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Cold Active Lipases Produced by *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149 Isolated from Nella Lake, Antarctica

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A B S T R A C T

Cold active lipase was investigated by bacteria and yeast isolates from the core sample of Nella Lake, Larsemann Hills region, East Antarctica. Among potential yeasts and bacteria producing lipases, best isolates were identified as *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149 by molecular technique. In order to enhance the lipase production capabilities, both the isolate again subjected for optimization processes using various physiological (temperature and pH) and chemical (Carbon, nitrogen, minerals and various substrates like oils and triglycerides) parameters. The results indicated that a supplement of 1% w/v fructose, 0.1% w/v KCl and 2% v/v tween 80 at pH 8.5 and 15°C enhances the lipase production by 9.81-fold (6.81 U/ml) using *Rhodococcus erythropolis* N149. In other hands, the activators are 1% w/v of galactose and peptone, 0.1% w/v KCl and 2.5% v/v ghee at pH 11.5 and 15°C enhances the lipase production by 4.01-fold (3.35 U/ml) using *Cryptococcus* sp. Y-32. The present study successfully produced cold-active lipases with novel properties like low temperature and high pH stability, which can be used in the degradation of lipid wastes in cold regions and also can be used for detergent formulation for cold temperature washing of delicate clothes.

Introduction

Microorganisms are ubiquitous. Even in extreme environment they can grow and reproduce. Microorganisms loving cold temperature are regarded as psychrophiles having maximum growth temperature ≤ 20°C whereas another group is present as facultative psychrophiles or psychrotolerants having maximum growth temperature in the mesophilic range in spite can grow at near to zero temperature (Moyer and Morita, 2007). The biology of Antarctica is dominated by microorganisms other than animals and plants with a high level of adaptation to withstand extreme conditions (Friedmann, 1993).

Previous reports suggest of getting more psychrotolerants from cold habitats like Antarctica than true obligate psychrophiles (Antony *et al.,* 2016; Hatha *et al.,* 2013; Vaz *et al.,* 2011). Over the last decades the Antarctic examined mainly for the presence of psychrophilic bacteria and archea, and more rarely for fungi (Gunde-Cimerman *et al.,* 2003). The chances of getting psychrophiles

and psychrotolerants are more obvious at polar environments and also can be found in high mountains, glaciers, deep-sea, and alpine soils (Maharana and Singh, 2018).

Lipases (EC 3.1.1.3, glycerol ester hydrolases) are the hydrolytic enzymes that act on carboxylic ester bonds of triglycerides and give diglycerides, monoglycerides, fatty acids and glycerol as end product. Lipases can be produced from various sources of animals, plants and microorganisms. However, for industrial applications, lipases from microorganisms are more focusing because of higher yield, better adaptability and can be easily manipulated genetically (Hasan *et al.,* 2006). Lipases have immense applications to various industries. Now-days cold active lipases are of more demand than that of neutral one because of their lower energy consumption and prevention of loss of volatile compound (Margesin, 2009). Cold active lipases have application in various processes like bio-remediation, and additives in food industries and detergents and also in bio-transformation processes (Joseph *et al.,* 2008).

Pseudomonas is regarded as a remarkable producer for cold active lipase (Maharana and Ray, 2013, 2014a, 2015b; Zeng *et al.,* 2004). Besides, other genera for better lipase production are belonged to the genera of *Acinetobacter*, *Aeromonas*, *Bacillus*, *Microbacterium*, *Moraxella*, *Psychrobacter*, *Staphylococcus* etc. (Joseph *et al.,* 2008). Yeasts like *Rhodotorula*, *Cryptococcus*, *Yarrowia lipolytica* etc. have maximal potentiality to produce cold active lipases (Singh *et al.,* 2014a, b; Taskin *et al.,* 2016; Maharana and Singh, 2018). Besides, Maharana and Ray (2014c) reported psychrotolerant micro-fungi like *Absidia*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Coccoides*, *Fusarium*, *Microsporum*, *Mucor*, *Penicillium*, and *Rhizopus* producing cold active lipases.

Microbial growth depends on many physical and chemical factors like temperature, pH, salinity, substrates, carbon and nitrogen sources, minerals and even if some amino acids. Enzyme production is directly proportional to the growth of the microorganisms but in some cases, there is maximum enzyme production in the medium by the microorganisms which are in an inactive form, resulting in lower enzymatic activity (Maharana and Ray, 2015a). This condition achieved due to variations in temperature, pH and nutrient availability. In addition to this, there are many other molecules that show feedback inhibition of enzyme production. Besides, varied microorganisms showed varied affinity towards substrates employed for the production and have their own mechanism for enzymatic hydrolysis. Therefore, optimization for the production of enzymes is essential for various microorganisms and should be compared for the potent.

The present study focused on the production optimization of cold active lipase by yeast *Cryptococcus* sp. Y-32 and actino-bacterium *Rhodococcus erythropolis* N149 isolated from Lake Nella, East Antarctica, which is the first attempt on Nella lake microbial isolates producing cold active lipase as per the best of our knowledge.

Materials and Methods

Sample collection and isolation of bacteria and yeasts

Current study was on Lake Nella (76°22′ S, 69°24′ E), an ultra-oligotrophic lake located at an altitude of 15 m a.s.l. in the Broknes peninsula, Larsemann Hills region, East Antarctica. The lake measures 0.157 km^2 in area and has a maximum water depth of 8.2 m. Core samples from Lake Nella were collected and cut off into small pieces from 0.5 cm to 90

cm, which were brought back to Polar Biology Lab, NCAOR with polar packs and kept at -20°C to investigate further. All core samples were subjected for the isolation of bacteria and yeasts. Samples were serially diluted by 10-fold dilution technique and spread over various media viz. Antarctic Biological Medium (ABM), ABM/10, Nutrient Agar (NA), NA/10, Zobell Marine Agar (ZMA), ZMA/10, Potato Dextrose Agar (PDA) and PDA/10.

Plates were incubated for 1-2 weeks at 1, 5, 15 and 22°C and cultures after visible were streaked on the same agar plate for further purification done by microscopically (Epi-fluorescence research microscope, BX51 Olympus, Japan) and purified isolates were stored at 1°C (Refrigerated incubator, MIR-554-PE, Panasonic). Glycerol stocks were prepared in their respective broth with 20% glycerol (v/v) and kept at -20°C (Biomedical freezer, MDF-U537D, Sanyo).

Screening and selection of potential lipase producers at cold temperature

Qualitative assay

All the purified isolates were subjected for screening of extracellular lipase using tributyrin agar base (Hi-media) having tributyrin oil. Those plates were incubated at various temperatures viz. 4, 15, 22 and 35°C, and zone of clearances were determined by subtracting the culture diameter with whole diameter including zone of clearances.

Quantitative assay

For quantitative assay submerged fermentation technology was implemented. Initially for inoculum preparation, selected isolates were inoculated separately in respective broth and incubated at 15°C for 48 h at 150 rpm in a shaking incubator (Refrigerated incubator shaker, IS-971RF, Jeio tech, Korea) for agitation. For production of lipase freshly prepared inoculum was used. The production was done by using mineral salt medium (gl^{-1}: yeast extract, 1.0; NaCl, 2.0; $MgSO_4$, 0.4; $(NH_4)_2SO_4$, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.3; $CaCl_2.2H_2O$, 0.1; gum arabic, 2.0; $NaNO_3$, 0.5; olive oil, 1% v/v; pH 8.0) as recorded by Lee *et al.,* (2003) with slight modifications. The production medium was incubated at 15°C and 150 rpm after addition of 5% (v/v) inoculum for 72 h.

About 5 ml medium was taken and extraction was done by centrifugation at 12,000 rpm (Refrigerated table top centrifuge, 5810, Eppendorf, Germany) for 20 min at 4°C. The supernatant was regarded as crude lipase enzyme and subjected for lipase assay.

Lipase activity was measured spectro-photometrically (UV-Vis spectrophotometer, Specord S 205, Analytik Jena, AG Germany) using *p*-nitrophenyl palmitate (*p*-NPP) (Sigma-Aldrich Co.) as substrate and formation of *p*-nitrophenol (*p*-NP) was measured at 400 nm (Gupta *et al.,* 2002) with slight modifications noted by Maharana and Ray (2015b). One unit of lipase activity is defined as the amount of enzymes releasing one micromole of *p*-NP per minute per milliliter under assay conditions.

Identification of potential isolates

Molecular identification of the potential isolates producing cold active lipases were done by using ITS-D1/D2 gene and 16s rRNA gene sequencing based molecular technique. The isolates were outsourced to MTCC, Chandigarh, India for molecular identification. The gene sequence was used to carry out BLAST with the nr-database of GenBank database. The consensus sequence of sample was submitted at BankIt, GenBank, and NCBI for the accession numbers.

Partial characterization of potential isolates

The potential isolates were subjected for different biochemical and sugar utilization tests. Besides, for physiological characterization, a loopful of culture was inoculated in flask containing 50 ml of broth and the flask were incubated at 5, 15, 22 and 35°C at 150 rpm in a shaking incubator. About 50 ml of broth with varied pH values (3, 5, 7, 9 and 11) and NaCl concentrations (0, 1, 5, 10 and 20% w/v) were tested for the growth of isolates at 15°C and 150 rpm in a shaking incubator. In each case, growth was analyzed by spectrophotometer at 600 nm in the interval of 24 h. Furthermore, potential isolates were screened for other hydrolytic enzymes like protease, amylase and cellulase.

Production optimization strategy for lipase

Production optimization for lipase was done by "one factor at-a-time" (OFAT) method. Factors investigated were incubation period (24-120 h), substrate concentrations (0-15% v/v), temperature (5, 10, 15, 25, 30 and 35°C), inoculum size (0.1-20% v/v), initial pH (3.5-12.5), nitrogen (1% w/v: yeast extract, beef extract, peptone, $NaNO_3$, KNO_3, KNO_2, $Ca(NO_3)_2.4H_2O$, $(NH4)_2SO_4$ and NH_4NO_3) and carbon sources (1% w/v: glucose, maltose, fructose, lactose, galactose, sucrose, carboxymethyl cellulose (CMC) and xylose), and minerals (0.1% w/v: NaCl, KCl, $MgCl_2$, $MnCl_2$, $CaCl_2$, $BaCl_2$, NH_4Cl_2, $HgCl_2$ and $ZnCl_2$) and substrates (5% v/v: olive, tributyrin, ghee, mustard, sesame, sunflower, coconut oil, palmolein, tween 20 and tween 80). In each experiment, the optimized factor was implemented with the control medium and experiments were done in triplicates.

Statistical analysis

The data recorded during the investigation were subjected to significance testing by t-test, analyses of variance (ANOVA) and Pearson's correlation coefficient using Microsoft excel 2007. Statistical significance was set at $p<0.05$, and for high significance set at $p<0.001$. Results were denoted as mean±standard error of triplicate experiments.

Results and Discussion

Screening and selection of potential lipase producers

All the bacterial and yeast isolates were investigated for lipase production by plate assay method. Among them best three from yeasts and five from bacteria were selected for further quantification using two substrates i.e. olive oil and tributyrin at 15°C for 5 days. Figure 1 denotes lipase production by potential isolates, where it was found that olive oil was regarded as a good substrate for lipase than tributyrin and maximal lipase were produced by Y-32 (yeast) and N149 (bacteria). ANOVA reveals a highly significant variation ($p<0.001$) in lipase activity among the yeast and bacterial isolates with respect to olive oil and tributyrin.

Identification and partial characterization of Y-32 and N149

From molecular identification it was found that Y-32 is identified as *Cryptococcus* sp. Y-32 (GenBank accession no. KY887681) and N149 as *Rhodococcus erythropolis* N149 (GenBank accession no. KY783363) and phylogenetic tree is represented in Figure 2. Isolate Y-32 showed closest similarity (99.9%) with *Cryptococcus sp.* RY-21 (GenBank accession no. AB259936) which was isolated from permafrost, Russia (Fattakhova *et al.,* 2017, Unpublished). The isolate N149 showed closest similarity (99.9%) with *Rhodococcus erythropolis* Pi3 (GenBank accession no. AM905947) which was isolated from alkaline ground waters

contaminated by benzene, toluene, ethylbenzene, xylenes (BTEX) from the SIReN aquifer, UK and had the ability for benzene-degrading (Fahy *et al.,* 2008).

Figure 3 shows the macro- and micro-morphology and lipase activity at 4 and 15°C by both the isolates. *Cryptococcus* sp. Y-32 can utilize various sugars as carbon sources viz. D-glucose, raffinose, D-gluconate, D-ribose, D-arabinose, lactose, glycerol, DL-lactate, myoinositol, D-xylose, maltose, cellobiose, melezitose, D-glucoronate, mannitol, D-galactose, rhamnose and L-arabinose. Between nitrogen sources, Y-32 utilizes nitrate, nitrite, lysine, tryptophan and creatinine. *Rhodococcus erythropolis* N149 is an aerobic, Gram positive rod actino-bacterium and was tested for many biochemical analyses. It is positive for catalase and oxidase tests and negative for MRVP, indole, citrate utilization, esculinase and phosphatase tests. Besides, it can utilize various sugars as carbon sources viz. D-xylose, dextrose, D-galactose, melibiose, sucrose, mannose, adonitol, arabitol and malonate. Optimum temperature for the growth of both the isolate is 15°C, followed by 5°C (Fig. 4). It was found that there is almost no growth at 35°C. It denotes psychrotolerant nature of *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149. Both Y-32 and N149 have the ability to tolerate a wide range of pH, i.e. pH 3-11 and 5-9, respectively. But the optimum was pH 11 and 7 for Y-32 and N149, respectively (Fig. 4). Y-32 showed the ability to grow at 10% w/v salt concentrations while N149 hardly showed growth at 10%. But both can grow at 1/10th strength of their respective broth medium (Fig. 4). Y-32 is negative for other enzymes like amylase, cellulase and protease while N149 can degrade various substrates apart from tributyrin i.e. skim milk (cold active protease) and carboxymethyl cellulose (cold active cellulase) and negative for amylase.

Effects of production parameters on lipase by N149 and Y-32

Figure 5 reveals that maximum lipase was produced at 15°C by both the isolates and after 25°C lipase activity decreased, which confirm that cold temperature is needed for them to produce lipase. ANOVA reveals that there is a highly significant variation (p<0.001) in lipase activity among the isolates. There is a negative correlation between both the isolates with incubation temperatures, which denote with an increase in temperature the lipase activity decreases significantly. Significant test also confirmed by t-test where both the isolate has significant variation along the temperature at 2-tail (p<0.05).

Inoculum sizes ranging from 0.1-20% (v/v) were taken for lipase production. From Figure 6 it was found that there is a variation in inoculum size requirements for lipase production by both the isolates. The optimum inoculum size was 2% v/v and 15% v/v by *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149, respectively. The t-test reveals a significant variation among the inoculum tested with the respective isolates at p<0.05 while there is a positive correlation between the inoculum sizes with that of lipase activity by N149 significantly (p<0.01). There is a negative correlation between the inoculum sizes with lipase activity by Y-32 significantly at p<0.001.

Olive oil ranging from 0-15% v/v was investigated for lipase production at 15°C. Figure 7 reveals the maximum lipase was produced due to the application of 2.5% v/v and 2% v/v olive oil in the production medium by *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149, respectively. The t-test reveals that there is a significant variation among the population means of both the isolates with respect to different olive oil concentrations at p<0.05.

Fig.1 Lipase production by potential isolates at 15°C. The results are the means of 3 independent experiments and the bars correspond to standard errors

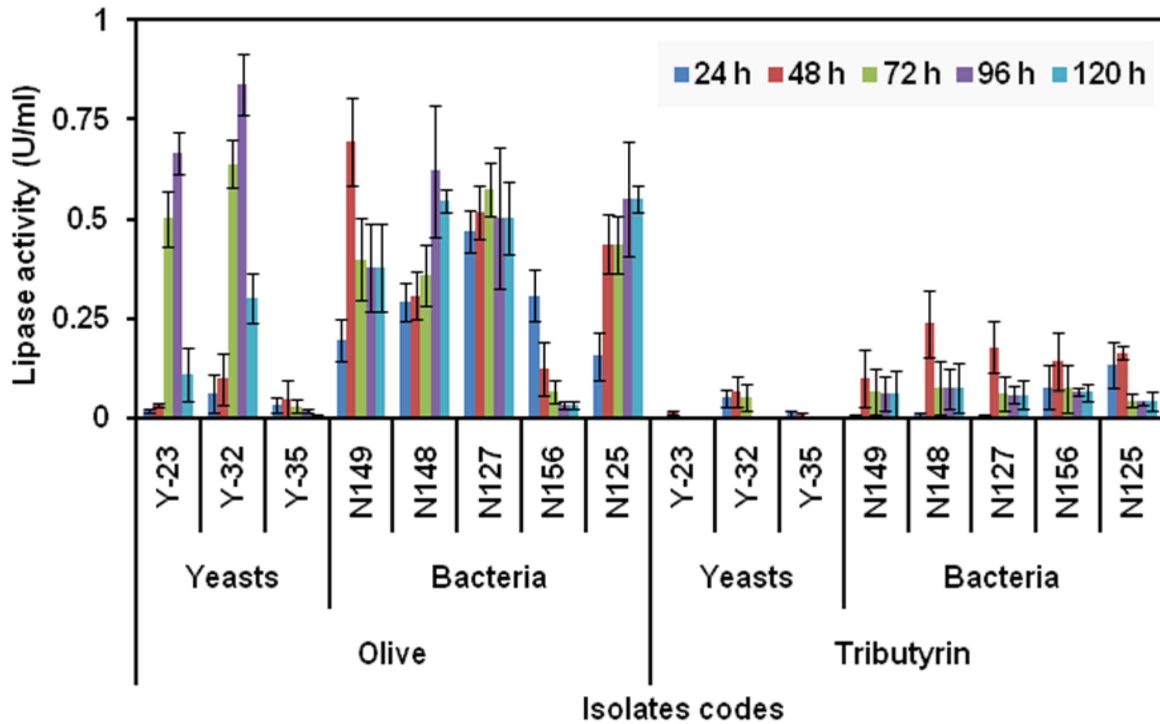


Fig.2 Molecular phylogenetic analysis of A. N149 and B. Y-32. Figure showing the position of current strain in relation to the other genera. GenBank accession numbers for the sequences are shown in parentheses. Numbers at nodes denote bootstrap values based on 1000 replicates. Evolutionary analyses were conducted in MEGA5

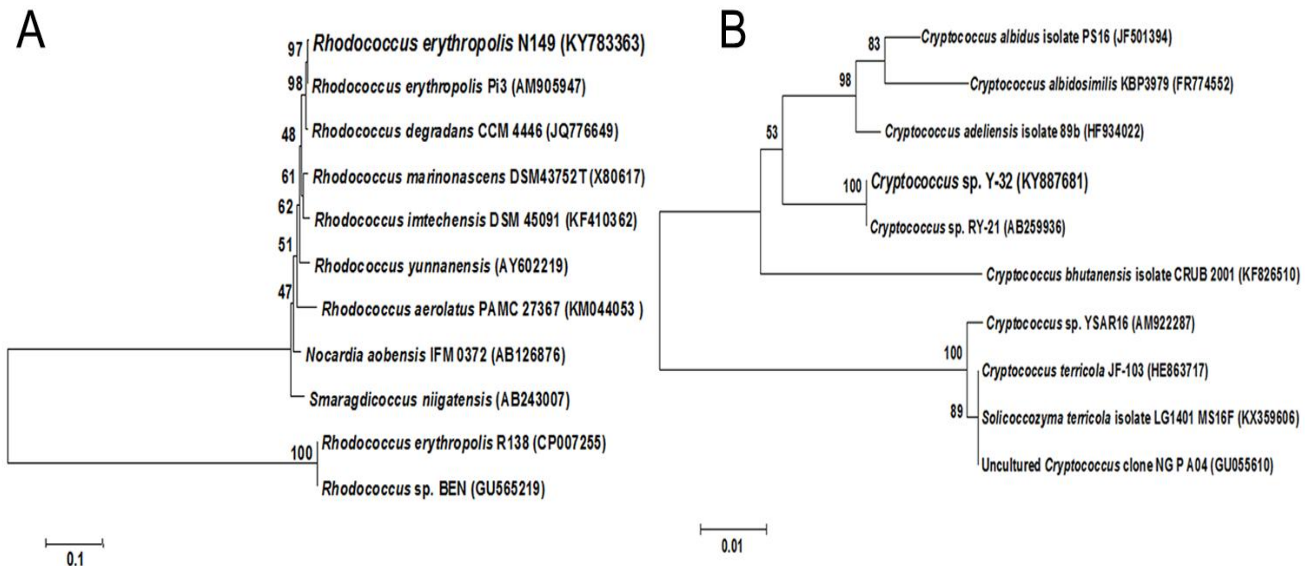


Fig.3 A. N149 on NA, B. micro-morphology of N149 observed under 60X, C. Y-32 on PDA and D. micro-morphology of Y-32 observed under 100X. Lipase plate assay at different temperatures, E. 4°C by N149, F. 15°C by N149, G. 4°C by Y-32 and H. 15°C by Y-32 on tributyrin agar plates

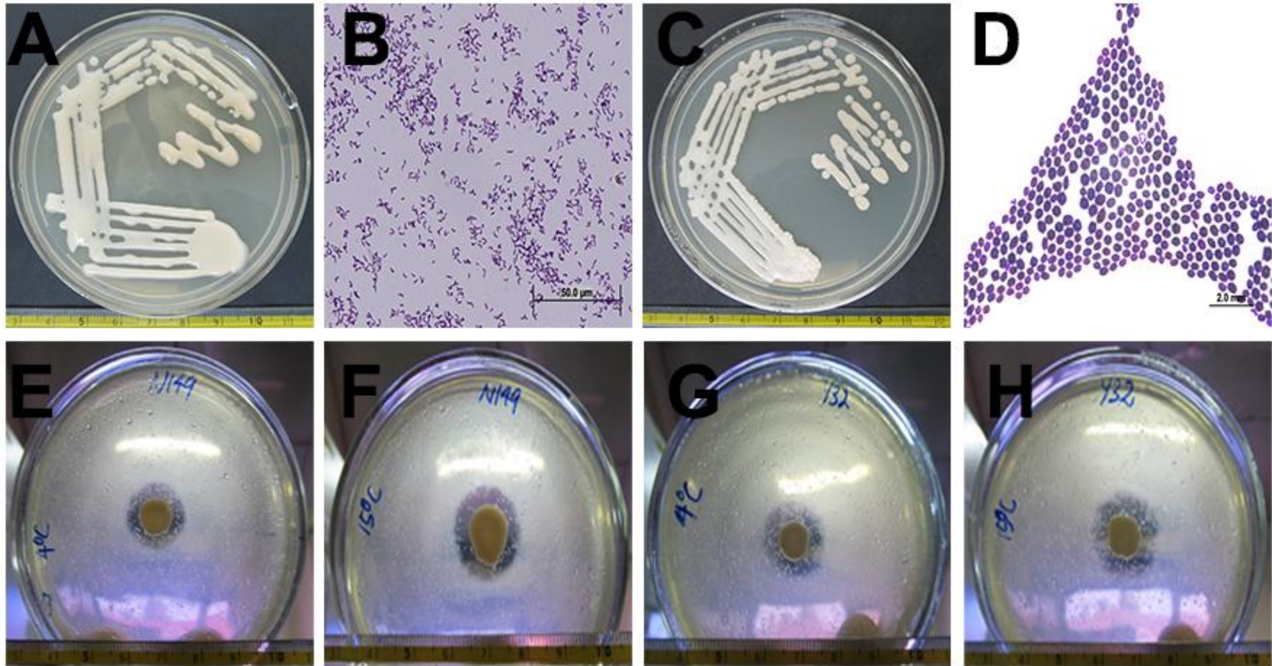


Fig.4 Effects of temperature, pH and salt concentrations on growth of Y32 and N149. PDB: Potato dextrose broth

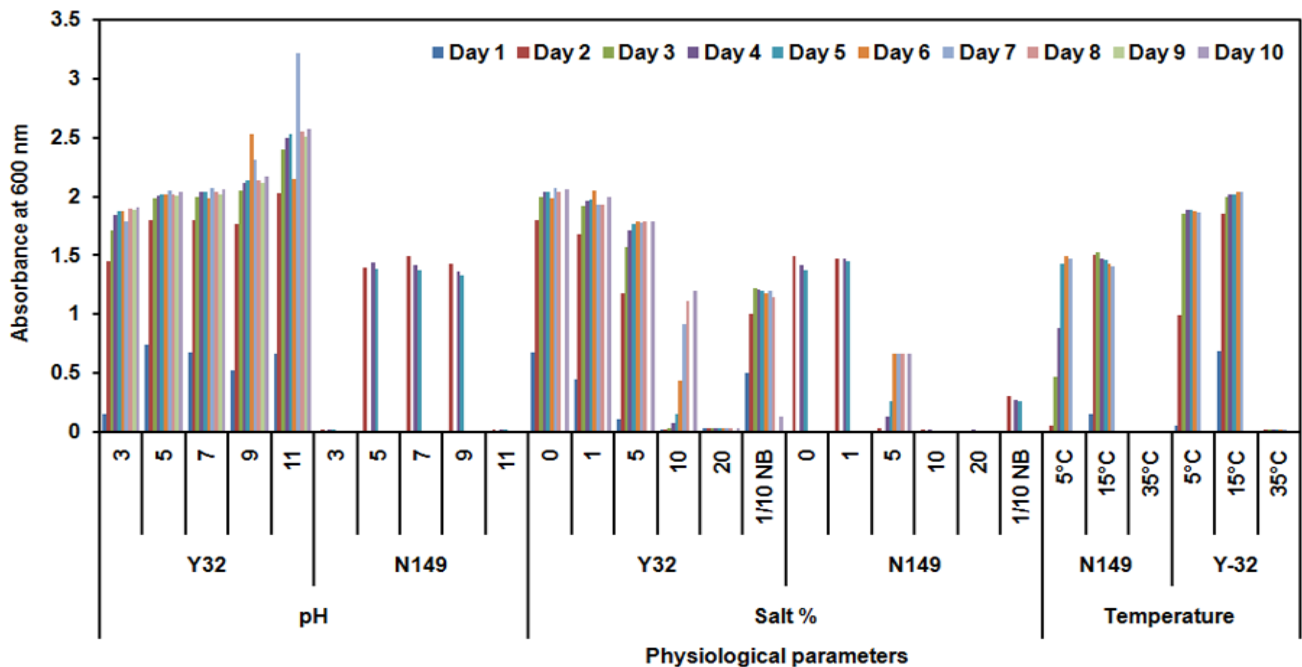


Fig.5 Effects of various temperatures on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

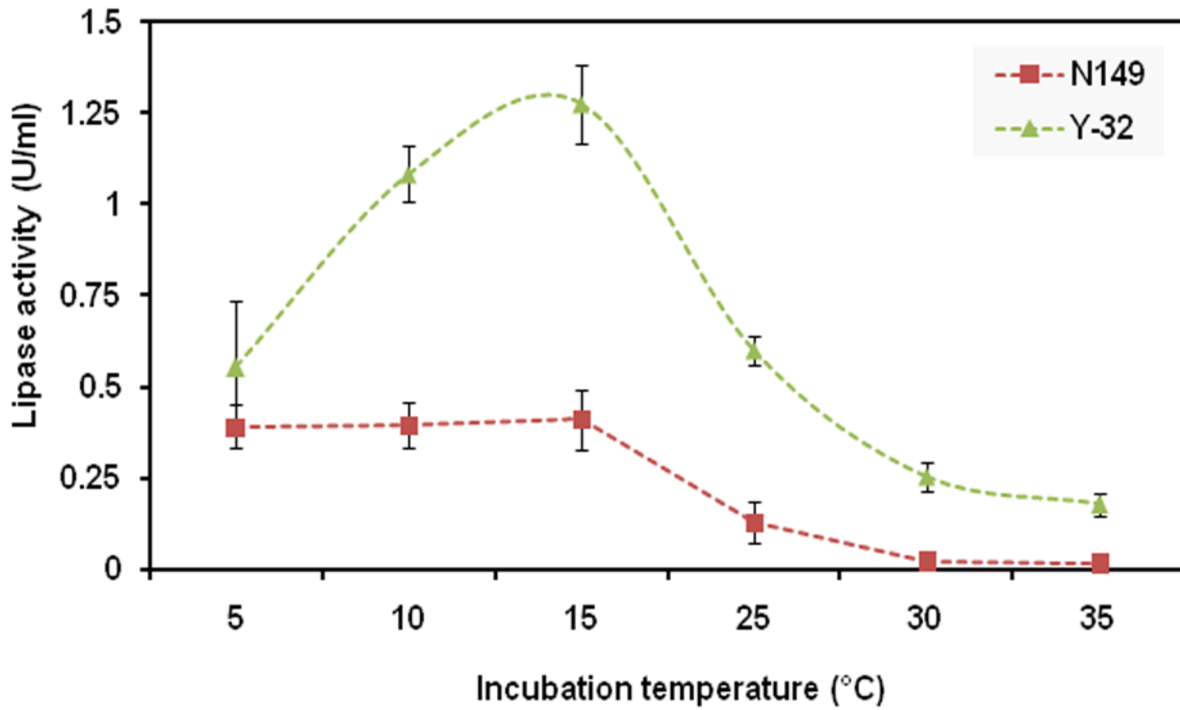


Fig.6 Effects of inoculum size on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

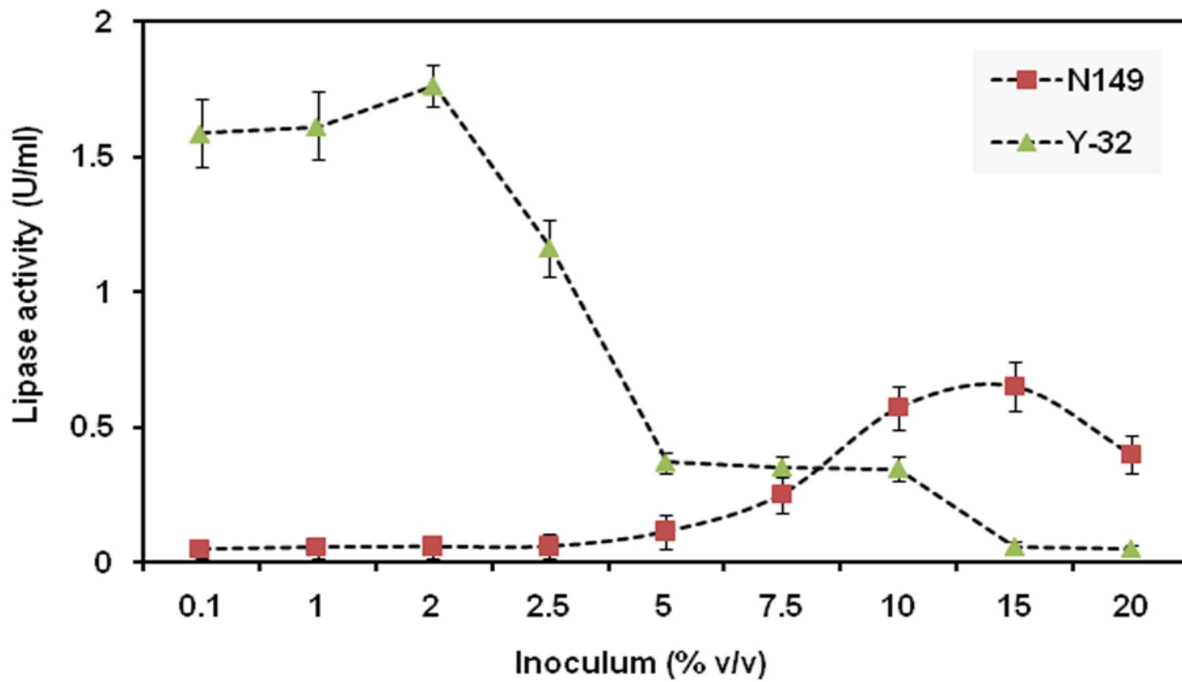


Fig.7 Effects of olive oil concentrations on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

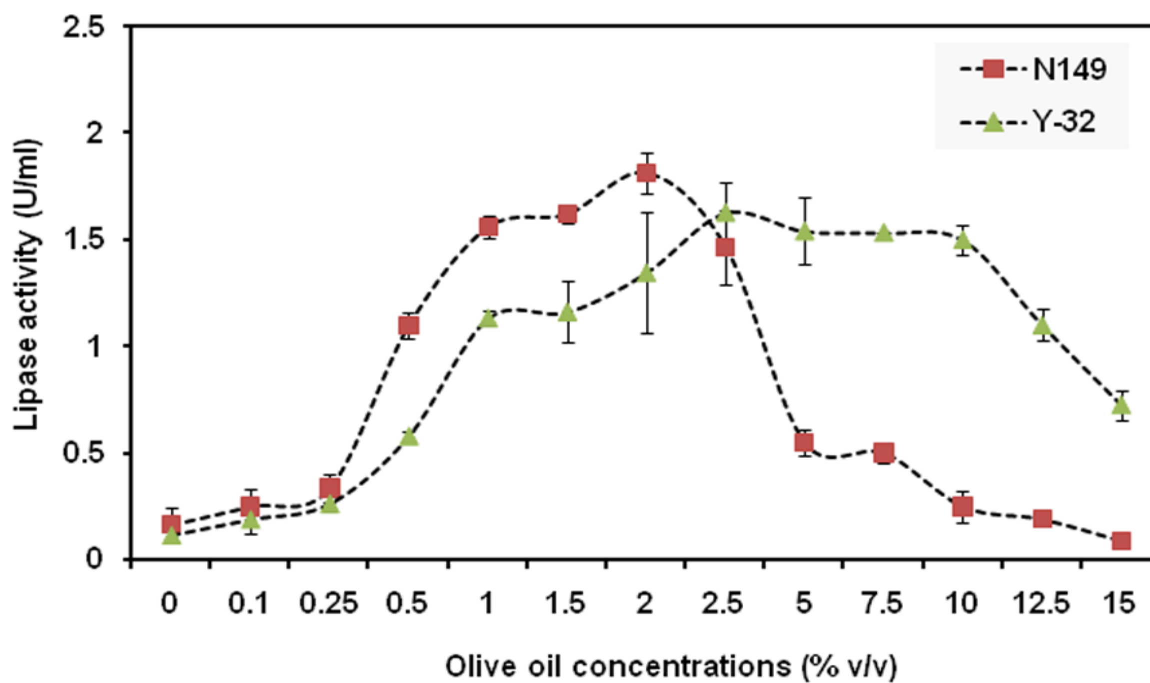


Fig.8 Effects of initial pH on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

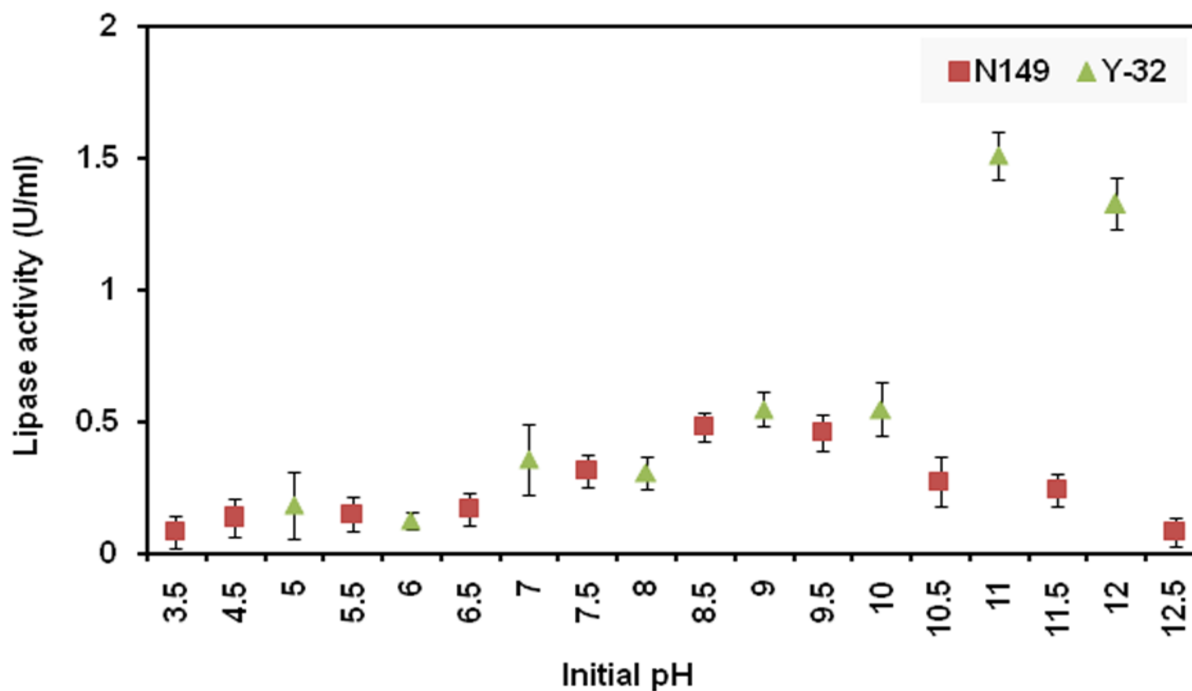


Fig.9 Effects of carbon sources on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

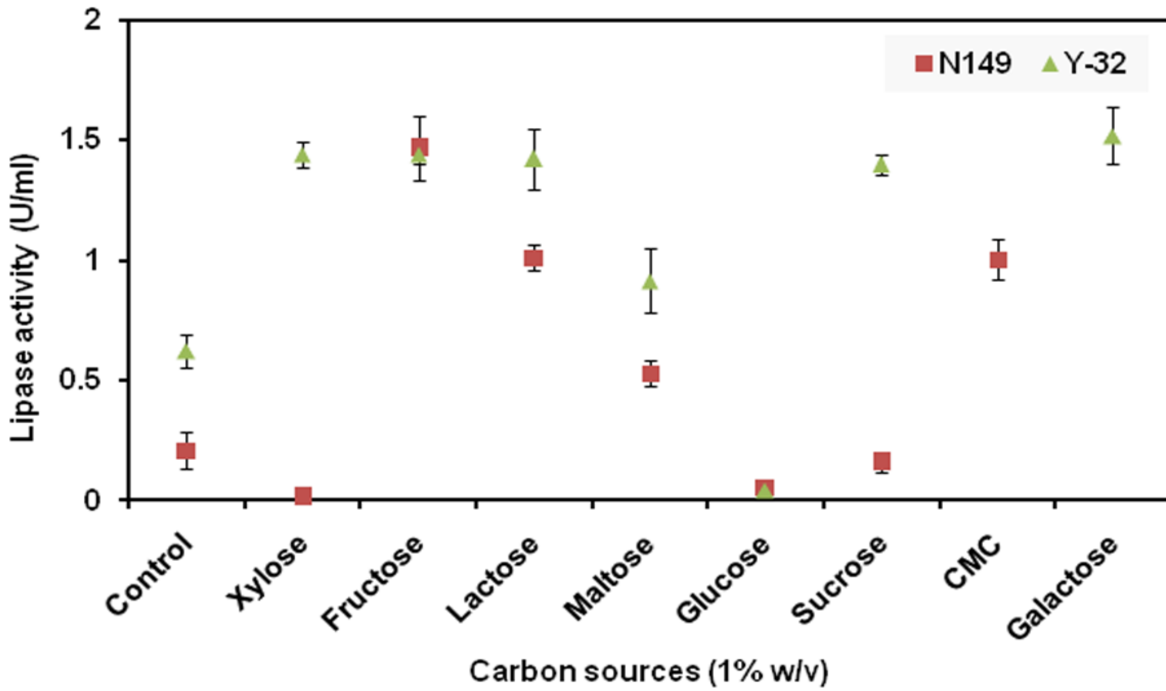


Fig.10 Effects of nitrogen sources on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

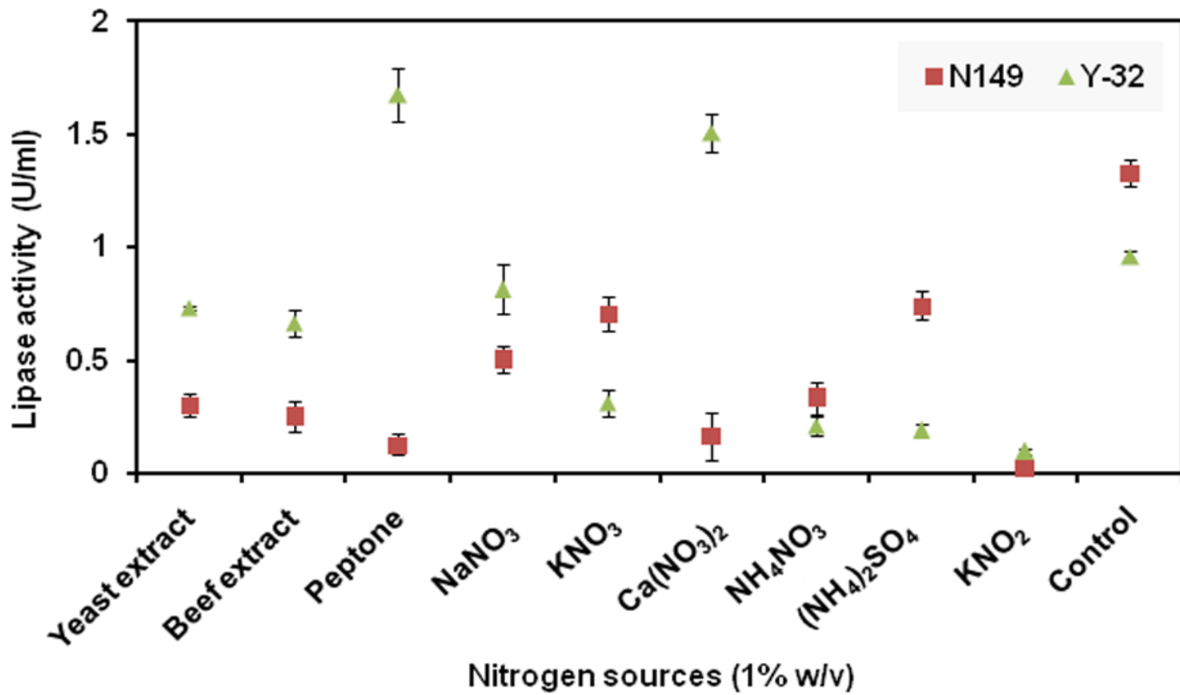


Fig.11 Effects of minerals on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors

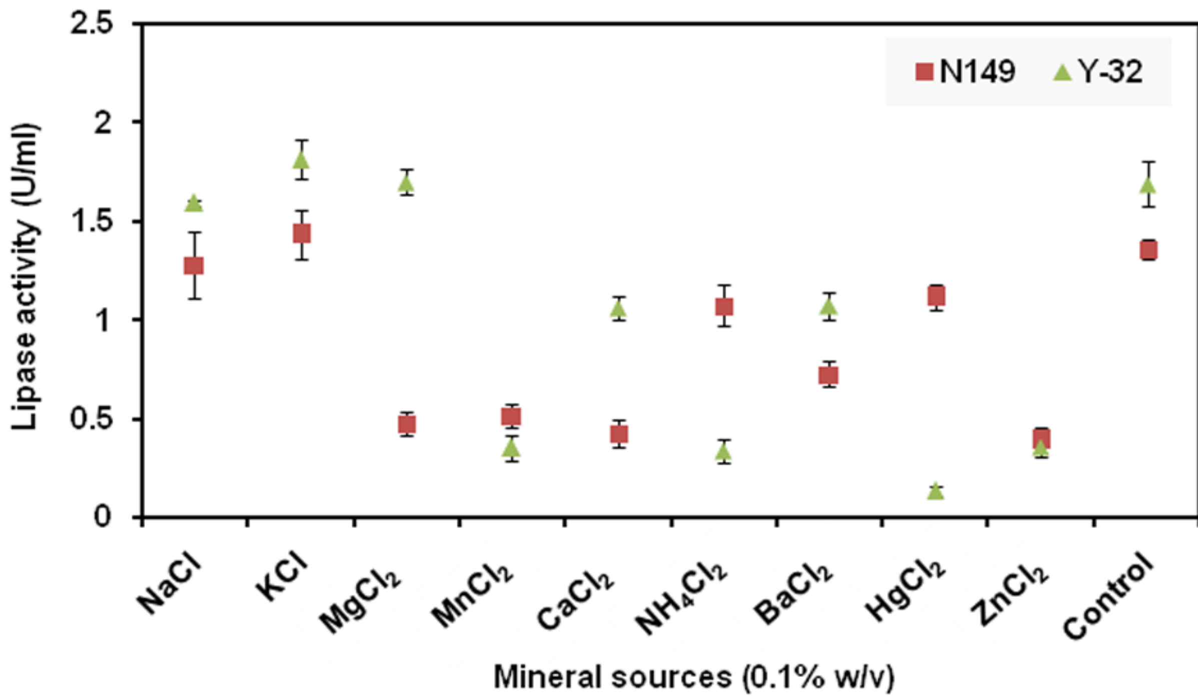
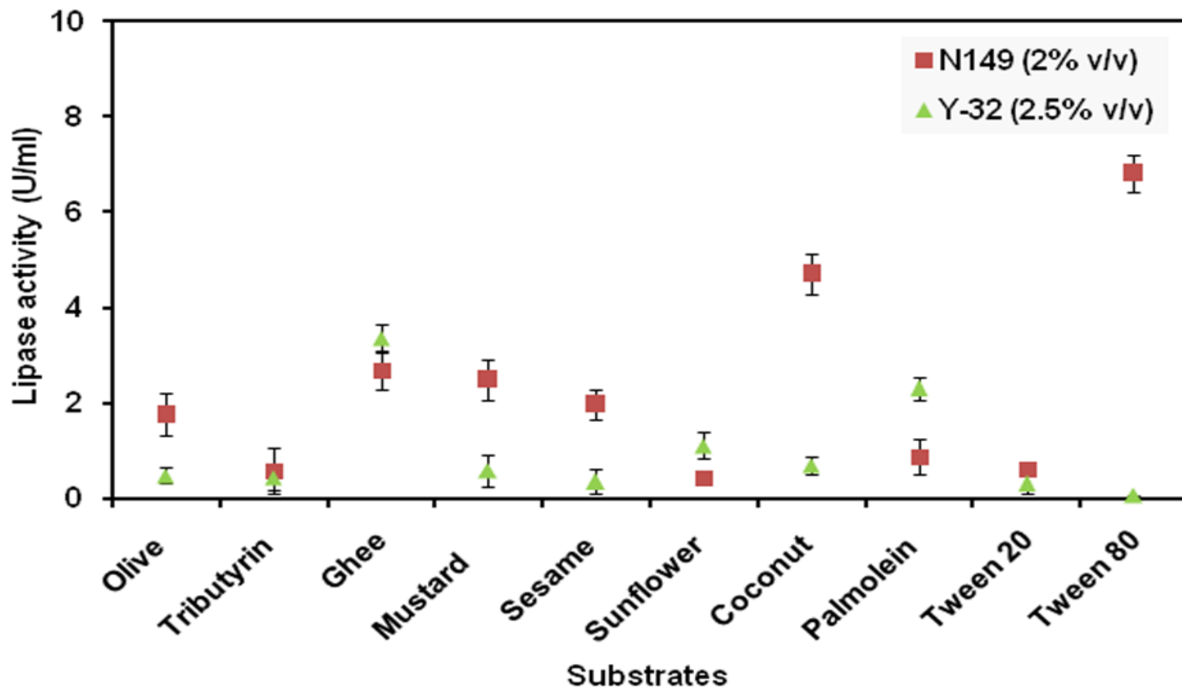


Fig.12 Effects of different substrates on lipase production by N149 and Y-32. The results are the means of 3 independent experiments and the bars correspond to standard errors



The optimum pH for lipase production was 11.0 and 8.5 by *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149, respectively (Fig. 8). The t-test reveals that there is a highly significant variation among the population means of both the isolates with respect to different pH at $p<0.001$. There is a highly significant and positive correlation between the initial pH with that of lipase activity by Y-32 ($p<0.001$) denotes with an increase in pH lipase production increases.

Figure 9-11 show the effects of carbon, nitrogen and mineral sources on lipase production by both the isolates at 15°C. In case of yeast Y-32 galactose regarded as the most potent inducer among other carbon sources investigated whereas glucose acts as a potent inhibitor. Likewise, N149 showed maximum production with fructose 1% w/v while potential inhibitors were xylose, glucose and sucrose (Fig. 9). Amongst nitrogen sources, potent activator was peptone by Y-32 whereas N149 did not require any additional nitrogen sources while compared with the control which may be due to the presence of yeast extract 0.1% w/v in the initial production medium which was enough for the growth of N149 to produce lipase (Fig. 10). All the minerals except KCl contributed as the in-activators for lipase production by both Y-32 and N149 while compared with the control (Fig. 11).

Due to specificity nature of the enzymes-substrate reactions lipase was produced by using different types of oils and triglyceride by Y-32 and N149 at 15°C. Figure 12 reveals the maximal production was found when media were employed with ghee (3.35 U/ml) and tween 80 (6.81 U/ml) in case of *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149, respectively. The t-test reveals a significant difference between the means of the lipase activity of both the isolates ($p<0.05$).

Present study emphasizes on lipase production in cold temperature conditions. Therefore, cold temperature isolates were isolated from sediments samples of Nella Lake and screened primarily on tributyrin agar plates and then the potential isolates were selected on the basis of the zone of clearances and submerged fermentation technique. From Figure 1 it was concluded N149 and Y-32 were the potent bacterium and yeast for hyper lipase production respectively. This figure also unfolds the potential substrates among olive and tributyrin. The present study is in contrast with the study of Joseph *et al.,* (2012) who reported that tributyrin induced lipase production by *Microbacterium luteolum* isolated from Gangotri glacier, western Himalaya. But there is a report of getting *Rhodotorula* sp. able to produce cold active lipase using olive oil (Rapp and Backhaus, 1992; Divya and Padma, 2015). There are many reports of getting cold active lipase by bacteria and fungi isolated from various regions like Antarctica (Vaz *et al.,* 2011; Carrasco *et al.,* 2012; Antony *et al.,* 2016), Arctic (Hatha *et al.,* 2013; Singh *et al.,* 2012b, 2014a, b, 2015) and other deep sea and temperate regions (Buzzini and Martini, 2002; Jeon *et al.,* 2009; Joseph *et al.,* 2012; Maharana and Ray, 2014a, b, 2015b).

The occurrence of *Cryptococcus* sp. in the present study is in accordant with that of *Cryptococcus* yeasts isolated from snow and ice in Antarctica (Buzzini *et al.,* 2012; Zalar and Gunde-Cimerman, 2014). Cold active lipase produced by *Cryptococcus* spp. (Carrasco *et al.,* 2012; Hatha *et al.,* 2013; Vaz *et al.,* 2011) were also reported from various sources but all of them reported lipase in qualitative method only. There are reports of lipase producing *Rhodococcus* from cold habitats (De Santi *et al.,* 2014). There is no report of getting cold active lipase from *Rhodococcus erythropolis* and this is the first

report of getting the same genus from Nella Lake, Antarctica. However, *Rhodococcus* spp. was dominant in glacial snow and soil. There are reports of getting *Rhodococcus* spp. from Pindari glacier (Shivaji *et al.,* 2011), Antarctic sediments (Muangchinda *et al.,* 2014), cold desert of the Himalayas (Mayilraj *et al.,* 2006), and Arctic glacier (Reddy *et al.,* 2009). Pham *et al.,* (2014) reported on the role in bioremediation at cold habitats by *Rhodococcus* strains. *R. erythropolis* T7-2 was isolated from the oil-contaminated mud of Bohai Sea, northern China, capable of degrading diesel oil at 15°C (Huang *et al.,* 2008). Cold-tolerant alkane-degrading *Rhodococcus* species isolated from near Scott Base, Antarctica (Bej *et al.,* 2000).

Optimization of process parameters is necessary for hyper production of enzymes. Therefore, different physical and chemical parameters investigated. Temperature is an important factor that not only alters the growth of microorganisms but also indirectly controls enzyme production due to thermo labile nature of enzymes. *Cryptococcus* sp. Y-32 produced maximum lipase production as compared with *Rhodococcus erythropolis* N149 and both have optimum growth temperature of 15°C. The isolates are psychrotolerant in nature. Therefore, lipase production was maximum at these conditions as lipase production is directly proportional to the growth of the organisms. *Cryptococcus* spp. isolated from lake sediments of King George Island, the sub-Antarctic region were able to produce lipase more at 4°C than 20°C (Vaz *et al.,* 2011) but they mentioned only qualitative data for lipase at lower temperature by plate assay method.

Initial inoculum size is also a vital factor that affects the enzyme production. The variation between the present isolates in small and large inoculum size by Y-32 and N149, respectively can be defined in terms of genus variations and initial incubation periods. The exhaustion of nutrients, productions of inhibitors and depletion of oxygen causes less lipase production in larger inoculum size (Abo-State *et al.,* 2010). Singh *et al.,* (2012a) also reported that with the increase in inoculum size enzyme production decreased due to increase in the duration of the initial lag phase.

For optimal production of any enzyme, there is a requirement of suitable substrate in the appropriate amount which not only will be utilized by the concerned microorganism for their growth but also can help to enhance the enzyme production effectively. The present study revealed maximal production using olive oil as compared with tributyrin, which is in accordance with the study of others (Rapp and Backhaus, 1992; Divya and Padma, 2015).

Alkaline range denotes the alkali tolerant nature of both the microorganisms Y-32 and N149. From pH tolerance study, it was found that the optimum growth pH for both is 5.0 to 9.0 (Fig. 4). Therefore, maybe there is a chance of getting lipases that tolerate higher pH efficiently. Inappropriate pH in the production medium alters the 3-D shape of the protein which in turn reform protein recognition and the enzyme might become inactive. In other senses, pH also affects the growth of microorganism. The acidic pH was inhibiting the lipase production which might be due to the fact that microorganism's growth decreased because of acidic medium or the medium became more acidic due to release of more fatty acids to the production medium from olive oil which in turn show feedback inhibition mechanism (Maharana and Ray, 2015b).

For growth, all microorganisms need energy sources. Therefore, different kinds of carbon, nitrogen and mineral sources were employed

in the production medium for maximal production of lipase. Carbon, nitrogen and mineral sources stimulate lipase production indirectly by giving additional nutrients to the microorganisms for growth which in-turn enhance the enzyme production in same proportion.

Besides, different kinds of oils and triglycerides were investigated to test the affinity of the microorganism for the production of lipase at cold temperatures. From Figure 12 it was found that almost all the oils have induced effect on lipase production besides, Ghee and tween 80 was regarded as the best substrate for lipase production by Y-32 and N149, respectively.

Both the isolate have the ability for the hydrolysis of different vegetable oils investigated, which are industrially and economically important in the production of bio-diesel.

Lipase produced from *Cryptococcus* sp. S-2 showed maximum activity on 120 h at 25°C with pH 5.6 and have capability to hydrolyze sardine oil, soy bean oil and triolein (Kamini *et al.,* 2000). Huang *et al.,* (2008) investigated on *Rhodococcus erythropolis* having diesel oil degradation capabilities which was induced by the application of $(NH_4)_2SO_4$ and yeast extract.

In summary, the present study successfully optimized the production medium enhancing the lipase production effectively from un-optimized conditions, which confirmed from the fold increase in lipase by 9.81 using *Rhodococcus erythropolis* N149 and 4.01-fold using *Cryptococcus* sp. Y-32. Besides, to the best of our knowledge, our study is the first report on *Cryptococcus* sp. and *Rhodococcus erythropolis* isolated from Nella Lake, Antarctica producing cold active lipase. Therefore, lipases produced from above cold tolerant microorganisms can be used in various fields of science like detergent formulation for cold temperature washing of clothes and lipid contaminants removal from waste water in cold and temperate regions due to their alkaline and cold tolerance capabilities. Present studied cold active lipase may provide new insights into the potential biotechnological applications of these isolates in Antarctic environments.

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