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Exploration of newer substrate for fibrinolytic enzyme production by solid state fermentation using *Penicillium chrysogenum* SGAD12

Authors:
Gopinath SM,
Suneetha TB,
Ashwini patil GM.

Institution:
Department of Biotechnology, Acharya Institute of Technology, Soldevanhalli, Bengaluru-560090, Karnataka, India.

Corresponding author:
Gopinath SM

Email:
gopinath@acharya.ac.in

ABSTRACT:

Rice chaff - a polished substance extracted from *Oryza sativa* L. cv. Devamallige was used as a novel substrate for the production of the fibrinolytic enzyme. This vital enzyme is used in thrombolytic therapy, as a clot buster. The production was done by solid state fermentation of rice chaff by *Penicillium chrysogenum* SGAD12, locally isolated from vegetable markets. Of the 28 strains isolated and screened, *Penicillium chrysogenum* SGAD12 was found to give an inhibition zone greater than 2 mm. Hence it was identified as the potential organism showing maximum fibrinolytic activity under specified culture conditions. Activity optimization was done under the parameters: Time, Inoculum ratio, Moisture, particle size. The fibrinolytic activity was favourably maximized at 104 hrs, 7% (v/v) inoculum ratio, 35-45 % (v/w) moisture content and 500µm particle size.

Keywords:
Rice chaff, Fibrinolytic enzyme, Solid state fermentation, ***Penicillium chrysogenum*** SGAD12.

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INTRODUCTION

Now-a-days thrombolytic diseases are responsible for significant incapacitation and morbidity. Fibrinolytic enzymes have apparent significance in thrombolytic therapy in human being (Haber et al, 1989). Despite most of their wide spread use, the currently available fibrinolytic enzymes have a number of significant limitations (Collen and Lijnen, 1991). Therefore great attention has been directed towards a search of thrombolytic agents of various orgins, particularly through microbial activity.

Fibrinolytic enzymes occur in bacteria, earthworm, and snake venom, from fermented foods (Collen and Lejnin, 1993) (Sumi et al, 1987), but upto now there have been very few evidences for production of fibrinolytic enzyme from fungi (S. A. El-Aassar et al, 1990)(Sun Tao et al, 1997). Solid state fermentation (SSF) process indicates significant difference in comparision with submerged state fermentation (SMF). Its main advantage is that it is a simple technique, it utilizes less amount of water, and it has a low operating cost and high productivity. Fungi have been widely utilized by SSF in the production of enzyme (Lonsane et al, 1992) (Pandey, 1992). This study reports the production of fibrinolytic enzyme by *Penicillin chrysogenum* SGAD12, locally isolated from vegetable markets and new substrate rice chaff extracted from *Oryza sativa* L. cv. Devamallige collected from local regions of Karnataka, India.

MATERIALS AND METHODS

Penicillium chrysogenum SGAD12, a fibrinolytic enzyme producer was isolated from soil of vegetable market and was identified. It was found to produce fibrinolytic enzyme on fibrin plate. This potential trait was used for further production on solid state fermentation. Culture was maintained on Czepek Dox Agar at 4°C and sub cultured fortnightly. The basic medium contained rice chaff (variety: Devamallige) (20g), KH_2PO_4 (0.5g), $MgSO_4.7H_2O$ (0.5g), $MnSO_4.7H_2O$ (0.001g), $ZnSO_4.7H_2O$ (0.002g), $FeSO_4.7H_2O$ (0.0005g) and 9 ml water. After autoclaving at 121˚C for 30 minutes, the medium was adjusted to a moisture content of 45% (v/w) and inoculum size of 7% (v/v). It was then inoculated with spore suspension of 10^6 spores/ml and incubated at 28˚C.

Enzyme Extraction

The fresh moldy pith in each flask was soaked in distilled water and incubated in a rotary shaker at 130 r.p.m at 28 ˚C for 1 hour. The extracts were obtained by filtering through filter paper. For 1 g of dry substrate taken 2.8 ml of filtrate was recovered.

Assay method

Fibrin plate analysis method

The original fibrin plate method (Astrup and Mullertz, 1952) with slight modification was used for measurement of the fibrinolytic activity of the test preparation along with streptokinase. Petri dishes containing 9 ml of 0.2% fibrinogen solution (pH 7.8) were placed on a horizontal glass plate. To each of these Petri dishes, 0.2 ml of plasminogen (10 units) was also added and mixed well. Clotting was induced by the addition of 0.2 ml of thrombin solution (20 units). In order to speed up the clotting process, the plates were incubated at 28 ˚C for 20 mins. Plates were prepared afresh every time. Known quantities of the enzyme solution and standards were placed as small droplets on the surface of the fibrin clot. The plates were incubated at 28 ˚C for 2 h and visually inspected for liquefaction. The area of the digested fibrin was considered as a quantitative measure of the fibrinolytic activity of the enzyme.

RESULTS AND DISCUSSION

Enzyme production profile

Time course

Good fungal growth was supported by rice chaff. The spores germinated within 20 h, giving rise to mycelium formation, whose density was seen to increase with time. Visual examination showed that the substrate was fully impregnated

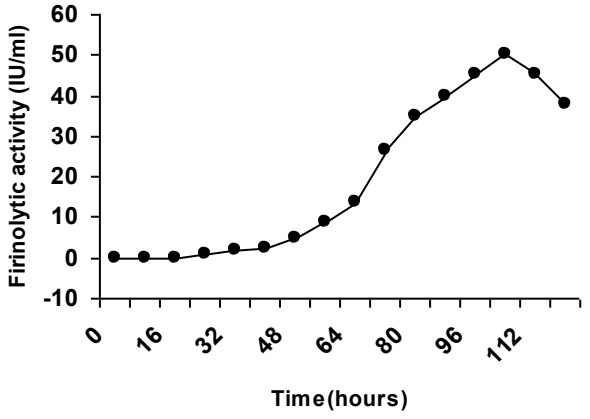

Fig. 1. Time course of enzyme production by *Penicillium chrysogenum* SGAD12 on rice chaff (variety: Devamallige). With fermentation temperature 28 ˚C, Inoculums size: 7% v/v. Moisture level: 45%, Particle size: mixture of sizes

with mycelium in about 40 h while the uninnoculated control plate showed no detectable change. During initial 24 h no fibrinolytic activity was seen, thereafter the enzyme activity increased reaching a maximum at 104 h. With further incubation the enzyme activity decreased.

Effect of Inoculum Ratio:

While optimization for inoculum ratio it was found that 7% to 14% (v/v) (based on the volume of mineral solution) elicited the best enzyme activity, with 7 %(v/v),as adopted for this experiment, giving the maximum result (fig 2). Any inoculum size beyond the optimal range showed lower activity. An inference can be drawn that larger inoculum sizes containing more amount of water led to decrease in the enzyme activity.

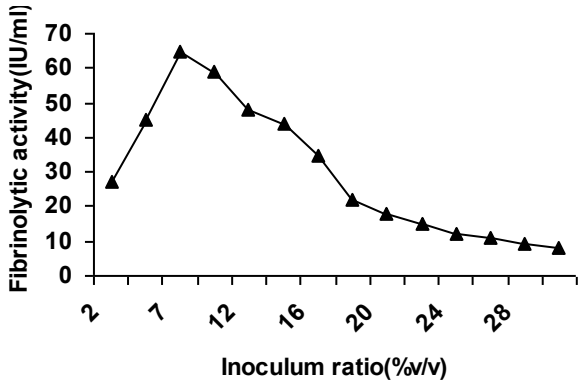

Fig. 2. Effect of inoculum size on production by *Penicillium chrysogenum* SGAD12 on rice chaff. (variety: Devamallige) Fermentation time: 104 hours. Temperature: 28 ˚C. Particle size: Mixture of different sizes.

Effect of moisture level:

Water has a profound effect on productivity and hence used in limited amount in solid state fermentation (Lonsane et al, 1992). Moisture level between 35% - 45% (v/w) (fig3) results in the maximum enzyme production. Any value beyond this was unable to give increase in production. A conclusion can be drawn that lower moisture level leads to dry culture, sparse growth and hence reduced production. Higher moisture concentration also creates an unsuitable environment for solid mycelium growth and hyphal diffusion, leading to less production of enzyme.

Effect of substrate particle size:

The effect of specific surface area is of high importance in solid state fermentation. Maximum production was obtained at a particle size of 500µm

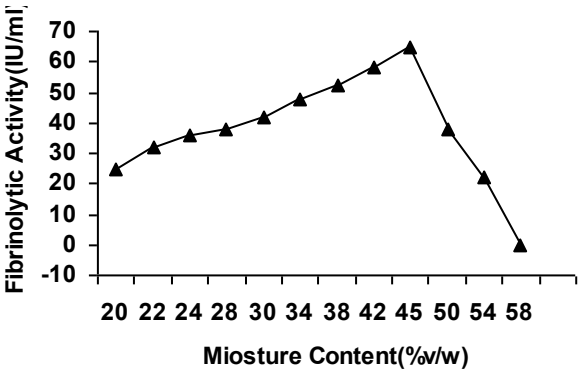

Fig. 3. Effect of moisture level on the productivity by *Penicillium chrysogenum* SGAD12on Rice chaff (variety: Devamallige). Fermentation time: 104 hours. Temperature: 28 ˚C. Particle size: Mixture of all sizes.

(fig4). Through visual observation it was inferred that larger particle size provided less surface area hence productivity was less. Whereas significantly smaller particles though gave large surface area but the porosity was decreased to an extent that the filamentous fungi could not reach deep inside to the substrate particles leading to decrease in production.

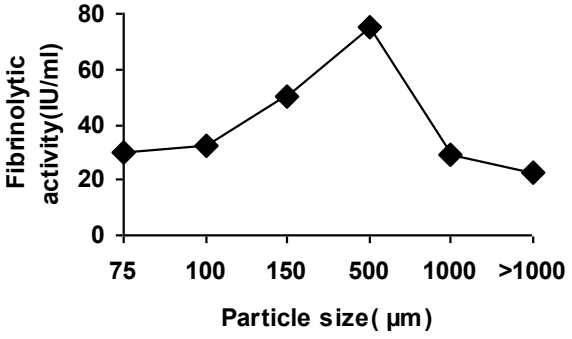

Fig. 4. Effect of substrate particle size on production by *Penicillium chrysogenum* SGAD12. Fermentation time: 104 hours, temperature: inoculum size-7% (v/v), moisture content 45 %(v/w).

CONCLUSION

The above work indicates that Rice chaff (variety: Devamallige) can be used as a potential substrate for production of economically important fibrinolytic enzyme with *Penicillium chrysogenum* SGAD12. This substrate is easily available and economically feasible. *Penicillium chrysogenum* SGAD12 was identified as the potential organism

showing maximum fibrinolytic activity under specified culture conditions. Time, Inoculum ratio, Moisture content, Particle size; were found to be ideal parameters for the activity optimization. The fibrinolytic activity was favourably maximized at 104 hrs, 7% (v/v) inoculum ratio, 35-45 % (v/w) moisture content and 500µm particle size.

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