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RESEARCH ARTICLE

Isolation of novel gras fungi for phospholipase A_2 and fermentation studies

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Abstract

The aim of the present research work was to identify a Novel GRAS Fungus for Phospholipase A_2 and conduct fermentation studies. The soil samples were subjected to standard microbiological methods for the isolation of individual Fungus colonies. The strain MRL 85 strain show 3.63 units/ml and 7.84units/ml of phospholipase activity in SSF and SMF. The fermentation studies showed that the enzyme activity was higher in Submerged Fermentation than the Solid State Fermentation. It has been proved that the strains were PL producer.

INTRODUCTION

Phospholipase A_2 is one of the most intensively studied membrane proteins which hydrolyze phospholipids at the sn-2 position to form fatty acid and lysophospholipids products. Solid state fermentation, Submerged and are age-old techniques used for the preservation and manufacturing of food products. Solid state fermentation, Submerged and are age-old techniques used for the preservation and manufacturing of food products.(**Gwen Falony** *et al* ., 2006). Compared to submerged fermentation processes, solid state fermentation is more cost-effective by using smaller vessels, less water consumption, reduced wastewater treatment costs and lower energy consumption **(Durand,** 2003 ; **Pandey** 1999**).**

Materials and Methods

Oil contaminated areas (**Dharmsthiti & Luchai** 1999), (Kanwar *et al.,* 2002) (Labuschange *et al.,*1997) (**Adhinarayana** *et al.,* **2004**) were selected to pick out the Phospholipase producing Fungi. Samples were mixed together and the quadrant part of the sample was taken for the serial dilution as per the standard microbial methods.

Media for culture

These samples are grown on selection and screening media composed of soya lecithin, rhodamine (**Wang** *et al* .,1995) as an indicator for the deduction of phospholipase activity and selective antibiotics. The plate was prepared by standard microbiological methods. The pH of the media is checked and adjusted to 6.5.(**Sarat Babu Imandi,** *et al* .,2010).

Observations

Around 93 colonies are enumerated in this study. Based on the physical investigation for the appearance of the halo like regions that diffuse on rhodamine, then its disappearance suggested the possibilities to shortlist single strain for the production of PL.

It was observed that the strains MRL 85 producing the halo like appearances that enhances the disappearance of rhodamine layer by diffusion in the selection medium that contains lecithin from soya. MRL85 was stained by using saffronin. The stained slide was observed under microscope and the image was captured.

Solid State Fermentation:

Inoculum development

The broth media was prepared and the culture was inoculated in seed flask. Then the flasks were incubated for 2-3 days at room temperature. After incubation it was submerged into the production media.

Koji Harvest:

The solid state fermentation was done by using wheat bran, an agro industrial waste (**Ashok , 2003**). Koji was prepared as follows, 15 gm of wheat bran was weighed separately. To this each plate 3 ml of deionized water was added and mixed thoroughly. Exactly 15 gm of this Koji mix was weighed and packed in the sterile plates. The plates were autoclaved at 121^0C at 15 lb for 30 minutes. Then 10 ml of inoculum was added in the Koji plates under Laminar Air Flow chamber. The plates were labeled and kept for incubation. It gives a mass of microbial growth after two days. (Microcore Research Laboratory India, Erode).

Submerged Fermentation

The inoculum was added in broth and kept for agitation at 265 rpm at 27^0C for 5 days. Soya lecithin 1.5(g/L), Glycerol 1.5(g/L), Sunflower oil 7(g/L), Soya peptone 2(g/L), $MgSo_4$ 0.05(g/L), NaCl 0.05(g/L), $CaCo_3$ 0.1(g/L) was used as phospholipid and carbon source respectively.

Then the crude enzyme was separated from that colonies by centrifuge at 5000 rpm for 10 minutes(**Gwen Falony 2006**).

Biomass for phospholipase assay

The 3g of biomass was taken from SSF. It was ground in 25 ml Deionized water and filtered through muslin cloth. It was centrifuged at 5000 rpm (**Adhinarayana , 2004**) (**Sarat Babu Imandi ,2010**) (**K.S.S. Rekha ,2012**) . Supernatant, the crude enzymes from SSF and SMF were taken for phospholipase assay.

PhospholipaseA2 assay

Substrate preparation for assay (for 200ml)

By emulsion method 1g of phospholipid source soya lecithin was added to 100ml of deionized water then vortexes (**K Adhinarayana**) (**Mustranta A**, 1992); 25ml of 0.32M $CaCl_2$ was added. The pH was maintained at 7 by using 0.5M KOH. Then 50ml of Triton X-100 (**Dennies,1973; Al-Asheh** & **Duvnjak**, 1994; **Ebune** *et al*.,1995; **Yu** *et al* 1990; **Sharma** *et al* 2001) and 25ml of 0.003M Citric acid was added and vortexes. (Microcore Research Laboratory, Erode, India.)

Assay procedure

25ml of substrate was taken and the temperature of substrate was maintained at 40^0C for 1 minute. By adding of 0.01M KOH the pH was adjusted to 9. Then 100µl of crude enzyme was added. Now there was drop in pH. The pH was adjusted to 10 by using 0.01M KOH. It was noted that required time to adjust the pH from 9 to 10 and the amount of KOH to be taken. By using this formula the activity of PLA_2 units/ml was calculated.

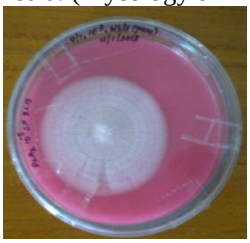
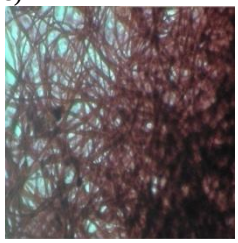
$$\text{Units/ml} = \frac{\Delta\ (\text{Volume of KOH}) \times \text{Concentration of KOH} \times 10}{\Delta T \times CS \times V}$$

CS- Concentration of substrate (0.5ml)
V – Volume of substrate
ΔT –Difference in time interval
Δ - Different volume of KOH consumed

Result and Discussion

Microscopic observation

Morphology discription of MRL 85 was found to be a *Aspergillus* species spongy like structure in culture media. (Mycology online)

Activity in SSF

The MRL 85 shows high activity. The success of SSF depends on several factors such as temperature, pH, substrate moisture, aeration, and inoculums concentration, type of substrate and microorganism species. The MRL 85 shows 3.63 Units/ml phospholipase activities.

Activity in SMF

The enzyme activity of MRL 85 in SMF was 7.84 Units/ml. The enzyme activity in SMF was higher than the SSF. The reason is the SMF contains the high nutritive media than the SSF. The phospholipase was propagated in lab scale solid state fermentation and submerged fermentation. In lab scale solid state fermentation the agro-industrial waste wheat bran was used as substrate. The higher activity was observed in MRL 85 strain that shows 3.63 units/ml and 7.84units/ml of phospholipase activity in SSF and SMF. It was proving that the strains were Phospholipase producer.

Conclusion

The fungi isolated from oil contaminated areas were produced phospholipase A_2. The enzyme produced by SSF by using wheat bran without any other source shows high activity 3.63 Units/ml. In submerged fermentation the activity is 7.84 units/ml due to the sources added.

Due to the fact that this microorganism was generally recognized as safe for food, brewing and pharmaceutical application, more research is necessary to optimize the fermentative process in order to obtain higher phospholipase production through this strain.

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