

**EFFECT OF SOLID SUBSTRATE AND MOISTURE CONTENT ON PRODUCTION OF FUNGAL LIPASE IN SOLID STATE FERMENTATION**

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**ABSTRACT**

The present study has been designed to investigate the effect of solid substrates and moisture contents on the production of extra cellular lipase enzyme. High yield of enzyme activity 281.70 U/gss was obtained with rice bran and minimum activity 162.37 U/gss was observed with coconut cake. In such case of moisture content maximum lipase production was obtained 365.00 U/gss with 5ml moistening whereas minimum was obtained at 3ml moistening solid substrate. The study will be helpful in defining moisture content and solid substrate for higher production of lipase which are helpful in industries for commercial applications.

Figures : 02

References : 20

Table : 00

KEY WORDS : *A. blakesleeana*, Lipase, Moisture content, Solid State fermentation, Solid substrate.

**Introduction**

Lipases are ubiquitous enzymes of considerable physiological significance and industrial potential<sup>6</sup>. Due to the versatility of the molecular structure and catalytic properties, these enzymes have potential application in different industrial sectors such as food, waste water treatment, cosmetics, oleochemical, pharmaceuticals, detergents<sup>4</sup> and in the fuel sector, which applies lipase as catalyst for synthesis of esters and for transesterification of the oil for the production of biodiesel<sup>13,18</sup>. Cost effective large scale applications are made possible by the capacity for producing novel enzymes in large quantity through biotechnology<sup>8,11,12</sup>. Lipases are produced by animals<sup>15</sup>, plants<sup>20</sup> and microorganisms<sup>3,8</sup> but only microbial lipases are commercially significant<sup>5,14</sup>. Lipases can be used to accelerate the degradation of fatty wastes<sup>9</sup> and polyurethane<sup>17</sup>. In the last decades, the interest in microbial lipase production has increased. Solid state fermentation (SSF), a current development in biotechnology holds tremendous potential for the production of enzymes certain valuable chemicals, fungal toxins by using agricultural wastes such as rice bran, wheat bran, maize bran, soybean bran etc<sup>1,2,7,10,19</sup>. These substrates provide a rich and complex source of nutrients, which may or may not need to be supplement. Such substrates selectively support mycelial organisms<sup>16</sup> which can grow at high nutrient concentrations and produce a variety of extracellular enzymes.

In this paper, the effect of moisture contents and different solid substrates for higher production of lipase has been studied. It can be seen from the result that rice bran was the best solid substrate for isolate *A. blakesleeana* with lipase activity of 281.70 U/gss. Further addition of 5 ml of moistening medium for each 5 gram of solid substrate gives optimum maximum production of lipase (365.00 U/gss) at 96 h

**Materials and Methods**

Lipase producing fungi were isolated by enrichment culture technique using glucose – yeast extract - peptone (GYP) medium (pH 6.5) and incubated at 37°C for 4 -6 days. Evaluation of fifty isolates for their lipolytic activity was carried out by point inoculation on a Tributyrin Agar plate. The pH of the medium was adjusted to 6.5 and was autoclaved at 15 psi for 15 minutes.

The inoculated plates were incubated at 37°C±1°C and observations were recorded upto 240 h at the regular intervals of 24 h. Spores of fungi were harvested from the slant (96 h old culture) by adding 5 ml of sterilized distilled water. The slants were vortexed and conidial suspension thus obtained was filtered to ensure the absence of any hyphal fragment.

**Effect of solid substrate**

5ml of mineral growth medium was dispensed in 250 ml Erlenmyer flask, containing 5g of rice bran as solid substrate. The flasks were sterilized and inoculated with approximately 5x10<sup>7</sup> spores/ml which were counted

by using hemacytometer (Neubauer, Feinoptik, and Kenburg). Flasks were incubated in bacteriological incubator at 37°C. The samples were harvested after every 24 h up to 240 h. The effect of different solid substrates (wheat bran, rice bran and coconut cake) for production of lipase was studied. The flasks were inoculated with test organism and incubated at 37°C. The enzyme activity was determined at the end of incubation period.

#### Effect of initial moisture content

Various initial moisture content values from 3-8 ml/ 5 g of solid substrate were tested for the lipase production.

### Results and Discussion

Fifty fungal strains isolated from different habitats were screened for lipase production on Tributyrin Agar plate at 37°C. Among these isolates only 30 showed lipolytic activities. All the strains demonstrated diverse level of clear zone around the colony. On the basis of

diameter of clear zone, isolates were selected for further study. The isolated culture was identified as *Absidia blakesleeana* at Indian Type Culture Collection division of plant pathology, Indian Agriculture Research Institute, Pusa, New Delhi, India.

#### Effect of solid substrates

Influence of different solid substrates on lipase production was studied and the results are presented in Fig. 1. It is clear from the result that rice bran was the best solid substrate for isolate *A. blakesleeana* with lipase activity of 281.70 U/gss.

#### Effect of initial moisture content

*A. blakesleeana* was grown at various initial moisture content values from 3 to 8 ml under the condition optimized. Maximum lipase production 365.00 U/gss was observed at 5.0ml moisture content. A good amount of lipase activity was also obtained even at 6.0 ml moisture content. Further increase in moisture content did not

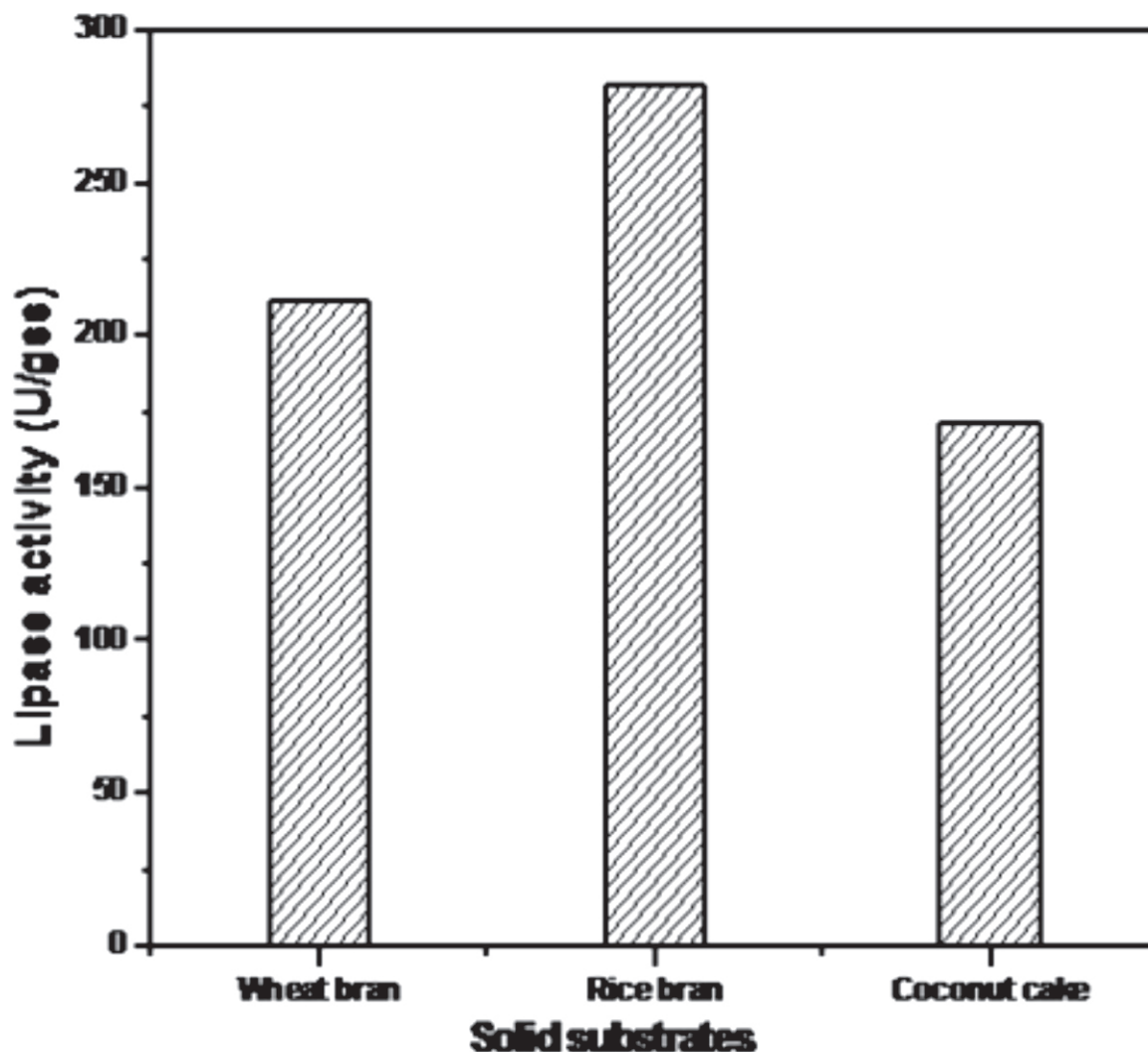


Fig.1 : Effect of different solid substrate on lipase production at temperature 37°C and pH 6.5

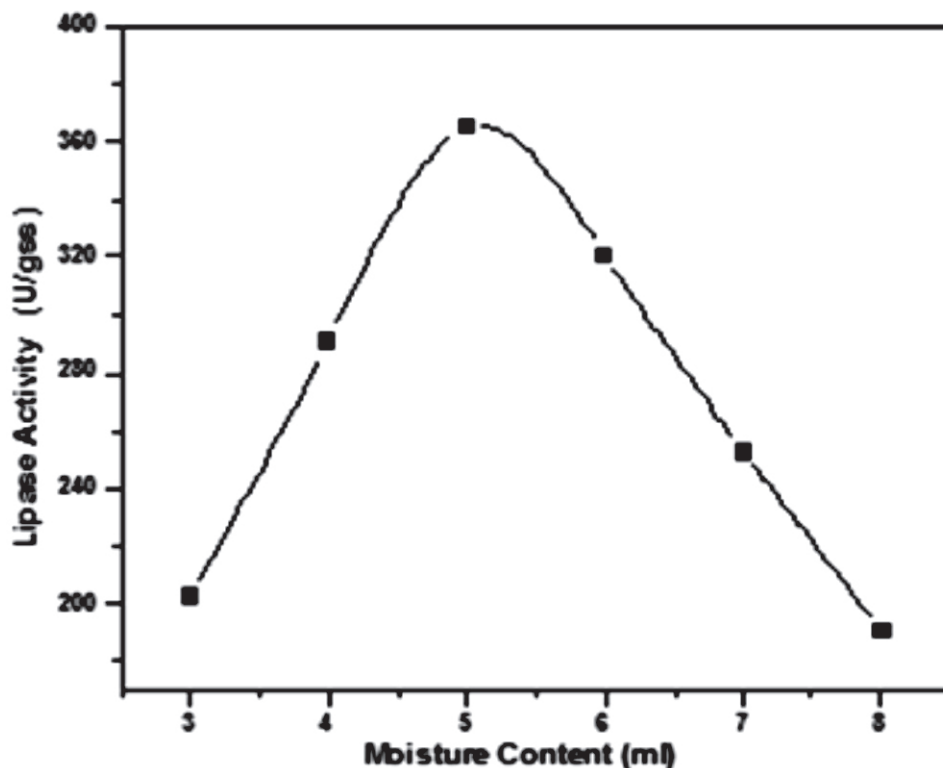


Fig.2 : Effect of moisture content on lipase production at temperature 37°C and pH 6.5

support lipase production (Fig. 2.).

From the result it was observed that addition of 5 ml of moistening medium for each 5 gram of solid substrate was optimum to obtain maximum production of lipase (365.00 U/gss) at 96 h. For this reason it can be demonstrated that the lipase obtained using SSF is more concentrated, making it an attractive feature from an economic standpoint.

### Conclusion

The effect of different solid substrates and moisture contents on the production of extra cellular lipase enzyme has been investigated. The enzyme activity 281.70 U/gss were obtained with rice bran which is high as compared to the minimum activity 162.37 U/gss with coconut cake. Among different initial of moisture contents, maximum lipase production 365.00 U/gss were obtained with 5ml moistening per 5 g.

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