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Alkalilipophilic Actinomycetal Diversity of Kadi Region of Gujarat

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Abstract

Actinomycetes are having different kinds of features and extensive commercial importance. A diversity of Actinomycetes was studied from soils of various sites of oil industries throughout the Kadi region (District – Mehsana, Gujarat). After enrichment, the soil suspensions were spread on Bennet's Agar medium with alkaline pH and incubated at 35ºC for 5 days. After getting the growth further purification was done using same medium. A total of 40 isolates of Actinomycetes with distinct characteristics at pH 10 and 11 were isolated. The primary screening of alkaline lipase producing Actinomycetes can be performed based on clear zone formation on Tri butyrine agar plates. On the basis of zone diameter six strains were selected for checking lipase production in submerged culture. The best Actinomycetal isolate AP-11/18 producing higher lipase was further studied for cultural characterizations on different nutrient media and biochemical characterization.

Key words: Lipase, oil industries, enrichment, spot culture technique

Introduction

Actinomycetes are a group of Gram-positive bacteria (Class *Actinobacteria*) characterized by a high G + C content that perform a wide array of important functions in various habitats[1]. *Thermoactinomyces* and other alkalithermophilic actinomycetes genera with very resistant endospores, grow in compost, rotten hay, manure, and soil[2]. Alkalithermophilic actinomycetes, exceptionally well adapted for dispersal, are the most common etiological agents of hypersensitivity pneumonitis[3].

Different kinds of alkaliphilic microorganisms, including bacteria belonging to the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, *Streptomyces,* and *Thermoactinomyces,* and eukaryotes, such as yeasts and filamentous fungi, have been isolated from a variety of environments. Alkaliphilic microorganisms coexist with neutrophilic microorganisms. The frequency of alkaliphilic microorganisms in neutral ordinary soil samples is 10^2 to 10^5 per gram of soil, which corresponds to 1/10 to 1/100 of the population of the neutrophilic microorganisms[4]. Microbial lipases form a versatile tool in biotechnology and in recent years they have become an important class of enzymes. Their applications include the addition to detergents, manufacture of food ingredients, pitch control in pulp and paper industry, production of aromas, production of insecticides, synthesis of drugs such as naxopren and ibuprofen, and as a biocatalyst of stereoselective transformations[5].

Actinomycetes have proved their importance as biocontrol agents, lignocellulose decomposers[6], antivirals producers[7], antibiotics producers, as well as also in production of agroactive compounds. They also possess chitinolytic[8],

fungicidal activity[9] and antidermatophytic activity[10]. Therefore, the current study was undertaken to study alkalilipophilic Actinomycetal diversity of Kadi region (having large number of edible industries) to screen lipase-producing actinomycetes.

Experimental

Soil sample and Enrichment

The soil samples used in these studies were collected from 20 different oil industry units of Kadi region (Dist. Mehsana, Gujarat, India). Soils were enriched with $CaCO_3$ for 5 days.

Isolation of Actinomycetes

Tenfold serial dilution of soil samples was made using sterile distilled water. The soil suspensions were plated using Bennet's agar (Casein enzyme hydrolysates-1.0, meat extract-0.5, yeast extract-0.5, glucose-1.0, agar agar-3.0 gm% respectively). The pH of the medium was adjusted to alkaline (8.0, 9.0, 10.0, and 11.0). Bacterial and fungal contaminant can be avoided by the addition of antibacterial (nystatin) and antifungal (cyclohexamide) agents respectively. The plates were incubated at 35^{0}C for 5-6 days. Morphologically different types of 40 isolates were picked up separately. These 40 isolates were further screened for lipase production ability on Tri-butyrine agar (TBA) plates (Peptone-0.5, yeast extract-0.3, tributyrene-1.0, agar agar-3.0 gm% respectively, pH 8.0) by spot culture technique.

Culture Maintenance

Actinomycete isolates were maintained on Bennet's agar slants. The pH of the medium was adjusted to respective alkaline pH and culture was incubated at 35^{0}C for 5-6 days and stored thereafter in refrigerator at ~ 4^{0}C. Subculturing was carried out once in every 2-3 months.

Morphological characterization

The morphology of the isolates was studied by light microscopy. A small peripheral portion of well-grown mature parts of the colony was picked using a sterile inoculation loop. Then transferred to a glass slide and stained using gram's staining technique and observed in microscope under oil immersion lens of 100 X.

Cultural and Biochemical characterization

The highest lipase producing strain AP 11/ 18 thus obtained were cultured on Bennet's agar, Calcium malate agar, Czapex sucrose agar (sucrose-3.0, sodium nitrate-0.3, K_2HPO_4-0.1, $MgSO_4.7H_2O$-0.05, potassium chloride-0.05, ferrous sulphate-0.05, agar agar-3.0 gm% respectively, pH 8.0), Glucose aspargine agar (glucose-1.0, aspargine-0.05, yeast extract-0.05, K_2HPO_4-0.05, $MgSO_4.7H_2O$-0.025, agar agar-3.0 gm% respectively, pH 8.0), Glycerol aspargine agar (glycerol-1.0, aspargine-0.05, yeast extract-0.05, K_2HPO_4-0.05, $MgSO_4.7H_2O$-0.025, agar agar-3.0 gm% respectively, pH 8.0), Nutrient agar (peptone-1.0, meat extract-0.3, sodium chloride-0.5, agar agar-3.0 gm% respectively, pH 8.0) and Potato dextrose agar (starch soluble-2.0, peptone-1.0, meat extract-0.3, sodium chloride-0.5, agar agar-3.0 gm% respectively, pH 8.0) as suggested for monosporic actinomycetes by various researchers[11-13].

The strain was subjected to biochemical characterization by testing the utilization of sugars or substrates, antibiotic sencitivity and production of enzymes as described in literature[12, 14].

Inoculum preparation and Production medium

50 ml of sterilized Bennet's broth in 250ml Erlenmeyer flasks was inoculated with 6 mm disc culture and incubated at orbital shaker at 140 rpm and 40^{0}C for 96 hrs. When the optimum growth (O.D 2.0 at 600 nm) was achieved, 1.0% of this growth culture was used for inoculating 50 ml lipase production Bennet's broth medium (Tri-butyrine oil, 1% v/v) in 250 ml flasks, keeping composition and growth conditions the same as in growth phase. Lipase produced in broth filtrate was estimated by colorimetric assay using p-nitro phenyl palmitate (pNPP)[15]. One lipase unit (U) is equal to the amount of enzyme that liberated 1 μ mole p-nitro phenol per 60 min.

Results and Discussion

Cultural and Morphological characterization of Actinomycetes

40 different strains were grown at pH 10.0 and 11.0 on Bennet's agar. Out of the 40 actinomycetes isolates, 16 isolates with distinct characteristics, showed extracellular lipase production on TBA and the diameter of clear zone of lipid hydrolysis were measured in mm (Table 1). Out of 16 isolates, 6 isolates were grown at pH 10.0 and 10 isolates were at pH 11.0, confirming their Alkalilipophilic nature. Their cultural and

Table.1 Morphological and Cultural Characteristics of Various Actinomycetal Isolates, along with their Lipolytic ability

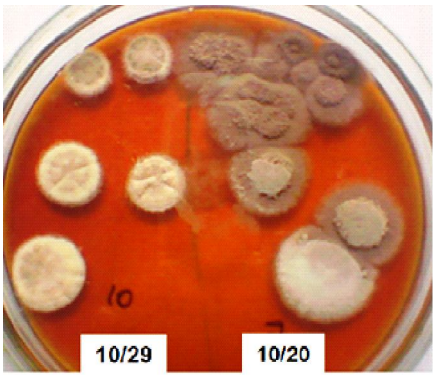
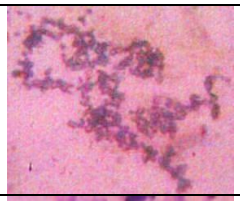
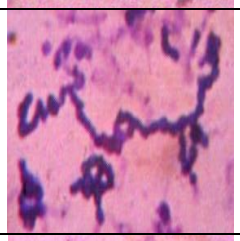
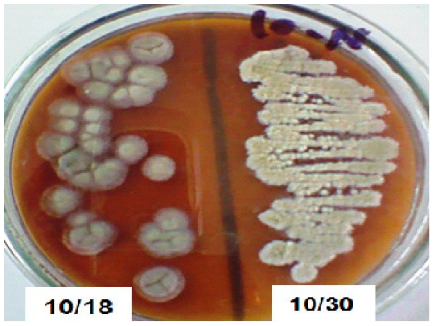
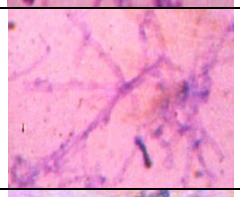
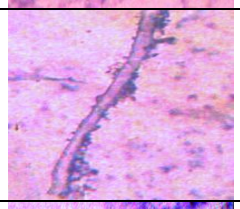
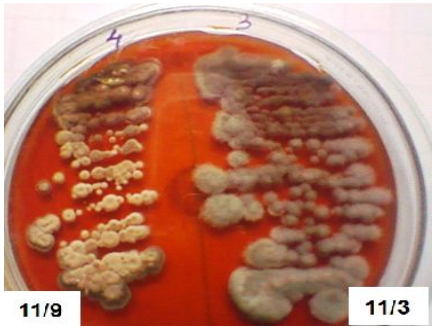
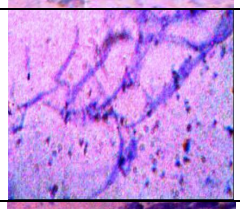
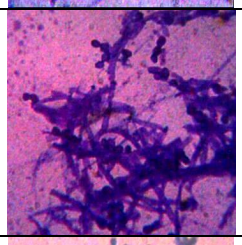
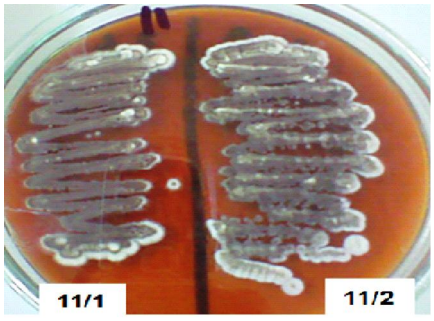
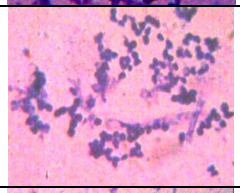
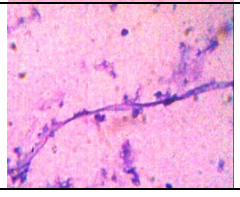
Growth of actinomycetes on Bennet's agar medium.	Cultural characteristics of Isolates	Microscopic observation/ Morphological characteristics	Zone of lipid hydrolysis in mm
	**10/29:** Yellow centered, periphery white, bottom dark black centered.		15
	10/20: Powdery, filamentous white periphery, grayish after aging.		15
	**10/18:** Yellow centered, White filamentous at periphery.		20
	10/30: Flat, Yellowish brown centered, White powdery periphery.		14
	**11/9:** Powdery, Cream centered with Brown filamentous periphery.		15
	11/3: Powdery, Grey centered with filamentous periphery.		18
	**11/1**: Flat, Brown centered, White periphery.		21
	11/2: Fluffy, Brown centered White periphery.		20

Table 1. continued.....

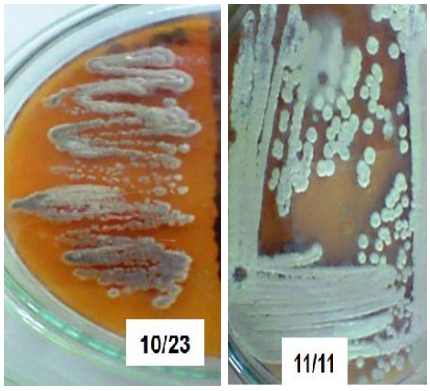
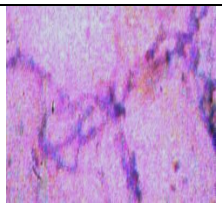
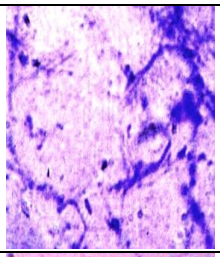
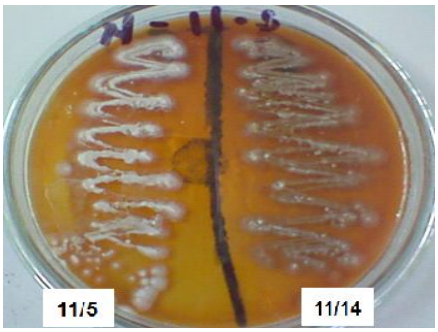
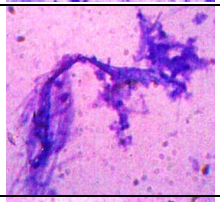
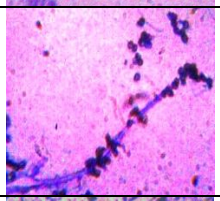
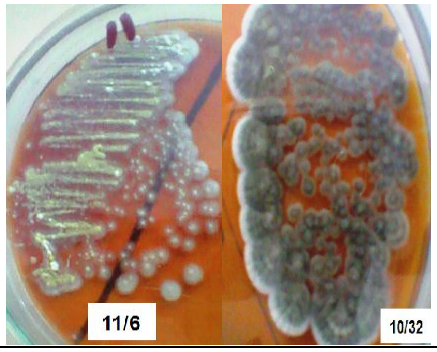
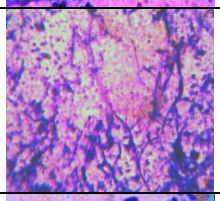
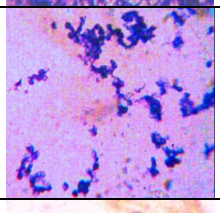
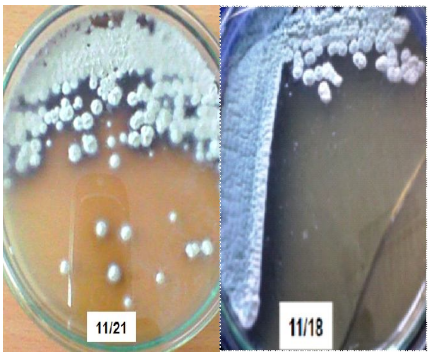
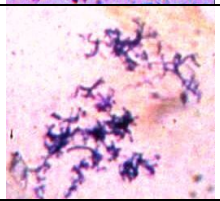
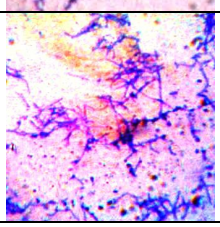
Growth of actinomycetes on Bennet's agar medium.	Cultural characteristics of Isolates	Microscopic observation/ Morphological characteristics	Zone of lipid hydrolysis in mm
	**10/23:** Powdery, Green centered, deeped in medium.		13
	11/11: Flat, Powdery White, Pitch in color after aging, deeped in medium.		12
	**11/5:** White, filamentous at periphery, Small in size.		23
	11/14: Light yellow, deeped in medium & bottom is black centered .		14
	**11/6:** Fluffy, Yellow centered, White periphery.		20
	10/32: Dark grey centered, White filamentous periphery.		14
	**11/21:** Choky white center colony with dark reverse.		25
	11/18: Small colorless mycelium initially, bluish grey sporulation after 72 hrs. Dark reverse.		33

Table 2. Cultural characteristics of Actinomycetal isolate AP-11/18 on different media

Medium	Growth	Mycelium colour	Reverse	Soluble Pigment
Bennet's agar	+++	Dark greenish grey	Dark blackish	Black
Czapex sucrose agar	+	Light White	Colourless	None
Glucose aspargine agar	+++	Dark white	Light pale Yellowish	None
Glycerol aspargine agar	++	White gray	Grayish white	None
Nutrient agar	+	Whitish gray	Yellowish gray	None
Potato dextrose agar	+++	White	Pale Yellowish	None
Calcium malate agar	++	White	Colourless	None

morphological characterizations are shown in Table 1.

Screening of actinomycetes for the production of extracellular lipase in submerged condition

Six strains out of the 16 actinomycetes isolates (AP11/1, AP11/2, AP11/5, AP11/6, AP 11/18 & AP11/21) exhibited zone of lipid hydrolysis on TBA agar plates at 96 hours incubation greater than 20 mm. AP11/18 showed highest lipase activity (172U/ml) when fermented in Bennet's broth medium. The next highest was AP11/21 yielding only 75 U/ml (Fig.1).

Morphological and Cultural characterization of Actinomycetal isolate AP 11/18

Highest extracellular lipase producing isolate AP 11/18 was selected for further studies. actinomycete AP-11/18 was found to be gram positive with light bluish grey aerial mycelium and dark reverse on Bennet's agar medium. Microscopic observations confirmed sessile, small and round spores with smooth surface attached singly on the conidiophores (monosporic).

The Table 2 reflects morphological characteristics of Actinomycetal isolate AP 11/18 on various nutrient media. The sparse aerial mycelium on most media was white till greenish grey sporulation covered the growth. This strain did not produce any diffusible pigment on all tested media except Bennet's agar. The reverse growth

Table 3. Biochemical characterization of Actinomycetal isolate AP-11/18.

Character	Utilization	Character	Utilization
Carbon source utilization		**Enzyme production**	
Arabinose	-	Catalase	+
Dextrose	+	â-galactosidase	+
D-fructose	+	Urease	+
Galactose	+	Amylase	+
Glucose	+	Protease	+
Maltose	-	Lipase	+
Mannose	+	Alkaline Phosphatase	+
Mannitol	-	Nitrate reduction	+
Melibiose	+	Citrate utilization	+
Raffinose	+	Arginine utilization	+
Sucrose	+	ONPG utilization	+
Trehalose	+	Malonate utilization	-
Xylose	-	Acetoin production	-
Lactose	-		

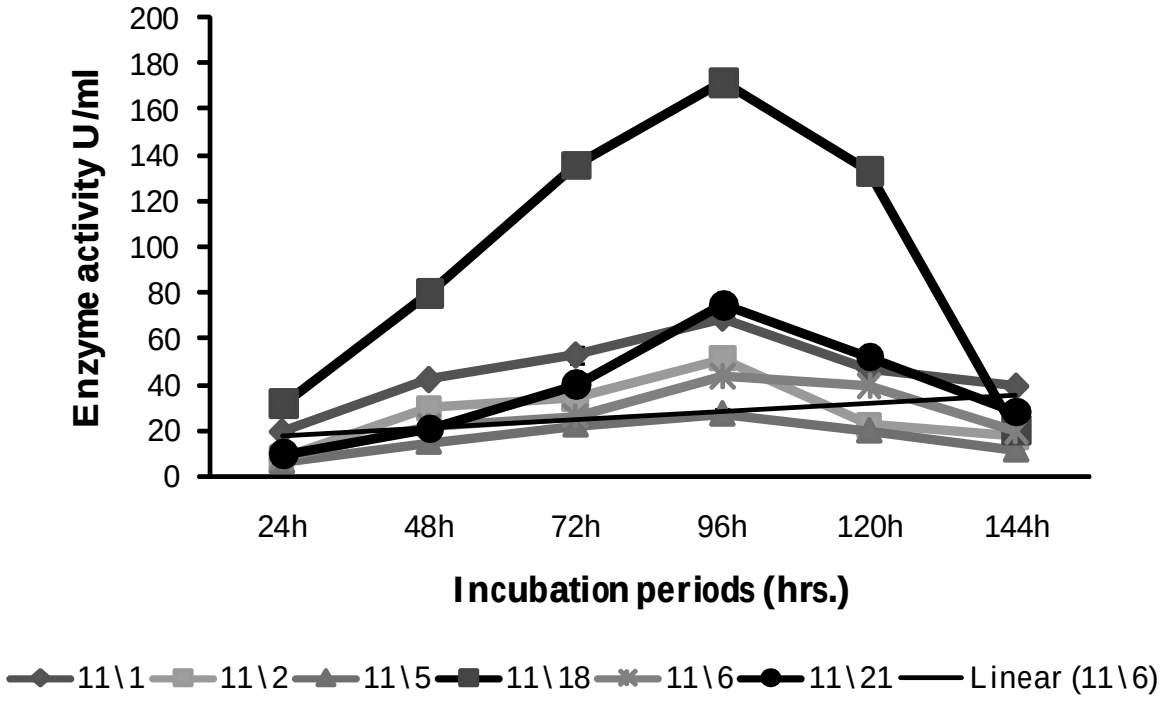


Fig.1 Time scale production curve for lipase production by prominent actinomycetal isolates

characteristics of the isolates on different media are also reported in Table 2.

Biochemical characterization of Actinomycetal isolates AP 11/18

The Table 3 shows the results of biochemical characterization of actinomycete isolate AP 11/18. The carbohydrates dextrose, fructose, galactose, glucose, mannose, melibiose, raffinose, sucrose and trehalose have been utilized. No growth observed with arabinose, maltose, mannitol, lactose and xylose. Phospatase, urease, catalase, amylase, protease, lipase and β galacosidase production were observed by the culture. Citrate utilization, nitrate reduction, ONPG utilization, arginine utilization were positive while malonate utilization and acetoin production were negative. Isolate shows resistance against antibiotics niastatin and streptomycin at the concentration of 50 µg/ml.

Conclusion

This study reveals the existence of a wide variety of alkaliphilic actinomyctes (growing well even at pH 10 and 11) in soil of Kadi region of District Mehsana in Gujarat state. Many of the actinomycetal isolates also exhibited lipase production ability suggesting their alkalilipophilic nature having ability to utilize oils at alkaline pH.

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References

1. Das M, Royer TV and Leff LG. 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. *Applied and Environmental microbiology*, 73(3): 756 - 767.

2.Srinivassan MC, Laxman RS and Deshpands MV. 1991. Physiology and nutritional aspects of Actinomycetes: an overview. *World Journal of Microbiology and Biotechnology*, 7: 171 - 184.

3. Reponen TA, Gazenko SV, Grinshpun SA, Willeke K and Cole EC. 1998. Characteristics of airborne Actinomycete spores. *Applied and Environmental Microbiology*, 64: 3807 - 3812.

4. Horikoshi K. 1999. Alkaliphiles: some applications of their products for biotechnology. *Microbiology and Molecular Biology Review,*63: 735 - 750.

5. Jaeger KE and Reetz MT.1998. Microbial lipases form versatile tools for biotechnology. *Trends in Biotechnology,* 16: 396 – 403.

6. Niladevi KN and Prema P. 2005. Mangrove Actinomycetes as a source of lignolytic enzymes. *Actinomycelogica*, 19: 40 – 47.

7. Mohamed SH and Galal AM. 2005. Identification and antiviral activities of some halotolerant *Streptomycetes* isolated from Qaroon lake. *International Journal of Agriculture and Biology*, 7 (5): 747 – 753.

8. Gadelhak G, El-Tarabily KA and Al-Kaabi FK. 2005. Insect control using chitinolytic soil Actinomycetes as biocontrol agents. *International Journal of Agriculture and Biology*, 7 (4): 627 – 633.

9. Doumboul CL, Salove MKH, Crawford DL and Beaulieu C. 2002. Actinomycetes, promising tools to control plant diseases and to promote plant growth. *Phytoprotection*, 82 (3): 85 - 102.

10. Laxmipathi DT and Krishnan K. 2009. A report on antidermatophytic activity of Actinomycetes. *International Journal of Integrative Biology*, 6 (3): 132 - 136.

11. Runmao H. 1987. *Saccharornonospora azurea* Sp. Nov. A New Species From Soil. *International Journal of Systematic Bacteriology*, 37 (1): 60 - 61.

12. Runmao Hu, Cheng Lin and Guizhen W. 1988. *Saccharomonospora cyanea* Sp. Nov. *International Journal of Systematic Bacteriology*, 38 (4): 444-446.

13. Xiang Jint Li-Hua Xu, Tsong-Hsiung Hseu and Cheng-Lin Jiang. 1998. Description of *Saccharomonospora xinjiangensis* sp. Nov. Based on chemical and molecular classification. *International Journal of Systematic Bacteriology*, 48 : 1095-1099.

14. Al-Zarban SS, Abbas I, and Kroppenstedt RM. 2002. *Saccharomonospora halophila* sp. nov., a novel halophilic actinomycete isolated from marsh soil in Kuwait. *International Journal of Systematic and Evolutionary Microbiology*, 52: 555–558.

15. Savitha JS, Jagat S, Payal R , Priyanki P, Rashmi S, Roshini K T and Shantal YM. 2007. Identification of potential fungal strain(s) for the production of inducible, extracellular and alkalophilic lipase. *African Journal of Biotechnology*, 6 (5): 564-568.

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