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Original Research Paper

Utilization of agro-industrial wastes for the production of lipase from *Stenotrophomonas maltophilia* isolated from Arctic and optimization of physical parameters

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ABSTRACT

In the present work production of lipase by Arctic sediment isolate *Stenotrophomonas maltophilia* was investigated, and focus was on the lipase production using agro industrial waste. Optimization of physical parameters which enhance the lipase production in agricultural waste such as groundnut cake, rice bran, wheat bran, neem cake, and coconut cake was achieved and significance was validated by ANOVA. The crude lipase was concentrated by precipitation with acetone and further purification was carried out by ion exchange chromatography followed by SDS-PAGE. When screened for extracellular lipase production, 54 isolates showed positive results and significant activity was expressed by sediment isolate KS 46 which was phylogenetically identified to be *S. maltophilia*. The groundnut cake supported maximum enzyme production (74.117 U/ml) followed by coconut cake (61.911 U/ml), neem cake (58.737 U/ml), wheat bran (49.614 U/ml) and rice bran (28.165 U/ml). Characterization of the enzyme revealed the optimum temperature to be 28 °C and optimum pH to be 6 for groundnut cake, neem cake and coconut cake while pH 7 for rice bran and wheat bran. Maximum lipase production was attained at 60% moisture for all substrates except for neem cake (70%). The enzyme was purified to 2.9 fold and molecular weight was found to be 49.1 KDa. The results suggest that agro wastes such as groundnut cake has the potential to be used as alternative cost effective substrate for lipase production and optimum conditions vary for the same enzyme when different substrates are utilized.

1. Introduction

Lipases (Triacylglycerol acylhydrlases, EC3.1.1.3) catalyze reactions such as partial or complete hydrolysis of triacylglycerol and lipid esterification, transesterification and interesterification (Colla et al., 2010). These varied catalytic properties generated interests in high production of lipases in various industries for their applications as additives in food industry, fine chemical synthesis, pharma, detergents, cosmetics, leather processing, biodiesel production, biomedical assays and waste water treatments (Colla et al., 2010; Burkert et al. 2004; Elibol and Ozer 2000; Kamini et al., 2000). Despite cold active lipase producing bacteria and yeast are reported from around the globe, production of cold active lipases is under explored (Joseph et al., 2007). The ability of cold active enzymes to catalyze reactions at low or moderate temperature provides industrial and biotechnological potential (Gomes and Steiner, 2004). Cold active enzymes have additional advantage that they can be inactivated by treatment at low temperature for short time periods (Margesin et al., 2002) reducing energy consumption and cost.

Although all agree that enzymatic processes are favorable to the environment than traditional processes, the economic viability of replacing chemical methods with enzymatic processes still poses doubts (Abdelmoez et al., 2013; Jagannathan and Nielsen, 2013). Thus exploitation of agricultural residues for enzyme production and other value added products would be of greater significance in future biotechnologies mainly due to their low cost, accessibility and nutrient content (Salihu et al. 2012). One of the main area in lipase research now involve the use of different microbes, supplements and substrates to obtain the best combinations for high value lipases by operational conditions that offer low production cost at industrial scale (Rigo et al., 2010).

Agro industrial waste and their complex organic contents constitute a significant source of residual nutrients which serve as a rich media for microbial growth and production of the enzymes (Alonso et al., 2005). Solid state fermentation had been successfully used for bacterial lipase production using agricultural and oil industries waste (Kapoor et al., 2001; Kashyap et al., 2001; Sabu

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et al., 2006; Virupakshi et al., 2005). Various reports suggest that apart from the nature of solid substrate (water activity and particle size), physical parameters (temperature, pH, and heat conductivity) also greatly influence the production of lipase on agricultural waste (Barrios- Gonzalez et al., 1993; Echevarria et al., 1991; Liu and Tzeng, 1999; Pandey et al. 1994). Thus during this study – the effect of physical parameters on lipase production by the same bacteria on different substrates was investigated.

Even though agricultural residues are produced in large quantities in developing countries, they are mainly utilized as animal feed and landfills (Salihu et al., 2012). India is world's second largest fruit and vegetable producer and encounters waste close to 25% worth of produce. It is also significant that 40% of loses occur at post harvest and processing levels while in industrialized countries loses happen only at retail and consumer levels (NIAM, 2012). In recent times, agricultural wastes have been made to use in biotechnological processes such as production of value added compounds and substrates for microbial isolation. These help in disposal problems which otherwise lead to pollution (Graminha et al., 2008; Mussatto, 2009; Pandey and Soccol, 2000). Oil cakes from different oil extraction industries have been utilized for fermentative lipase production mainly because; the residual oil content serves as inducer for lipase production (Salihu et al., 2012; Singhania et al., 2008). During the present study-groundnut cake, rice bran, wheat bran, neem cake, and coconut cake were tested for fermentative lipase production. Further, development of proper physical parameters for bioprocess and partial purification of lipase was performed.

2. Materials and methods

2.1. Isolation, identification and screening

Samples were collected from 4 predetermined stations at Kongsfjord (Fig. 1) Arctic; sediments were collected by Van Veen Grab and water by Niskin bottle deployed from Research Vessel Teisten. Samples were then transported to the laboratory on shore within four hours of collection and were then serially diluted and plated on to ZoBells Marine Agar (ZMA) for isolation of psychrotrophic bacteria by incubating at 15 and 20 °C. These heterotrophic isolates were streaked on tributyrin agar (TBA) plates for screening of extracellular lipase production. Lipase producers show a clearance zone around the colony that is attributed to butyric acid release upon tributyrin hydrolysis.

2.2. Identification and culture media

Single isolate showing the highest activity was selected for further studies and the isolate was identified using conventional as well as 16S rDNA based molecular technique (Giovanoni et al., 1991). DNA was isolated using the Bacterial Genomic DNA (prep) Kit (Chromous Biotech, India) and was checked for purity by agarose gel electrophoresis. 16SrDNA primers 27F (5′ AGAGTTTGATCATGGCTCAG 3′) and 1492R (5′ ACGGTTACCTTGTTACGACTT 3') were used to amplify the gene in isolated genomic DNA. The amplified PCR product was sequenced and these sequences were subjected to BLAST similarity analysis and aligned using CLUSTAL-W to identify the nearest taxa with NCBI GenBank data base. The accession number obtained for the identified isolate was deposited under the number JX262392 in GenBank. Phylogenetic tree was constructed using Neighbor-joining (NJ) method using Mega version- 6.

The identified isolate was inoculated (OD = 1.0 at 620 nm) into sterilized modified Glucose Yeast Peptone (GYP) medium constituting glucose 1 g/L, yeast 5 g/L, peptone 10 g/L, $MgSO_4$ 0.3 g/L and $MnSO_4$ 0.1 g/L and olive oil 10 ml/L and incubated at 20 °C (to provide psychrotrophic conditions) with agitation at 150 rpm (Orbitek, India) for 3 days. After the incubation period, 1 ml of this culture was used as mother culture to inoculate agro-waste media.

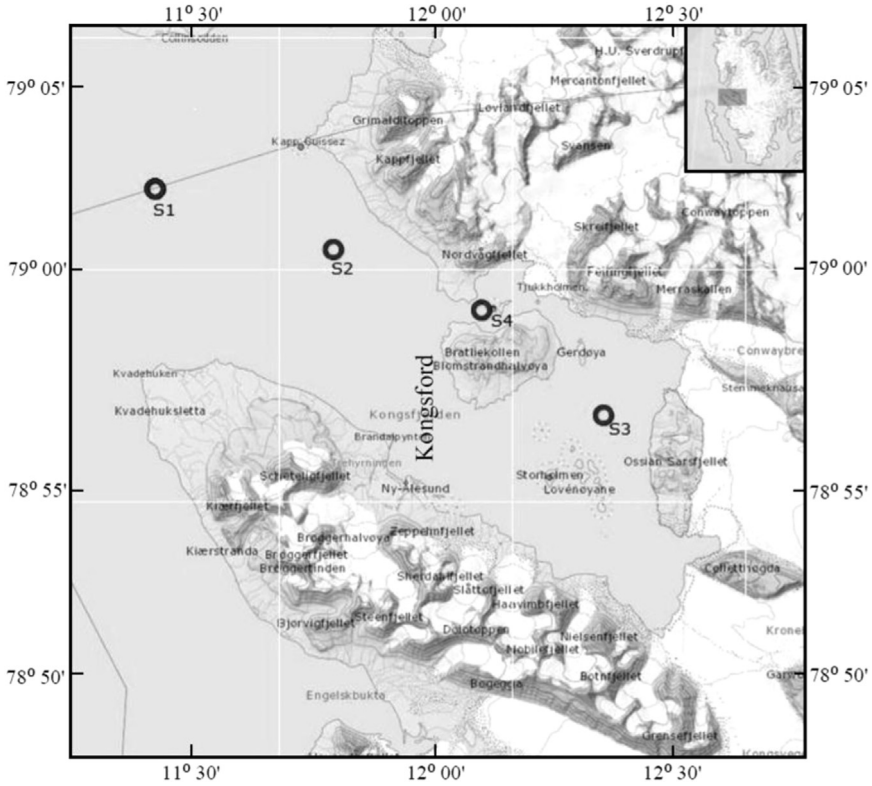

Fig. 1. Map showing location of sampling sites (S1, S2, S3, and S4) in Kongsfjord, Arctic.