determination

The mycelium from each flask was filtered and then washed. The washed mycelium was dried in Gallenkamp oven at 110^OC to a constant mass and the mass was determined using an automatic electronic balance

2.6 Statistical analysis

Experiments were performed in triplicate and the results were analyzed 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 (Duncan, 1955).

3. Results

Utilization of carbon source on growth of lipolytic fungal strains is shown in Table 1. The carbon source had a profound effect on the growth of the isolates. Olive oil was found to be the best utilizable carbon by the strains. Sucrose and xylose was best utilized for growth by *Trichoderma virens* and *Hypocrea patella*. There was variation in growth at different incubation time (days). Utilization of carbon source for lipase production by the strains is shown in Table 2. The strains have the capacity to utilize all the carbon sources for growth and lipase production. Carbon source significantly affects lipase

production of the isolates. Coconut oil was best utilized for lipase production by 90% of the isolates. Tween80 was best utilized by *T. viriens* (2.80^{a}U/ml) for lipase production.

Utilization of nitrogen source for growth by the lipolytic fungi is shown in Table 3.The growth of the selected isolates ranged from 0.000^{e} – 0.035^{a}, 0.000^{f} – 0.051^{a} and 0.00^{f} – 0.031^{a} for *Hypocrea patella, Aspergillus* sp. and *Trichoderma virens* respectively.

Ammonium dihydrogen phosphate was the best utilizable nitrogen source for growth of all the strains. There was a significant difference in the growth pattern of the selected isolates at different incubation period in which the highest growth was observed at day 14 after incubation.

Table 1: Effect of carbon source on the growth of the selected fungal isolates

Carbon Sources	Lipase Production (U/mL)								
	Fermentation time (days)								
	3			**7**			**14**		
	Hypocrea patella FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3
Glucose	0.029^{h}	0.000^{j}	0.009^{h}	0.000^{e}	0.040^{c}	0.000^{d}	0.000^{f}	0.036^{c}	0.112^{a}
Sucrose	0.062^{b}	0.042^{e}	0.025^{c}	0.000^{e}	0.022^{d}	0.047^{a}	0.053^{b}	0.002^{i}	0.077^{b}
Xylose	0.032^{g}	0.076^{c}	0.020^{e}	0.034^{b}	0.000^{f}	0.004^{c}	0.137^{a}	0.049^{b}	0.073^{c}
Tween80	0.042^{e}	0.037^{f}	0.001^{i}	0.000^{e}	0.000^{f}	0.000^{d}	0.007^{e}	0.020^{e}	0.000^{f}
Starch	0.056^{c}	0.079^{b}	0.056^{a}	0.043^{a}	0.042^{b}	0.000^{d}	0.000^{f}	0.054^{a}	0.001^{e}
Lactose	0.044^{d}	0.052^{d}	0.000^{j}	0.028^{c}	0.000^{f}	0.000^{d}	0.000^{f}	0.030^{d}	0.000^{f}
Olive-oil	0.085^{a}	0.084^{a}	0.021^{d}	0.000e	0.002^{e}	0.000^{d}	0.035^{d}	0.007^{h}	0.000^{f}
Ground nut oil	0.039^{f}	0.023^{h}	0.029^{b}	0.021^{d}	0.000^{f}	0.000^{d}	0.036^{c}	0.000^{j}	0.000^{f}
Palm kernel oil	0.021i	0.032^{g}	0.012^{f}	0.000^{e}	0.046^{a}	0.018^{b}	0.000^{f}	0.011^{f}	0.009^{d}
Coconut oil	0.000^{j}	0.020^{i}	0.010^{g}	0.000e	0.000^{f}	0.000^{d}	0.000^{f}	0.010^{g}	0.000^{f}

Means followed by different superscript within a column are significantly different (P≥0.05).

Table 2: Effect of Carbon Sources on Lipase production by the Selected Fungal Isolates

Carbon Sources	Lipase Production (U/mL)								
	Fermentation time (days)								
	3			**7**			**14**		
	Hypocrea patella FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3
Glucose	1.11^{d}	1.44^{b}	1.70^{b}	1.30^{g}	1.80^{c}	1.60^{f}	1.05^{e}	1.05^{g}	1.00^{e}
Sucrose	1.70^{b}	1.45^{b}	1.10^{e}	1.70^{e}	1.90^{b}	1.80^{e}	1.10^{de}	1.30^{b}	1.20^{c}
Xylose	1.55^{cd}	0.50^{g}	1.32^{c}	2.00^{c}	1.80^{c}	1.90^{c}	1.10^{de}	1.00^{h}	1.10^{d}
Tween80	1.13^{d}	1.25^{c}	1.10^{e}	2.10^{b}	0.90^{g}	2.80^{a}	1.15^{cd}	1.25^{c}	1.10^{d}
Starch	1.10^{d}	1.25^{c}	1.35^{c}	1.70^{e}	1.90^{b}	1.60f	0.90^{f}	1.10^{f}	1.30^{b}
Lactose	1.15^{d}	1.25^{c}	1.20^{d}	2.00^{c}	1.55^{e}	1.55^{g}	1.20^{bc}	1.20^{d}	1.11^{d}
Olive oil	1.30^{cd}	1.10^{e}	1.00^{f}	1.80^{d}	1.60^{d}	1.40^{h}	1.24^{b}	1.15^{e}	1.15^{cd}
Ground nut oil	1.10^{cd}	1.15^{d}	1.36^{c}	1.40^{f}	1.50^{f}	1.90^{c}	1.05^{e}	1.15^{e}	1.15^{cd}
Palm kernel oil	1.19^{d}	0.90^{f}	1.20^{d}	1.38^{f}	0.77^{h}	1.85^{d}	1.05^{e}	1.20^{d}	1.15^{cd}
Coconut oil	10.00^{a}	6.00^{a}	1.80^{a}	2.20^{a}	2.1^{a}	2.35^{b}	2.15^{a}	2.70^{a}	2.70^{a}

Means followed by different superscript within a column are significantly different (P≥0.05).

Table 3. Effect of Nitrogen Sources on Growth (Dry cell weight) of the Selected Fungal Isolates

Nitrogen Sources	Growth (G)								
	Fermentation time (days)								
	3			**7**			**14**		
	Hypocrea patella FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3
KNO_3	0.010^{d}	0.049^{b}	0.028^{b}	0.009^{d}	0.000^{d}	0.000^{d}	0.005^{e}	0.093^{b}	0.020^{c}
NH_4SO_4	0.013^{c}	0.000^{f}	0.025^{c}	0.000^{g}	0.000^{d}	0.000^{d}	0.000^{f}	0.026^{f}	0.011^{d}
$NH_4H_2PO_4$	0.035^{a}	0.002^{e}	0.001^{d}	0.039^{a}	0.019^{a}	0.022^{c}	0.048^{b}	0.043^{e}	0.041^{a}
CSL	0.027^{b}	0.039^{c}	0.000^{e}	0.031^{b}	0.016^{b}	0.026^{b}	0.051^{a}	0.046^{d}	0.035^{b}
$NaNO_3$	0.013^{c}	0.016^{d}	0.031^{a}	0.000f	0.000^{d}	0.029a	0.039^{c}	0.065^{c}	0.000^{f}
NH_4Cl	0.000^{e}	0.051^{a}	0.000^{e}	0.011^{c}	0.007^{c}	0.000^{d}	0.022^{d}	0.199^{a}	0.000^{f}
UREA	0.011^{d}	0.049^{b}	0.000^{e}	0.006^{e}	0.016^{b}	0.022^{c}	0.000^{f}	0.018^{g}	0.008^{e}

Means followed by different superscript within a column are significantly different (P≥0.05). CSL= Corn steep liquor.

Table 4 shows utilization of nitrogen source for lipase production by the lipolytic fungi. The lipase production by the isolates ranged from 0.90^{e} – 2.05^{a}, U/ml 1.25^{f} – 2.35^{a}U/ml and 0.30^{e}U/ml – 1.60^{a}U/ml for *H. patella*, *Aspergillus* sp. and *T. virens* respectively. Urea was best utilized for lipase production (2.35U/ml) followed in order by ammonium chloride (2.05U/ml) and ammonium sulfate (2.00U/ml).

Table 5 shows utilization of mineral salts for growth and lipase production by *Hypocrea patella*. The highest growth of the strain was observed on the day 14 after incubation. The combination of $MgSO_4.7H_2O$+ $CaCl_2$ + NaCl was best utilized for growth (0.066^{a}) followed in order by a combination of $MgSO_4.7H_2O$+ $CaCl_2$ and $CaCl_2$ respectively. The combination of $MgSO_4.7H_2O$+$CaCl_2$ was found to stimulate the highest growth of the isolate on the 3rd day of incubation and while on the 7th day of incubation, $MgSO_4.7H_2O$ stimulated the best growth. The combination of $CaCl_2$ + NaCl was least utilized by the isolates. The maximum lipase production was observed on the 3rd, 7th and 14th day of incubation when $MgSO_4.7H_2O$+$CaCl_2$ (1.70^{a}U/ml), $MgSO_4.7H_2O$+ $CaCl_2$ + NaCl (1.15^{a}U/ml) and $MgSO_4.7H_2O$+$CaCl_2$ (1.10aU/m) was best utilized by the isolates. The investigation on the effect of $MgSO_4.7H_2O$, NaCl, and $CaCl_2$ on growth and lipase production by the selected bacterial and fungal isolates done individually as well as in combination showed that addition of $MgSO_4.7H_2O$ and $CaCl_2$ at 2g/l was best utilizable for maximum lipase production by the fungal isolates.

Table 4: Effect of Nitrogen Sources on Lipase production by the Selected Fungal Isolates

Nitrogen Sources	Lipase Production (U/mL)								
	Fermentation time (days)								
	3			7			14		
	Hypocrea patella FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3	*Hypocrea patella* FAD1	*Aspergillus* sp. FISO11	*Trichoderma* virens FSU/AW3
KNO3	1.25^{d}	1.25^{f}	1.40^{b}	1.00^{e}	1.00^{f}	1.00^{e}	1.19^{a}	1.10^{b}	0.92^{d}
NH_4SO_4	2.00^{a}	1.70^{c}	1.40^{b}	1.30^{b}	1.30^{b}	1.20^{b}	1.19^{a}	1.05^{c}	1.30^{a}
$NH_4H_2PO_4$	0.90^{e}	2.10^{b}	1.30^{d}	1.30^{b}	1.20^{c}	1.55^{a}	1.15^{b}	1.05^{c}	1.20^{b}
CSL	1.60^{b}	1.40^{e}	1.40^{b}	1.03^{de}	1.05^{e}	1.10^{c}	1.20^{a}	0.90^{d}	0.85^{e}
NaNO3	1.40^{c}	1.50^{d}	1.35^{cd}	1.10^{c}	1.30^{b}	0.85^{f}	1.04^{c}	1.09^{b}	0.82^{e}
NH4Cl	2.05^{a}	1.70^{c}	1.60^{a}	1.35^{a}	1.40^{a}	1.20^{b}	1.15^{b}	1.25^{a}	1.10^{c}
UREA	1.35^{c}	2.35^{a}	0.30^{e}	1.05^{d}	1.10^{d}	1.05^{d}	1.00^{d}	1.10^{b}	0.90^{d}

Means followed by different superscript within a column are significantly different ($P \geq 0.05$). CSL= Corn steep liquor.

Table 6 shows utilization of metal ions on growth and lipase production by *Hypocrea patella*. Zn^{2+} was the best utilizable metal ions for growth (0.168^{a}) by the selected isolate on the 7th day of incubation. There was no significant difference in the utilization of Cu^{2+} and Mn^{2+} on the growth of the isolate as well as Fe^{2+} and K^{+}. Mn^{2+} was the least utilizable metal ion for growth. Zn^{2+} was the best utilizable metal ion for lipase production with a value of 1.25 U/ml followed in order by Mn^{2+} and Cu^{2+} with values of 1.10 U/ml and 1.09 U/ml respectively. There was no significant difference in lipase production with all the metal ions on the 14th day of incubation.

Table 5. Effect of Mineral salts on growth and Lipase Production by Hypocrea patella FAD1

Mineral Salts	Growth			Lipase production (u/ml)		
	Fermentation time (days)			Fermentation (days)		
	3	7	14	3	7	14
$CaCl_2$	0.033^{b}	0.050^{b}	0.053^{c}	1.00^{e}	1.00^{c}	0.90^{e}
$MgSO_4.7H_2O+CaCl_2$	0.049^{a}	0.026^{d}	0.055^{b}	1.70^{a}	1.00^{c}	1.10^{a}
$MgSO_47H_2O+Cacl_2+NaCl$	0.015^{c}	0.037^{c}	0.066^{a}	1.40^{b}	1.10^{a}	0.92^{d}
CaCl2+NaCl	0.000^{e}	0.000^{e}	0.000^{e}	1.20^{d}	1.20^{b}	1.08^{b}
$MgSO_4$-$7H_2O$	0.013^{d}	0.052^{a}	0.022^{d}	1.30^{c}	0.95^{d}	1.00^{c}

Means followed by different superscript within a column are significantly different ($P \geq 0.05$).

Table 6. Effect of Metal Ions on growth and lipase production by *Hypocrea patella* FAD1

Metal Ions	Growth			Lipase production (u/ml)		
	Fermentation time(days)			Fermentation time(days)		
	3	7	14	3	7	14
Cu^{2+}	0.069^{c}	0.031^{b}	0.035^{c}	0.90^{e}	1.00^{c}	1.09^{a}
Mn^{2+}	0.029^{d}	0.029^{b}	0.034^{d}	1.10^{b}	0.90^{d}	0.95^{a}
Zn^{2+}	0.075^{b}	0.168^{a}	0.035^{c}	1.25^{a}	1.09^{b}	1.10^{a}
Fe^{2+}	0.081^{a}	0.017^{c}	0.06^{a}	1.00^{c}	1.00^{c}	1.01^{a}
K^{+}	0.069^{c}	0.018^{c}	0.057^{b}	0.95^{d}	1.11^{a}	0.96^{a}

Means followed by different superscript within a column are significantly different ($P \geq 0.05$).

4. Discussion

Microbial lipases have generated increased attention among researcher due to their important as biotechnological enzymes and their wide application in food, dairy, detergent and pharmaceutical industries. Lipases (triacyl glycerol acylhydrolases E.C.3.1.1.3) are enzymes that catalyses the hydrolysis of fats into fatty acids and glycerol at the water-lipid interface and bring about reversing the reaction in non aqueous media (Hala *et al*. 2010). Many lipase producing microorganisms (bacteria, fungi and yeast) has been reported and they are characterized with different enzymological properties and substrate specificities (Rapp and Backhaus, 1992). Lipases are secreted into the medium by microorganisms during fermentation and production is dependent on the availability of nutrient in the medium , microorganism used and the type of lipase secreted (Nadia, 2001).

Ability of microbial isolate to utilize available nutrient in the medium supply for the secretion of this biotechnologically important enzyme is of paramount importance. During this study, It was discovered that lipid/oil substrate was best utilized and it supported maximum amount of enzyme produced by the isolates. The isolates utilizes different carbon source for growth and enzyme production. It has been reported that lipase production requires carbon and nitrogen sources as required by any fermentation process (Sangeetha *et al.* 2011). Utilization of coconut oil for maximum lipase production observed is in accordance with the report of Chaturedi *et al*. (2010). This could be due to easy penetration of the organisms into the substrate and as a result of the presence of lipolytic enzyme capable of metabolizing the substrate. Rohit *et al*, (2001) and Selva Mohan *et al.,* (2008) reported that olive oil supported high level of lipase activity by *Bacillus* strains.

Fadiloglu band Erkmen (2002) reported that olive oil in combination with other nitrogen sources enhanced the lipase production. Rajesh et al. (2010) reported that olive oil supported the highest lipase production by *Trichoderma Reesei* Lactose however gave the maximum lipase production when compared to other carbohydrate substrates. The observers that lipids were better carbon sources than carbohydrates for lipase production is in agreement with the work of Christakopoulos *et al*. (1992) and Rohit *et al*. (2001). Most of the lipase production studies do not use simple sugars as carbon sources rather use lipid substances as sole carbon sources. Sangeetha(2011) gave similar report.

Ammonium sulfate and ammonium chloride were the best utilizable nitrogen source, this is in agreement with the report of Guptal *et al*. (2004) and Christakopoulos (1992). In a similar report Rohit *et al*. (2001) reported that lipase yield and stability could be improved by supplementing the preferred organic nitrogen source with ammonium. Urea was best utilizable for both growth and lipase production, this is in agreement with the report of Sharma *et al*. (2001) that urea stimulates lipase production in both bacterial and fungal isolates.

Utilization of metal ions for lipase production, Zn^{2+} was observed to stimulate lipase production by *Hypocrea patella*. A stimulatory effect of divalent ions on lipase production has been reported (Sangeetha *et al*. 2011).

Utilization of Zn^{2+} as the best metal ions for lipase production by the fungal strains is in contrast to the report of Gupta *et al*. (2004) who reported that calcium and magnesium ion had the best stimulation effect on lipase production by *Burkhloderia* sp.. Similarly he reported that Fe^{2+} plays a critical role in lipase production of *Pseudomonas* sp.

5. Conclusion

The findings from this research work may be considered as an additional knowledge to showcase the ability of lipolytic fungi to utilize different nutrients for the production of valuable extracellular lipases of industrial importance. Utilization of these nutrients for the cultivation of the strain will enhance commercial production of the lipolytic enzyme which will increase their availability for use in biotechnological applications and organic chemistry. From the results it can be concluded that the lipolytic strains had the ability to utilize different nutrients for growth and lipase production. Olive oil was the best utilizable carbon for lipase production while Sucrose and xylose was best utilized for growth by *Trichoderma virens* and *Hypocrea patella*. 90% of the strains has the ability to utilize coconut oil maximally for lipase production. Ammonium dihydrogen phosphate and urea was the best utilizable nitrogen source for growth of all the strains. The combination of $MgSO_4.7H_2O$+$CaCl_2$ and Zn^{2+} were the best utilizable mineral and metal ions for growth and lipase production.

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