Advances in Bioresearch
Adv. Biores., Vol 12 (1) January 2021: 81-89
©2021 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.12.1.8189

Advances in Bioresearch

ORIGINAL ARTICLE

Isolation, Identification and Screening of Extracellular lipase Producing Yeasts from Mangrove Sediments of Kasaragod, North Kerala

[1]Vidya P. and [2]Sebastian C. D.*
Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala,
India 673 635
[1]Email: vidya_p31@yahoo.com
[2*]Email: drcdsebastian@gmail.com

ABSTRACT
The enzyme lipase is found to have significant physiological, industrial and ecological potential. Yeasts are one of active producers of extracellular lipase enzyme among micro organisms. The present study is aimed at screening yeasts isolated from the mangrove sediments for the hydrolytic activity of lipase and identifying them. The study area selected was the mangroves of Chandragiri region, Kasargod. The sediment samples were collected from two different parts of this region viz a polluted site and a non-polluted site. The lipolytic potential was tested on nutrient agar plates supplemented with 1% tributyrin as substrate and observed for a period of 7 days. Candida tropicalis was the most abundant and active lipase producing species found in the sediments. Our study showed that the yeasts isolated from polluted sediments showed early onset and faster rate of lipid hydrolysis compared to those from non polluted sites. A period of 72-96 hours was found to be the time at which yeasts showed maximum lipolytic activity after which it remained constant. Mangrove sediments due to its unique physico chemical properties tend to harbor microbial community with significant physiological potential. A stressed condition like pollution in these sediments act as a selective force for the microbes especially yeasts to be more potent to cope up with the prevailing changes. Thus, the results of our current work emphasis on the scope of using yeasts isolated from mangrove sediments as potential candidates for the commercial production of lipases used in industrial purposes and in bioremediation processes.
Keywords: Mangrove sediments, yeast, lipase, pollution, industrial applications, bioremediation

Received 11.11.2020 Revised 26.12.2020 Accepted 11.01.2021

How to cite this article:
Abhijit B. Shinde, Dhanraj B. Nakade. Decolourization of Distillery Spent Wash by *Bacillus coagulans* ABS012. Adv. Biores., Vol 12 (1) January 2021: 81-89

INTRODUCTION

Lipases (EC 3.1.1.3) are group of enzymes which are ubiquitously found in most of the micro organisms, plants and animals [7,16]. They act as catalysts in a wide range of reactions like hydrolysis, esterification, alcoholysis, acidolysis, aminolysis and so on [10]. Microbial lipases are of great interest in recent years due to their various industrial applications in the detergent, food, flavor, pharmaceutical, agrochemicals, cosmetics and perfumery industries [11,17,22]. They are also of large demand in diverse applications including making of fine chemicals, esters and amino acid derivatives, waste water treatment, medicine and bioremediation of polluted soils [6,15,25]. Fungi, in particularly yeasts, due to their extracellular lipase production are considered as the best source of lipase among microbes [24]. This is due to the fact that they are easy to grow and handle and is considered as natural and safe compared to bacteria [12]. Apart from these, yeast lipases can be produced in large scale by fermentation and they follow low cost extraction methods which make them technically favorable organisms [13,18].

Mangroves, despite their ecological significance, are facing destruction worldwide due to climate change, deforestation and other anthropogenic interventions. Pollution of mangrove sediments by both organic and inorganic contaminants is a serious issue that needs to be taken care immediately for the restoration of this ecosystem. One of the most suitable strategies that can be applied is bioremediation using extracellular enzymes produced by the microorganisms especially yeasts [21]. Contamination in

ecosystems may act as a strong selective force for microbial communities. As a result, the contaminated areas tend to be dominated by those organisms that can either tolerate or degrade the contaminants, hence directly affecting their diversity compared to non contaminated parts [8]. The microbial analysis and the metabolic reconstruction studies in mangrove sediments have shown that the existence of particular microbial community in them is modulated by the prevailing conditions [2].
Considering these facts, the present study is an attempt to screen the lipase activity and identify the yeasts isolated from polluted and non polluted sediments of mangroves of Kasaragod, Kerala. No such works have been reported before on the extracellular lipases from the yeasts of mangrove sediments of North Kerala, particularly from the current study area. Hence, this study would be helpful in building a baseline data for the future research on exploitation of yeast isolates from sediments of mangroves for the production of hydrolytic enzymes. Based on the results, the yeast isolates with best lipolytic activity can be used for industrial applications and bioremediation process after sufficient manipulations for maximum growth and enzyme production.

MATERIAL AND METHODS

For the present study, the mangroves of Chandragiri located in the Kasaragod district of Kerala were selected (Fig. 1). The sediment samples were collected from two different sites (~500 meters apart) of the same ecosystem during monsoon period (September 2019) of the year. The mangrove sediments of one site (KGD_P) (12°28'10" N 74°59'40" E) were polluted by anthropogenic activities, mainly due to dumping of food and house hold wastes while the other site showed little or no pollution (KGD_N) (12°28'13" N 74°59'34" E) (Fig. 2). Sub surface samples (10-20gms) were collected using hand core method, transferred aseptically into sterile polythene bags and transported to the laboratory in ice boxes. The subsamples from both the sites were then separately pooled and homogenized to composite sample and processed within 4 hours of collection. Physico-chemical characteristics of the sediments like pH, temperature and salinity were measured during the time of collection. The sediments were later air dried and analyzed for percentage organic matter and sediment texture using modified Walkley and Black method (23).
For the isolation of yeasts, plating of the sediment samples were done employing spread plate method in Wickerham's agar supplemented with 200 mg/l chloramphenicol (27). The plates were incubated at 18 ± 2°C for 7 days and the colonies developed were purified by quadrant streaking and transferred to malt extract agar slants for further studies. Isolates were stocked in malt extract agar vials overlaid with sterile liquid paraffin for long term storage.
The isolates were screened qualitatively for the production of extracellular lipase in solid medium supplemented with suitable substrate. Nutrient agar added with 1% tributyrin as substrate was used in our study to test the hydrolytic activity of lipase enzyme. The plates were spot inoculated and incubated at room temperature (28 ± 2ºC) for 7 days. Formation of clearance/halo zone around the colonies was considered as positive. The diameter of the clearance zones was measured and recorded at every 24 hours for a period of a week.
The yeast isolates showing lipolytic activity were identified up to genus level using morphological, physiological and biochemical methods (3). The species level identification was confirmed with the help of molecular techniques by extracting genomic DNA, sequencing of ITS region as per Harju et al. (9) and NCBI BLAST. ITS primers (Forward ITS 1: 5' -TCC GTA GGT GAA CCT GCG G- 3' and Reverse ITS 4 - 5' -TCC TCC GCT TAT TGA TAT GC- 3') by White et al. (26) which amplify a fragment of approximately 580 bp containing the ITS 1, 5.8 S and ITS 2 regions were used for the present study.

RESULTS

The number of colony forming units per milli litre (CFU/ml) of the diluted sediment sample from the two study sites (in triplicates) of Kasaragod mangroves was calculated. The CFU/ml of yeast in mangrove sediments from polluted site (KGD_P) was 200 while that from the non-polluted site (KGD_N) was 100. The sediment temperature, pH and organic matter (%) of KGD_P were 28 °C, 6.8, 2.59 ± 0.02 and KGD_N were 29°C, 6.8, 2.53 ± 0.01 respectively.
Yeast colonies showing varied morphological characteristics in Wickerham isolation media containing plates were selected. These colonies were then isolated, purified and used for screening the production of extracellular lipase. The growth of colonies and its hydrolysis of substrate (1% tributyrin) on plates qualitatively were monitored at every 24 hours for a week. The diameter of the clearance zone was measured and expressed in milli meters (mm) (Fig. 3). A total of 30 isolates which showed lipase activity in plates at 28 ± 2ºC were selected from both the sites (15 each from KGD_P and KGD_N) for the study. The samples were designated as KGD_P 1 - KGD_P 15 (isolates from polluted sediments) and KGD_N 1 – KGD_N 15

 © 2021 Society of Education, India

(isolates from non - polluted sediments). The results of the lipase enzyme activity of the yeast isolates were summarized in Table 1.

The isolates with lipase activity were characterized by both conventional and molecular techniques for their proper identification to the species level. *Candida tropicalis* (NCBI Accession no: MT 141122) was the most abundant yeast species found in the mangrove sediments studied. They were found to produce extracellular lipases with good hydrolytic activity against lipid substrates. *Candida dubliniensis* (MT 132376), *Kluveromyces siamensis* (MT 138586), *Trichosporon asahii* (MT 138568), *Debaromyces napelensis* (MT 138571) and *Saturnispora diversa* were the other species identified to have lipolytic activity. The isolate KGD_P 7 (*Candida tropicalis*) showed the highest zone of clearance among all.

The diameter of the zones of clearance of lipolytic forms from polluted sediments varied between 0-21mm (KGD_P) and those from non-polluted (KGD_N) varied between 0-12mm. Significant differences were noted in the lipolytic activities of yeasts isolates between polluted (KGD_P) and non polluted (KGD_N) mangrove sediments. In KGD_P the hydrolysis of lipid substrate by the isolates started early at 24 hrs and increased at a rate of 3-4 mm to maximum at every 24 hours for next 2 days. But in case of $KGD_{N,}$ the hydrolysis started late which was after 48hrs of incubation with the substrate containing media. The clearance zone was smaller in diameter and did not increase much even after 5 days of incubation. All the isolates showed maximum lipolytic activity around Day 4 (96 hrs) and then remained constant.

Discussion

In the present study, we have compared the lipolytic activity of yeast isolates from two different sites in terms of anthropogenic pollution of the same mangrove area of Kasaragod. The production of lipase by yeast is normally determined by measuring its enzyme activity which tests the ability of lipase to hydrolyze triacylglycerols into fatty acids and glycerol (4). Isolates from polluted sediments (KGD_P) showed faster rate of lipid hydrolysis compared to those from the non polluted (KGD_N) sediments. This shows the presence of highly active yeasts capable of producing extracellular lipases against pollutants in the sediments. The yeasts isolated from polluted environments would have enhanced enzyme production due to the presence of natural substrates in the pollutants. Such isolates with inherent potential can be exploited for the large scale production of hydrolytic enzymes for industrial uses and bioremediation. It has been recorded that *Candida* and *Pichia* are the most active group in producing lipase enzyme (14,19). *Candida tropicalis* has been already identified as a potential oil degrader (10). Most of the lipolytic isolates in our study also belongs to genus *Candida* particularly *Candida tropicalis.* The genus *Candida* has not only the ability to hydrolyze and synthesize various oil types and long chain esters (20) but also to act on non specific triacylglycerols widely found in nature (1). The isolates showed maximum hydrolysis during 72 hrs – 96 hrs after which the activity remained unchanged. Hence this period can be considered as most appropriate for the extraction of lipases from yeast for commercial purposes. Since the lipase activity is influenced by various environmental factors like the incubation period, pH, temperature, substrate concentration etc, they should be also taken into consideration during the large scale production of the enzyme (5). Development of a consortium of lipase active yeasts and optimization of their growth for maximum enzyme production would be a great step in the bioremediation of polluted environments. Also, the lipase production and hydrolytic activity of yeast isolates were under 30°C which make them potential candidates for commercial production and industrial applications.

CONCLUSION

Mangrove sediments, due to their unique ecosystem conditions, are found to be good source of potent microbes including yeasts. The extra cellular enzymes produced by them have not only industrial application but also biodegradation/bioremediation capacity. Our study proved the presence of highly active lipolytic yeasts belonging to mainly genus *Candida* in the mangrove sediments which has the capacity to degrade lipid substrates readily. It was also found that the yeasts isolated from polluted sediments showed higher hydrolytic potential than those from pristine environment. Hence, further research on maximizing the production and extraction of extracellular lipases from these yeasts would be promising for bioremediation of polluted environments and in industrial applications.

Table 1: Lipase enzyme activity of yeast isolates from mangrove sediments from polluted and non - polluted sites of Kasaragod, Kerala

No	Isolates	Species	Diameter of the Clearance/halo zone (mm)				
			24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
1	KGD_P -1	*Candida tropicalis*	-	4	8	15	16
2	KGD_P -2	*Candida tropicalis*	-	8	12	16	16
3	KGD_P -3	*Candida tropicalis*	8	12	15	17	18
4	KGD_P -4	*Kluveromyces siamensis*	10	10	10	10	10
5	KGD_P -5	*Candida dubliniensis*	10	15	17	20	20
6	KGD_P -6	*Candida tropicalis*	8	9	12	15	15
7	KGD_P -7	*Candida tropicalis*	9	13	17	20	21
8	KGD_P -8	*Candida tropicalis*	10	14	15	17	17
9	KGD_P -9	*Candida tropicalis*	10	12	13	14	14
10	KGD_P -10	*Candida tropicalis*	11	13	14	16	16
11	KGD_P -11	*Candida tropicalis*	10	11	11	12	12
12	KGD_P -12	*Candida tropicalis*	-	3	5	10	10
13	KGD_P -13	*Trichosporon asahii*	-	4	6	10	10
14	KGD_P -14	*Candida tropicalis*	9	12	13	15	16
15	KGD_P -15	*Debaromyces napelensis*	8	9	9	10	10
16	KGD_N -1	*Candida dubliniensis*	-	-	7	8	8
17	KGD_N -2	*Candida tropicalis*	-	-	8	10	10
18	KGD_N-3	*Candida tropicalis*	-	-	9	9	9
19	KGD_N -4	*Kluveromyces siamensis*	-	-	-	6	6
20	KGD_N -5	*Candida dubliniensis*	-	-	8	10	10
21	KGD_N-6	*Candida tropicalis*	-	-	-	5	5
22	KGD_N -7	*Candida tropicalis*	-	-	-	6	6
23	KGD_N -8	*Candida tropicalis*	-	-	8	9	9
24	KGD_N-9	*Candida tropicalis*	-	-	8	8	8
25	KGD_N -10	*Candida tropicalis*	-	-	7	7	7
26	KGD_N-11	*Candida tropicalis*	-	-	8	9	9
27	KGD_N-12	*Candida tropicalis*	-	-	-	9	9
28	KGD_N -13	*Candida tropicalis*	-	-	9	12	12
29	KGD_N -14	*Candida tropicalis*	-	-	9	9	9
30	KGD_N -15	*Saturnispora diversa*	-	-	-	6	6

Figure 1: Google earth image of sites of collection of mangrove sediments, Kasaragod. KGD_P – polluted site; KGD_N – non-polluted site. Scale bar = 1 Km

Figure 2: Sites of collection of mangrove sediments. A - KGD_N (non-polluted site); B - KGD_P (polluted site)

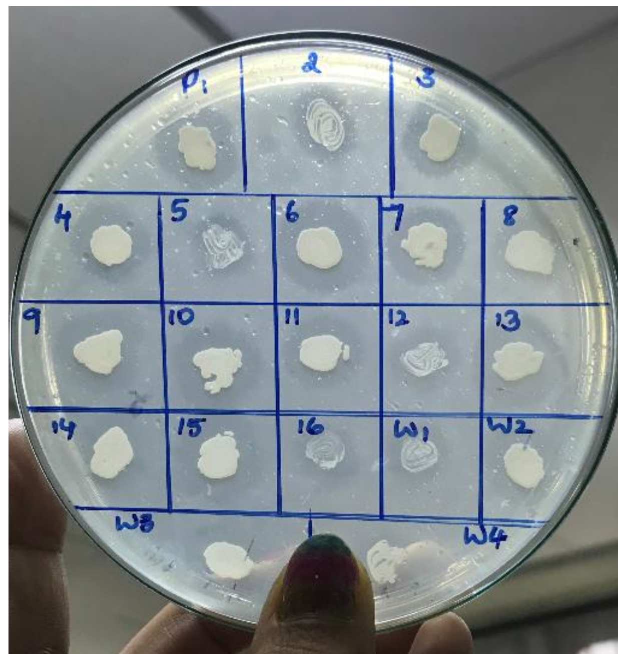

Figure 3: Nutrient agar plates (1%tributyrin) showing yeast isolates with lipase enzyme activity. Positive lipolytic forms shows clearance zones around the colonies.

REFERENCES

1. Alamia, N.H., Nasihah, L., Umar, R.L.A., Kuswytasari, Enny Zulaika, N.D. & Shovitri, M. (2017). Lipase production in lipolytic yeast from Wonorejo mangrove area. In: Proceeding of International Biology Conference, 2016, 1854:1-11.
2. Andreote, F., Jimenez, D., Chaves, D., Dias, A., Luvizotto, D., Dini-Andreote, F, et al. (2012). The microbiome of Brazilian mangrove sediments as revealed by metagenomics. PLoS ONE., 7(6): 1-14.
3. Barnett, J.A., Payne, R.W. & Yarrow, D. (2000) Yeast Characteristics and Identification. Cambridge University Press, London, pp.100-201.
4. Begam, M.S., Pradeep, F.S. & Pradeep, B.V. (2012). Production, purification, characteization, and applications of lipase from *Serratia marcescens* MMBBO5. Asian. J. Microbiol. Biotech. Env. Sc., 5(4): 237-245.
5. Bindiya, P. & Ramana, T. (2012). Optimization of lipase production from an indigenously isolated marine *Aspergillus sydowii* of Bay of Bengal. J. Biochem. Tech., 3(5): 5203-5211.
6. Emimol, A., Ganga, G., Parvathy, R., Radhika,G. & Nair G.M. (2012). Screening of Microbes producing extracellular hydrolytic enzyme from corporation waste dumping site and house hold waste for the enhancement of bioremediation methods. IOSR. J. Phar. Bio. Sc., 4(1): 54-56.
7. Facchini, F.D.A., Vici, A.C., Pereira, M.G., Jorg, J.A. & Polizeli, T.M. (2016). Enhanced lipase production of *Fusarium verticillioides* by using response surface methodology and waste water pretreatment application. J. Biochem. Tech., 6(3): 996-1002.

8. Grativola, A.D., Marchettia, A.A., Wetler-Toninia, R.M., Venancio, T.M., Gatts C.E.N., Thompson, F.L. & Rezende, C.E. (2017). Bacterial interactions and implications for oil biodegradation process in mangrove sediments panel. Marine. Poll. Bull., 118(2): 221-228.
9. Harju, S., Fedosyuk, H. & Peterson, K.R. (2004). Rapid isolation of yeast genomic DNA: Bust n' Grab.BMC. Biotech., 4(8): 1-6.
10. Hasan, F., Shah, A.A. & Hameed, A. (2006). Industrial applications of microbial lipases. Enz. Microb. Tech., 39(2): 235-251.
11. Jaeger, K.E. & Reetz, T.M. (1998). Microbial lipases from versatile tools for biotechnology. Trends Biotechnol., 16(9): 396-403.
12. Kademi, A., Lee, B. & Houde, A. (2003). Production of heterologous microbial lipases by yeasts. Indian J. Biotech., 2(3): 346-355.
13. Kumar, DS. & Ray, S. (2014). Fungal lipase Production by solid state fermentation – An overview. J. Anal. Bioanal. Tech., 6(1): 1-10.
14. Kutty, S.N. & Philip, R. (2008). Marine yeasts – A review. Yeast., 25(7):465-483.
15. Kutty, S.N., Damodaran, B. R. & Philip,R. (2014). Yeast isolates from the slope sediments of Arabian Sea and Bay of Bengal: Physiological characterization. Adv. Appl. Sci. Res., 5(5): 177-187
16. Mehta, A., Bodh, U. & Gupta,R. (2017). Fungal lipases: A review. J. Biotech. Res., 8(1): 58-77.
17. Nagarajan, S. (2012). New tools for exploring old friends microbial lipases. Appl. Biochem. Biotechnol., 168(5): 1163- 1196.
18. Ramos-Sanchez L.B., Cujilema-Quitio, M.C., Julian-Ricardo, M.C., Cordova, J. & Fickers, P. (2015). Fungal lipase production by solid state fermentation. J. Bioprocess. Biotechnol., 5(2): 1-9.
19. Sarkar, A. & Rao, K.V.B. (2016). Marine yeast: A potential candidate for biotechnological applications- A review. Asian. J. Microbiol. Biotech. Env. Sc., 18(3) : 627-634.
20. Sharma, R., Chisti, Y. & dan Banerjee, U.C. (2001). Production, purification, characterization and applications of Lipases. Biotech. Adv., 19(8): 627-662.
21. Sharma, S. & Shah, K.W. (2005). Generation and disposal of solid waste in Hoshangabad. In: Book of Proceedings of the Second International Congress of Chemistry and Environment, Indore, India, pp. 749–751.
22. Thakur S. (2012). Lipases, its sources, properties and applications: A Review. Int. J. Sci. Eng. Res., 3(7): 1-29.
23. Trivedi, R.K. & Goel, P.K. (1986).Chemical and biological methods for water pollution studies. Environmental publication, Karad, India, pp.104-248.
24. Vakhlu, J. & Kour, A. (2006). Yeast lipases: enzyme purification, biochemical properties and gene cloning. Elect. J.Biotech., 9(1):69-85.
25. Vasileva-Tonkova,E & Naturforsch, D.G.Z. (2003). Hydrolytic enzymes and surfactants of bacterial isolates from lubricant contaminated wastewater. Zeitschrift für Naturforschung C., 58(1):87-92.
26. White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols. A Guide to Methods and Applications [Eds. Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J] Academic Press, San Diego, CA, p.315–324.
27. Wickerham, L.J. (1951). Taxonomy of yeasts. U.S. Dept. Agric. Tech. Bull., 1029:1-19.

Copyright: © 2021 Society of Education. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.