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PRODUCTION OF MICROBIAL ENZYME TRIACYLGLYCEROL ACYL HYDROLASES BY *ASPERGILLUS SYDOWII* JPG01 IN SUBMERGED FERMENTATION USING AGRO-RESIDUES

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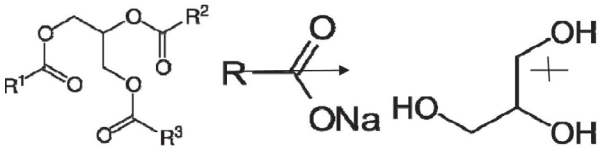
Keyword-Enzyme, Triacylglycerolacylhydrolase, Agro-residue, Soybean husk.

Abstract – Agriculture waste is one of the major concerns to environmentalist worldwide for a long time. This research work carried out with objective to utilize these agro-residues for production of the microbial enzyme. In this study wheat straw (WS), soybean husk (SH), barley straw (BS) and corn stover (CS) are utilized as a substrate for production microbial enzyme triacylglycerol acylhydrolase by *Aspergillus sydowii JPG01.* The comparative study of agro-residues indicates thatthe suitability order of agriculture waste for the enzyme production as SH>WS>CS>BS. Hence Soybean Husk is the better substrate in comparison to the other.

INTRODUCTION

Lipase (Triacylglycerol acylhydrolase, EC 3.1.1.3) are commercially important enzyme using in dairy product, detergent, biodiesel and textile industries. The synthesis, characterization and utilization of enzyme started in ancient time. Among 4000 known enzymes, only around 200 enzymes were used for the commercial purpose (Sharma *et al.,* 2001). Lipase is a type of hydrolytic enzyme utilized as commercial enzyme worldwide for long times. It hydrolyzes during adsorption at the interface of oil-water and remains nonreactive in the bulk fluid (Martinelle *et al*., 1995). The various lipases are active in an organic solvent and catalyse esterification (Chowdary *et al.,* 2001; Hamsaveni *et al.,* 2001; Kiran *et al.,* 2001; Kiyota *et al.,* 2001; Krishna and Karanth, 2001; Krishna *et al*., 2001; Rao and Divakar, 2001). It is utilizing under various purposes like organic synthesis, fats (hydrolysis and modification) and improvement in the flavour of food during processing, analyzing the racemic mixtures and various another organic compounds (Sharma *et al.,* 2001).

It is widely available in animals, plants and microorganism; it hydrolyses triacylglycerol into



glycerol and acids (Parashar *et al*., 2018; Sahu and Martin, 2011; Bornscheuer, 2002; Gupta *et al.,* 2004). It is more stable than other similar types of enzyme. The microbial triacylglycerol acyl hydrolase considered better due to even effortless cultivation gives good yields (Hasan *et al*., 2006). The new methodology always preferred for the commercial production of triacylglycerol acyl hydrolases. Triacylglycerol hydrolase precisely a serine hydrolase which is an industrially relevant enzyme, thus studied widely within the last few decades. The various researchers suggested lipase production through various industries like agriculture waste (Salihu *et al*., 2012), vegetable (Watanabe *et al*., 2000), milk (Sorhaug and Stepaniak, 1997) and oil-contaminated clay (Sirisha *et al*., 2010). In this study, lipase production was carried out through agro-residues.

The biodiesel production from vegetable oil by chemical catalysis using transesterification reaction

has disadvantages, of the accumulation of soap. It occurs because of the availability of the free fatty acid oils at a higher temperature. Microbial lipases offers a wide range of activities such as tolerance to high temperature, pH and solvents with substrata specificity feature. Although lipases have low catalytic efficiency compared to the chemical catalyst, also have little water and energy requirements. Further application of immobilization methods, chemical modification and protein engineering can make them superior in the catalytic conversion transformation process (Tyagi and Gupta, 1998; Aires-Barros *et al*., 1991).

Enzymes are colloidal organic protein catalysts produced by viable cells having the ability to catalyze reactions independently in the absence of cellular activity (Rao *et al*., 1993). Microbial enzymes can be classified according to their field of application into three major categories (a) to synthesize useful compounds (b) to stereo specifically carry out important bioconversion reactions and (c) able to hydrolyze polymers into monomers (Rao *et al*., 1993).

MATERIALS AND METHODS

Medium and reagents

Czapek-Dox- Glucose-Coconut oil media was used as a basal medium for lipase production. The composition of medium defined as $NaNO_3$ (0.25% w/v), KCl (0.05% w/v), KH_2PO_4 (0.10% w/v), $MgSO_4$ (0.05% w/v), glucose (5.00% w/v) and coconut oil (10.00% v/v). The various buffer solutions were prepared in water for the experimental requirement. All the reagents were prepared in distilled water. Lipase assay solution comprised of solution A and B. The sol. A contains 0.04 g of the *p*-NPP in 12 mL of 2-Propanol, while sol. B prepared by 100mg of Acacia Gum and 400mg of Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$) in the 90 mL of distilled water.

Agro-residues namely wheat straw (WS), soybean husk (SH), barley straw (BS) and corn stover (CS) were obtained from local food-grain mills of Guna, MP, India (Srivastava, 2019).

Inoculum preparation

The preparation of inoculum was started by using old culture slant. In this process 10 mL sterile distilled water added in old (~3 days) culture slant and cell suspension developed by using a sterile loop. Around 100 mL inoculum medium used for 1 ml of the cell suspension (1.9-2.2 x 10^8 CFU/mL) for subsequent fermentation. The pour-plate count technique was used to assess the number of viable pores in inoculum medium.

Submerged Fermentation

In this study, enzyme production were carried out by submerged fermentation (SmF). It was performed by utilizing basal medium containing (g/ 100mL) K_2HPO_4 0.1; $(NH_4)_2SO_4$ 0.1; NaCl 0.1; $MgSO_4.7H_2O$ 0.1, pH 7.0. In the primary stage agriculture waste (with different particle size)were tested in a basal medium at the 1% (w/v). The culture containing 100 mL samples kept in the autoclave at 121 °C for the 20 min. at a pressure of 15 lbs and the further solution cooled down to room temperature. After this process, the medium was inoculated with 1% inoculum and further incubated at 303.15 K for the period of 48 h. The Whatman filter paper NO 1 used for the filtration of fermented biomass. All these centrifuged at 5000 g for 20 min. at 277.15 K. A 15 litre aerobic bioreactor was used in the experiment sterile air supply was maintained at 5/ minute, pH was maintained at 7.5, the temperature was maintained at 30 °C. Supernatant used as enzyme solution and stored at 4 °C till further use. Residual solid material kept in the oven till achieved constant weight and weighed for further calculation.

Partial purification of the enzyme was done using precipitation by $(NH_4)_2SO_4$ at various concentrations up to 90% saturation under stirring at 4 °C for 1 h. The precipitate received after centrifugation and dissolved into 10 mL of Tris- HCl buffer solution. Precipitated enzyme further purified by dialysis with the same buffer for 24 h and used for enzyme assay.

Determination of enzyme activity

The activity of the enzyme was assessed through UV/Vis Spectrophotometer at 410 nm using p-NPP as a substrate (Garlapati and Benerjee, 2010). All analysis was carried out in triplicates.

RESULTS AND DISCUSSION

Strain Characterization

In this study, almost complete 16S rRNA gene sequence (1150 nucleotides) for strain JPG01 was assessed. This sequence was subjected to similarity searches against public databases to infer a possible relationship of strain. It was analyzed from this study that the strain JPG01 a member of the genus

Table 1. Composition of lignocellulosic biomass % dry weight. Lipase activity by *Aspergillus sydowii* JPG01 in presence of agroindustrial residues in basal medium.

Agriculture Residue	Cellulose	Hemicellulose	Lignin	Protein	Lipase Activity (U/mL)
Soyabean husk	33	14	-	5	128
Corn stover	38	26	19	5	113
Wheat straw	38	29	15	4	120
Barley straw	42	28	-	7	105

Aspergillus. The comparative analysis of 16S rRNA gene sequence yielded following closest fits: 98.28% (25 nucleotides differences out of 1492) *Aspergillus sydowii.* It is apparent from the genotypic data that strain JPG01 forms a distinct center of taxonomic variation within the genus *Aspergillus*. The taxonomical and Biochemical studies were carried out for this microorganism to be identified as the novel species of the *Aspergillus*.

Selection of substrate particle size

In submerged fermentation substrate particle size also plays an important role as based on morphology and growth characteristics problems may arise in proper oxygen transport through aeration and clogging may occur. Using different sieve sizes highest lipase production occurred using the small particle size of BSS-85 sieve; it is important to observe that the lowest enzyme activity was observed when optimum size BSS-25 sieve is used, this suggests that small particle size offers more surface area for fungi to grow (Fig. 1).

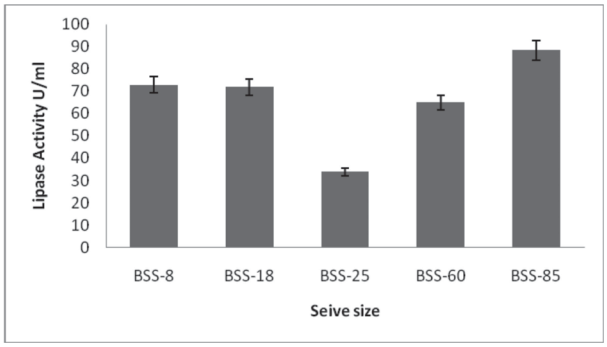


Fig. 1. Effect of sieve size on the triacyl hydrolase activity

Production of lipase by *Aspergillus sydowii* JPG03

In this study for the identification of suitable agriculture waste for the lipase synthesis through *Aspergillus sydowii JPG01*, the fermentation process has been carried out along with 1 % (w/v) wheat straw (WS), soybean husk (SH), barley straw (BS) and corn stover (CS) individually for each in place of soybean husk (SH). The results of the above study exposed that the suitability order of agriculture waste for the enzyme production as SH> WS> CS> BS(Fig 2). The cellulose and hemicelluloses contents are given in Table 1. The results indicate that soybean husk was comparatively suitable for lipase production (128 IU/mL) by *Aspergillus sydowii JPG01* (Fig 2). This study results also suggest that agriculture residue can be directly used for lipase production. The results indicate the production of lipase significantly connected to the optimum content of soybean husk and wheat bran in an approximate ratio of 3:01 ratio (Fig 3). It occurs because of preference in consumption of cellulose with protein during metabolic activities by

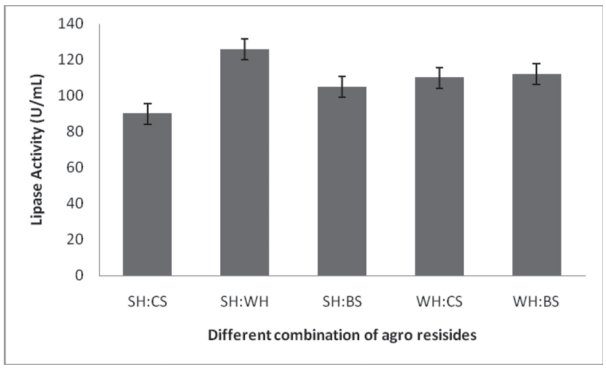


Fig. 2. Triacyl hydrolase activity in different agro-residues combination

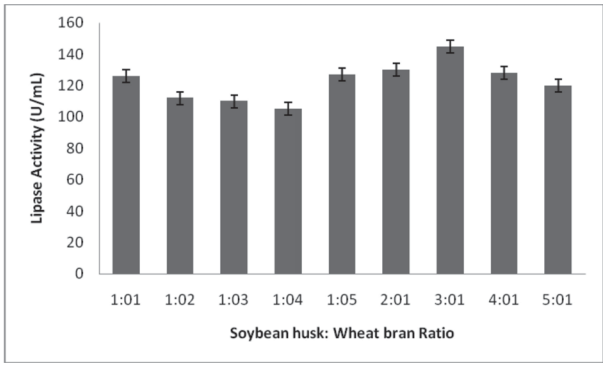


Fig. 3. Effect of Soybean Husk and Wheat bran ratio on triacyl hydrolase activity.

Aspergillussydowii JPG01.

CONCLUSION

Agricultural residues from agricultural activity, abundant in nature especially in agriculture based country like India. These residues usually burned to pose serious environment problems for a long time. This study concludes that agriculture waste has enormous potential for various useful byproducts. This study also finds that agriculture waste can be used for the single-step production of the lipase enzyme through the newly isolated strain of *Aspergillus sydowii JPG01* in SMF. The blend of soybean husk and wheat bran in a proportion of 3:1 was identified as the best substrate support in SMF under the laboratory level fermentations. The results concluded that the enzyme activity could be approached up to 145 (U/mL) on the substrate (carbon source) formed due to the blend of rice bran and wheat bran (3:01). The utilization of low-cost agricultural residue for triacyl hydrolase production seems to be a viable less-cost production of the industrially relevant enzyme. It is an attractive concept for the utilization of agriculture waste for the production of an industrially valuable enzyme.

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