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OIL CAKES AS SUBSTRATE FOR IMPROVED LIPASE PRODUCTION IN SOLID STATE FERMENTATION

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Abstract- Solid-state fermentation for lipase production from *Rhizopus oryzae* KG-10, using different low cost available oil cakes JOC (Jatropha oil cake), TOC (Teesi oil cake), MOC (Mustard oil cake), GOC (Groundnut oil cake) was carried and it was found that the fungus produced significant amount of lipase utilizing oil cakes as substrate. Among the four substrates used crude enzyme extracted from MOC medium showed highest activity of 170 IU. Activity of enzyme extracted from medium containing JOC, TOC and GOC were assayed to be 80 IU, 60 IU and 60 IU respectively. Total protein of the crude enzyme extracted from the different medium was estimated by Lowry's method. Total protein content of extracts from MOC, JOC, TOC and GOC medium were 32 mg/ml, 30 mg/ml, 31.2 mg/ml and 26.4 mg/ml respectively. Thus it could be seen that in about same amount of extracellular proteins the activity was maximum in case of MOC, suggesting it to be the best substrate.
Key words – Lipase, *Rhizopus oryzae*, oil cakes, solid-state fermentation

Introduction

Fungal lipases are known to be commercially used in various biotechnological industries. Lipases are reported in various microorganisms, animals, and plants and they breakdown lipids so they are used for various biotransformation reactions, catalysis industries and other industries e.g. detergents, dairy foods, bakery and beverages, health foods, pharmaceuticals [1,2]. These enzymes were well studied towards structural characterization and enzymatic action [3] but utilization of the given oil cakes as substrate for lipase production was not well reported. However, there is more emphasis towards lipase production in solid-state fermentation (SSF) because it has potential application in biotechnology industries [4]. However, SSF is most appropriate process due to its various benefits and bioconversion parameters. There are few more reports on lipase production by SSF of olive cake and sugar cane bagasse [5] and biotechnological potential of oil cakes [6]. Moreover some interesting studies on lipase production through supplemented soybean meals, statistical optimization, thermoactive alkaline lipase from *Talaromyces thermophilus* and optimization of lipase production in oil effluent has drawn the attention in recent years [7,8,9,10]. The present study gives a report of different low value oil cakes (oil cakes from Mustard, Jatropha, Groundnut and Teesi) on lipase production from *Rhizopus oryzae* KG10 through solid state fermentation.

Materials and methods

Microorganism and enzyme production

Rhizopus oryzae KG10 was taken for the study. It was grown on Potato-Dextrose-Agar (PDA) (Hi-media), incubated at 28°C for seven days and further it was stored at 4°C. Four low cost available oil cakes obtained from local farmhouse viz. JOC (Jatropha oil cake), TOC (Teesi oil cake), MOC (Mustard oil cake), GOC (Groundnut oil cake) were used as substrate for solid state fermentation. The production medium was prepared using mineral salt solution (20 ml) with peptone (15 g/L), NaCl (5 g/L), CaCl (1 g/L) and oil cakes (5 g) as substrate. This was inoculated with 1 ml of 10^4 spores of R. oryzae (24 hr grown fungal culture). This was further incubated for 7 days at 30 ˚C.

Enzyme extraction and protein estimation

To the fermentation product 50 ml of 0.1M Potassium phosphate Buffer pH 7.5 was added, stirred and mixed properly and enzyme was extracted by filtering the solution through whattman filter paper (No-1, Millipore, Carrigtwohill, Ireland). The culture filtrates obtained were centrifuged at 3000xg for 20 min and clear supernatant was collected and used as enzyme source.
The enzyme activity as amount of enzyme required liberating one micromole equivalent fatty acid per ml/min. (in triplicate) was measured by titrimetric method using phenolphthalein as indicator. Soluble protein concentration and fractionated protein concentration were determined by the method of Lowry using Bovine serum albumin as standard.

Concentration of Enzyme

The crude enzyme was precipitated with ammonium sulphate. The sample was separated into five fractioned based on the saturation percent of ammonium sulphate 30%, 55%, 75%, 85%. The precipitations were carried out at 4˚C under constant stirring and precipitated proteins were centrifuged at 15000g for 30 minute.

The fractions were dialyzed in 0.1M Potassium phosphate Buffer (pH 7.5) to remove the remaining salt.

SDS-PAGE

Sodium Dodecyl Sulpahte-Polyacrylamide gel electrophoresis (SDS-PAGE) 12% of crude and partially purified lipase was performed for the determination of molecular weight.

Results and discussion

Enzyme activity and total protein

Rhizopus oryzae KG-10 was able to produce lipase by solid-state fermentation with low value oil cakes viz. JOC, MOC, TOC and GOC upon incubation at 30°C for 7 days. Among the four substrates used crude enzyme extracted from MOC medium showed highest activity of 170 IU. Activity of enzyme extracted from medium containing JOC, TOC and GOC were assayed to be 80 IU, 60 IU and 60 IU respectively [Fig. 1]. Total protein of the crude enzyme extracted from the different medium was estimated by Lowry's method. Total protein content of extracts from MOC, JOC, TOC and GOC medium were 32mg/ml, 30 mg/ml, 31.2 mg/ml and 26.4 mg/ml respectively [Fig. 2]. It can be seen that mustard oil cake was the best inducer for lipase production from KG-10.

SDS-PAGE Profile

SDS PAGE profile of all different ammonium sulphate precipitate of enzyme extract from medium containing MOC has been shown in Fig 3.

Conclusion

There are other notable reports on lipase production through SSF using oil cakes, purification, statistical optimization and use in industries [7,8,9,10] and other notable reports on immobilizing the enzyme and natural selection for lipase producing microbial strains are available [11,12] but there are only few reports indicating utilizing low value oil cakes as substrates mentioned in the present work. It was thus reported that mustard oil cakes (MOC) could be utilized as better substrates over other oil cakes (JOC, TOC and GOC) for lipase production from *Rhizopus oryzae*. This study gives an idea on utilization of waste oil cakes for enzyme production through SSF and adds value addition to oil mill wastes.

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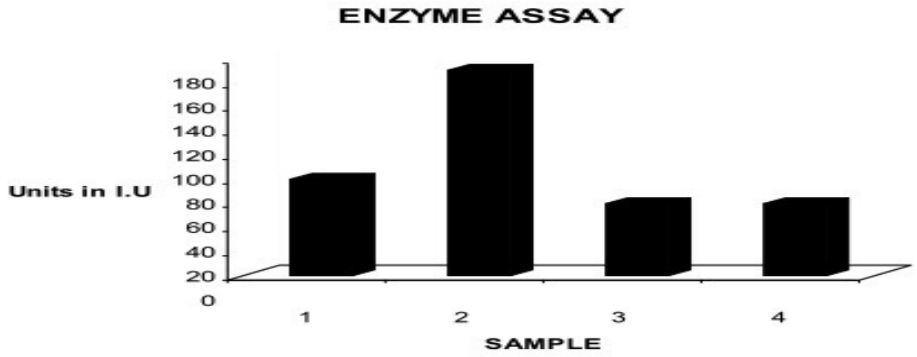

Fig. 1- Enzyme Activity (Unit/ml) for Jatropha oil cake (1); Mustard oil cake (2); Teesi oil cake (3); Groundnut oil cake (4)

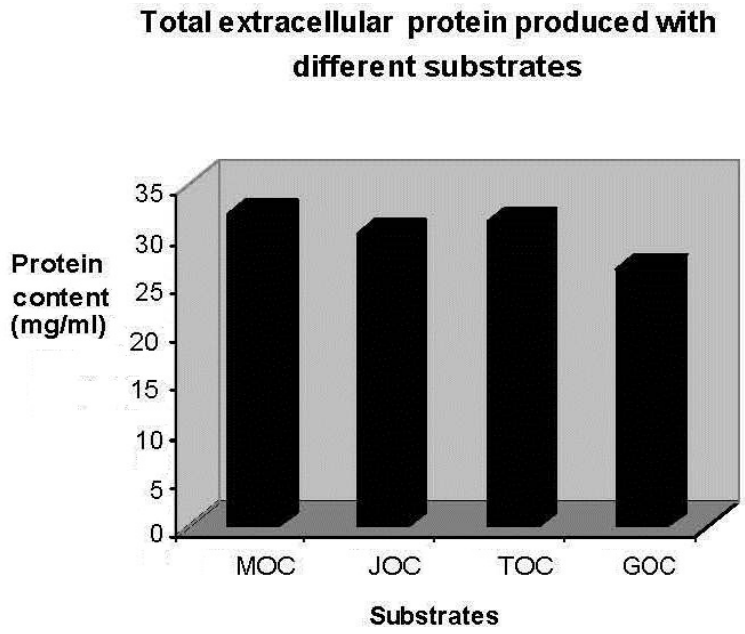

Fig. 2- Total Protein Content of the extracellular fluid on solid state fermentation with Mustard Oil cake (MOC); Jatropha Oil Cake (JOC); Teesi Oil Cake (TOC) and Groundnut Oil Cake (GOC).

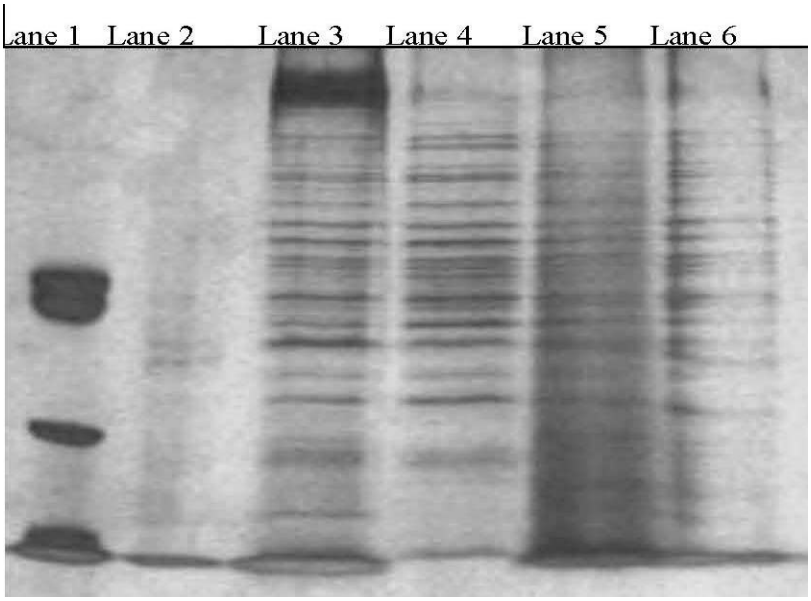

Fig. 3- SDS PAGE profile of crude and ammonium sulphate precipitate sample of Mustered oil cake (MOC). Lane1: molecular weight marker; Lane2 Crude sample, Lane 3: 30% precipitate; Lane 4: 55% precipitate; Lane 5: 75%, Lane 6: 85 %.