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Plausible exploitation of *Jatropha* de-oiled seed cake for lipase and phytase production and simultaneous detoxification by *Candida parapsilosis* isolated from poultry garbage

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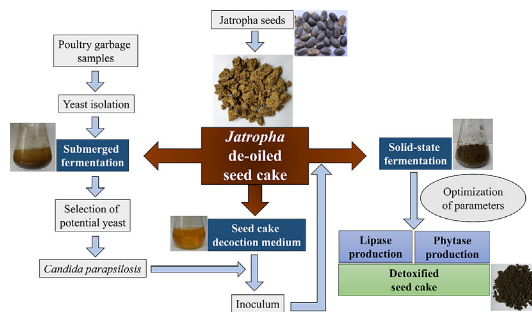
HIGHLIGHTS

- Lipase and phytase are produced from *Jatropha* de-oiled seed cake by *C. parapsilosis*.
- Seed cake decoction medium is employed for inoculum preparation.
- Toxins and anti-nutrients are reduced significantly from substrate.
- Phorbol esters are eliminated with 3 days incubation.

GRAPHICAL ABSTRACT



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ABSTRACT

Jatropha de-oiled seed cake was explored to utilize as a basic nutrient source for *Candida parapsilosis*, isolated from poultry garbage and selected based on the production of lipase and phytase enzymes under submerged fermentation. At optimized parameters under solid-state fermentation, lipase and phytase activities were recorded as 1056.66 ± 2.92 and 833 ± 2.5 U/g of substrate (U/g), respectively. Besides enzyme production, complete elimination of phorbol esters and significant phytate reduction from 6.51 ± 0.01 to 0.43 ± 0.01 g/100 g of seed cake were noted after 3 days incubation. Curcin and trypsin inhibition activity were reduced significantly from 26.33 ± 0.43 to 0.56 ± 0.02 mg/100 g and 229.33 ± 2.02 to 11.66 ± 0.28 U/g, respectively after 5 days incubation. Saponins were reduced from 5.56 ± 0.19 to 1.95 ± 0.01 g/100 g of seed cake after 7 days incubation.

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1. Introduction

The plant *Jatropha* belongs to the Euphorbiaceae family and is one of the best choices among oil seed bearing plants for the production of biodiesel (Kumar et al., 2012). After oil extraction from seeds, the resultant de-oiled seed cake contains 25–31% protein and 40–48% carbohydrates (Pasha et al., 2013), 0.6% fat, 4% fiber, and 9% ash (Rakshit et al., 2008). Unlike, other edible de-oiled seed cakes like ground nut, sunflower and soya, the use of *Jatropha* de-oiled seed cake is restricted for direct animal feeding and feed applications due to the presence of toxic phorbol esters, curcin and other anti-nutritional factors, such as phytate, saponins and trypsin inhibitors (Rakshit et al., 2008). However, biodiesel production from *Jatropha* seed oil generates large quantity of seed cake after oil extraction from seeds. Therefore, sub-sequential

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utilization of the *Jatropha* de-oiled seed cake must be addressed to improve the outcome value of *Jatropha* biodiesel production.

Physical, chemical and biological methods have been reported by various researchers for the reduction of toxins and anti-nutrients of *Jatropha* de-oiled seed cake (Joshi et al., 2011; Phengnuam and Suntornsuk, 2013). However, with the development in the area of fermentation technology using agro-industrial byproducts for the production of various value added products, some promising potential applications of *Jatropha* de-oiled seed cake have been opened, including its utilization as a convenient substrate for basic nutrient source and supporting the production of various enzymes such as lipases, proteases, phytases and xylanases (Mahanta et al., 2008; Veerabhadrappa et al., 2014; Joshi and Khare, 2011). Utilization of *Jatropha* de-oiled seed cake as a substrate for fermentation not only gives an opportunity for cost effective enzyme production, but also opens up the path for simultaneous degradation of toxins and anti-nutrients of the substrate (Veerabhadrappa et al., 2014; Joshi et al., 2011; Phengnuam and Suntornsuk, 2013). For microbial enzyme production, use of various edible and non edible de-oiled seed cakes have been reported, such as sunflower, mustard, palm, groundnut, canola and rapeseed oil cakes (Ramachandran et al., 2007). Utilization of non edible oil seed cakes for enzyme production makes the process of oil production cost effective to oil industry.

Lipases have their role in the conversion of plant seed oil into biodiesel (Kuo et al., 2015), detergent formulations, organic chemical processing, biosurfactants production, nutrition, cosmetics, dairy, oleochemical, agrochemical, paper and pharmaceutical industries (Pandey et al., 1999). Phytases have their promising role in feed applications. Inclusion of phytase in animal feed liberates phosphorus and other minerals from phytate, making these available for intestinal absorption and thereby reduce the usage of additional phosphorus supplements (Pandey et al., 2001). However, limitation of the usage of these enzymes for industrial and feed applications is mainly due to the high production cost, which can be overcome by replacing the conventional nutrient sources with less expensive raw materials, such as agro-industrial waste biomass/byproducts for microbial fermentation.

The lipase and phytase production by various yeast species have been reported, including lipase production by *Yarrowia lipolytica* (Fickers et al., 2011), *Candida rugosa, C. antarctica, C. lipolytica* and *C. cylindrica* (Bussamara et al., 2010) and phytase production by *Schwanniomyces castellii, Pichia anomala, Saccharomyces cerevisiae, Candida krusei* (Kaur et al., 2007). It is also clear that the yeast is capable of growing on solid substrates for the production of enzymes (Benjamin and Pandey, 1997). Therefore, in the present study, various yeast strains have been isolated to evaluate their efficiency for lipase and phytase production from *Jatropha* de-oiled seed cake. This study is aimed to develop a feasible approach for the production of commercially important and yet economically viable lipase and phytase enzymes using *Jatropha* de-oiled seed cake as a basic nutritional source and support for microbial growth and its detoxification as well.

2. Materials and methods

2.1. Substrate for enzyme production

J. curcas screw pressed de-oiled seed cake used in the experiments was obtained from Nandan Biomatrix Pvt Ltd, Zaheerabad, India. The seed cake was initially sun dried and later oven dried to remove the residual moisture. Material was ground using mortar and pestle to make fine particles and used as a substrate for fermentation studies. The dried and ground seed cake was evaluated for toxins, anti-nutrients and protein.

2.2. Isolation of yeast

Samples were collected in sterile polythene zip lock covers from various poultry garbage dump yards across the Hyderabad city, India. Samples were suspended individually in sterile distilled water. After uniform mixing, 2 ml solution was transferred to 50 ml sterile YEPD broth (Yeast extract 1%, Peptone 2% and Dextrose 2%) in a 250-ml Erlenmeyer flask and incubated at 30 °C, 150 rpm for 48 h. After incubation, 50 μl aliquots were inoculated on YEPD agar plates containing kanamycin (50 μg/ml) and incubated at 30 °C/48 h. The so obtained yeast and yeast like colonies were sub cultured and maintained on YEPD agar medium.

2.3. Screening for enzyme production

2.3.1. Primary screening

Primarily, the microorganisms were screened for enzyme activity on agar plates based on the methods described by Griebeler et al. (2011) for lipase activity and Gupta et al. (2014) for phytase activity. Initially, all the yeast isolates were inoculated into YEPD broth individually and incubated at 30 °C and 150 rpm for 24 h. After incubation, culture from each flask was inoculated on tributyrin agar and sodium phytate agar medium plates separately, and incubated for 96 h at 30 °C. Tributyrin was used as a substrate for lipase activity and sodium phytate was used as a sole phosphorus source for phytase activity. After incubation, the organisms showing transparent halo around the colony were selected to assess further enzyme activity.

2.3.2. Secondary screening

Yeast isolates showing lipase and phytase activities on agar plates were inoculated into the medium containing *Jatropha* de-oiled seed cake as nutritional source and submerged fermentation was carried out according to the modified method described by Phengnuam and Suntornsuk (2013). Each 250-ml Erlenmeyer flask containing 20 g seed cake suspended in 90 ml distilled water was autoclaved at 121 °C for 15 min and inoculated with 10 ml yeast culture suspension containing 10^{10} CFU/ml and the flasks were incubated at 30 °C and 120 rpm in a shaking incubator for 7 days. After incubation, a sample of 1 ml liquid portion from each flask was collected in a 2-ml eppendorf tube and centrifuged at 10,000 rpm for 10 min. The supernatant was collected for lipase and phytase activities and were expressed in U/ml of the sample. Similarly, 1 ml fermented sample from each flask was collected for the evaluation of microbial load. Total microbial load after submerged fermentation was estimated by standard plate count method described by Goldman and Green (2008) and represented as CFU/ml of sample. The seed cake material after fermentation was dried and used for estimation of toxins, anti-nutrients and protein.

2.4. Characterization of yeast

Based on the enzyme activity and ability to detoxify the toxins and anti nutrients of *Jatropha* de-oiled seed cake, yeast isolate OBB9 was selected and characterized by sequencing its 5.8S rRNA (Bhima et al., 2010). The genomic DNA was extracted using an Insta Gene Matrix (BIO RAD, California, USA) and amplified using universal primers (5′-TCCGTAGGTGAACCTGCGG-3′ and 5′-TCCTCCGCTTA TTGATATGC-3′). The PCR conditions used for amplification of DNA were denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and final extension step at 72 °C for 10 min. The amplicons were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA) and sequenced using PRISM BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, California, USA). The amplicons were added to Hi-Di

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