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Short Communication

SOLID STATE FERMENTATION OF RICE CHAFF AS SUBSTRATE (VARIETY: DEVAMALLIGE) FOR FIBRINOLYTIC ENZYME PRODUCTION BY *PENICILLIUMCHRYSOGENUM* AGSF16

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ABSTRACT
Rice chaff (Variety: Devamallige) is used as the substrate for the production of fibrinolytic enzyme by *Penicilliumchrysogenum* AGSF16 in solid state fermentation. The optimized moisture content of the medium is 35-45 %(v/w) an inoculumof 5-10% (v/v) and an average particle size of 400µm of the substrate were optimum for productivity and enzyme activity of 65-70 IU per ml filtrate was achieved after 96 hours of fermentation.The enzyme exhibited similar fibrinolytic activity as that of streptokinase on fibrin plates that were devoid of plasminogensuggesting that its fibrinolytic action is independent of plasminogen and it is not a plasminogen activator.

KEYWORDS: *Penicilliumchrysogenum* AGSF16, Rice chaff, fibrinolytic enzyme, Solid state fermentation, enzyme activity.

INTRODUCTION

Thrombolytic diseases are today a major cause of morbidity and mortality. Fibrinolytic enzymes have apparent significance in thrombosis therapy in man.Accounts of cardiovascular diseases has become the leading cause of death in the Western world (Haber et al, 1989). Many blood clot-dissolving agents, such as urokinase, streptokinase, and tissue plasminogen activator (t-PA), have been utilized in clinical treatments for cardiovascular diseases. Hemostasis is a complex process obtained through an optimal balance between bleeding and blood clot formation (Collen and Linjen, 1991). In an unbalanced state, fibrin clots may not be lysed resulting in thrombosis. Thrombolytic agents from various sources have been extensively investigated. Enzymes, such as urokinase, streptokinase and tissue plasminogen activators have been widely used in the treatment of thrombosis. However, these enzymes are often expensive, thermolabile and can produce undesirable side effects. Fibrinolyticenzymes occur in bacteria, earthworms and snake toxin, from fermented foods (Collen and Lejnin, 1993) (Sumi *et al*., 1987) but up to now, there have been few productions of fibrinolytic enzymes from fungi (S.A.El-Aassar et al, 1990)(Sun Tao et al, 1997).Solid state fermentation (SSF) has numerous advantages and by using rice chaff showed significant result(Gopinath et al, 2011). It can produce concentrated enzyme solutions, it is an alternative cultivation system for the production of high-cost microbial products and fungi have been widely utilized in SSF (Lonsane et al, 1992) in the practical production of enzyme or chemicals. In the present communication, we report the production of fibrinolytic enzyme by *Penicilliumchrysogenum* AGSF16 in SSF.

MATERIALS AND METHODS

Microorganism and growth conditions *Penicillium chrysogenum* AGSF16, a fibrinolytic enzyme producer, was grown in 300 ml flasks containing solid medium. The basic medium contained: rice chaff 20 g, (NH4) 2SO4 0.3 g, KH2PO4 0.2 g, CaCl2 0.05 g and 10 ml tap water. After autoclaving at 12°C for 30min and adjusted to different moisture levels by adding sterile water, the medium was then inoculated with a spore suspension (108 spores/ml) and incubated at 28°C.

Enzyme extraction

Fresh moldy pith from each flask was soaked in distilled water and shaken at 130 Rev min.–1 `28°C for 1 h. The enzyme extracts were obtained by filtering the mixtures through filter paper. For 1 g dry substrate taken, exactly 4 ml of filtrate was recovered.

Fibrin plate analysis method

Thrombin, 0.5 ml, (1.5 3 104 unit l–1 dissolved in 25 mM gelatin barbitoneNaCl (GBSB, pH 7.75)) was added to the mixture of 15 ml fibrinogen (0.85 g l–1, dissolved in 0.1 M phosphate buffered saline (PBS), pH 7.4) and 20 ml agarose(1%, also in 0.1 M PBS, pH 7.4). The above mixture was poured into 10 cm diameter petri dish forming the fibrin plates (Astrup and Mullertz, 1952). Wells with 1.5 mm diameter were cut into the fibrin plates and exactly 10 ml sample was placed in each well. After incubation for 12 h at 37°C, the fibrin was degraded to soluble low molecular peptides and ammonia acid resulting in translucent circles. The product of two perpendicular diameters of translucent circle was recorded (in square millimeters). The areas of translucent circles were converted into standard units by interpolation on a

reference curve of urokinase. The enzyme activities were expressed as IU per ml filtrate.

RESULTS AND DISCUSSION

The profile of enzyme production

The time course of enzyme production was explored. Rice chaff favoured good fungal growth. The spores germinated in about 20 h and were followed by the formation of mycelium, which gradually increased in density with time. Visual examination indicated that the mycelium impregnated the entire substrate in about 36 h, while uninoculated controls included in the study showed no detectable growth. Fibrinolytic activity was not detected during the first 24 h, thereafter; the enzyme levels increased reaching a maximum after 96 hours and gradually reduced with longer incubation times.

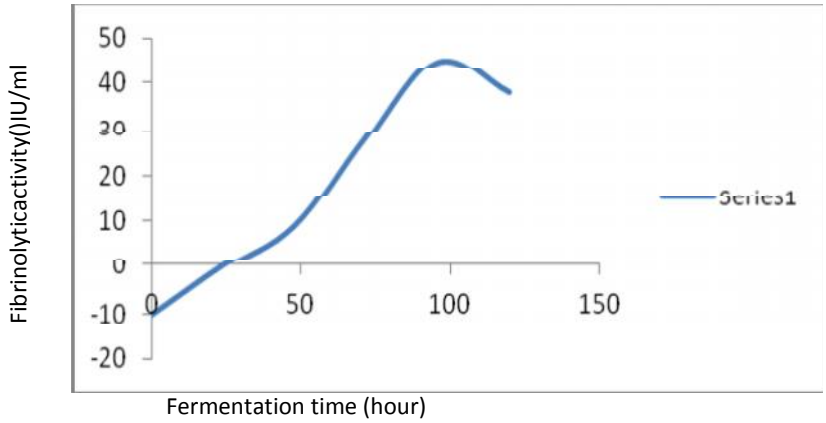

FIGURE 1: Time course of enzyme production by *Penicilliumchrysogenum* AGSF16 on Rice chaff (Variety: Devamallige). With fermentation temperature 31ºC, Inoculum size: 5%v/v, Moisture level: 40%, particle size: 400µm

The effect of inoculum ratio

Optimization of the inoculum ratio indicated that high enzyme productivity was obtained in flasks inoculated with 5% to 10% (v/v) inoculum (based on the volume of mineral solution), whereas enzyme activities were lower when inoculum sizes beyond this range. A conclusion may be drawn that high moisture content caused by large inoculum ratio could reduce productivity greatly. The inoculum size of 5% (v/v) was adapted in the following experiments.

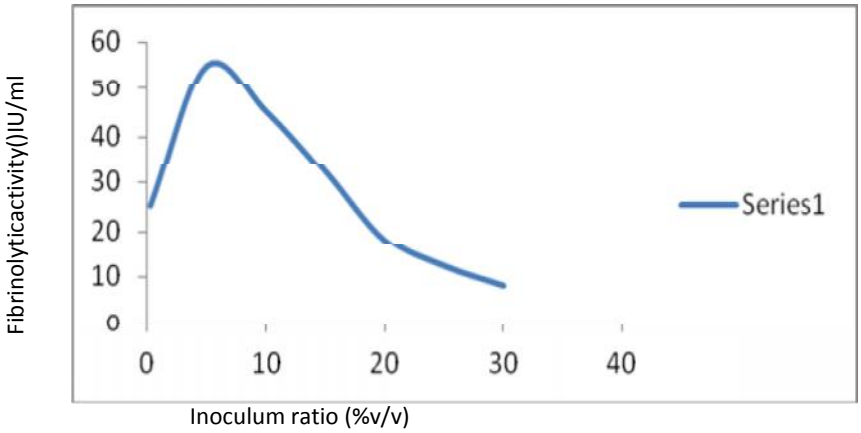

FIGURE 2 Effect of inoculum size on productivity by Penicillium chrysogenum AGSF16 on Rice chaff (Variety: Devamallige). Fermentation time: 96hours, Temperature: 31°C, particle size: 400µm

Effect of moisture level

Water is used only limited amounts in SSF and it has profound effects on the physicochemical properties of solids (Lonsane *et al,* 1992), which in turn affects process productivities. The correlation between enzyme production and moisture level was investigated and shown. Moisture level of 30–50% (v/w) resulted in maximal enzyme production after 4 days of fermentation. Beyond this range, there was no further increase in the enzyme level. The results indicated that a lower moisture level ledto dry culture, sparse growth with subsequent lower production of enzymes, while high moisture level decreased porosity, enhanced formation of liquid mycelium and created an additional barrier for hyphal diffusion into the substrate.

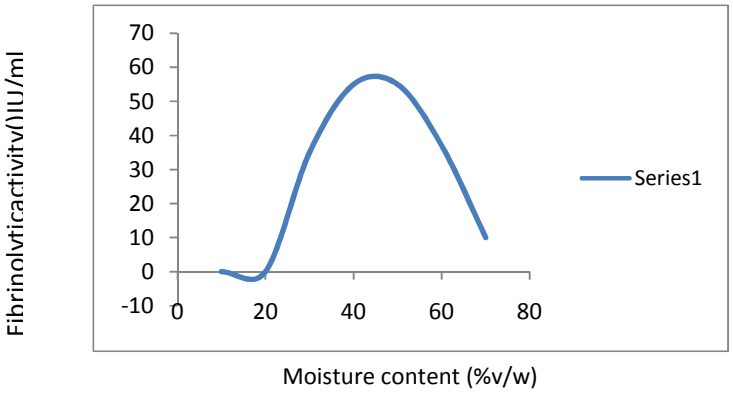

FIGURE 3: The effect of moisture level on the productivity of Penicillium chrysogenum AGSF16 on Rice chaff (Variety: Devamallige). Fermentation time: 96 hours, Temperature: 31°C, Particle size: 400µm.

Effect of substrate particle size

The effect of substrate particle size (and therefore the specific surface area) in SSF is important. Table 1 shows the effect of different particle sizes. Maximum enzyme productivity was obtained from 400 mm sized particles and was lower with bigger or smaller particles. With significantly smaller particles, the specific surface area was great but the porosity was less. The filamentous fungus could not penetrate deep into the pores and hence into the substrate particles. Visual observations showed that greater growth was stimulated but it was restricted mainly to the surface. With larger particle sizes, the growth was extended into the interparticle voids but the saturated surface area for growth was less and productivity was correspondingly less. The two opposing factors viz. decrease in surface area and increase in porosity may have compensated to approach a value corresponding to optimum growth and production at a particle size of 400 mm.

Table 1 Effect of particle size on productivity by *Penicilliumchrysogenum* AGSF16on Rice chaff (Variety: Devamallige)

Particle size(µm)	Fibrinolytic activity(IU)
50	20
75	25
100	46
200	58
300	66
400	69
500	60
600	26
Unsieved	Average [37]

Fermentation time: 96h. Inoculum size: 5 %(v/v). Moisture level: 40 %(v/w)

CONCLUSIONS

The present work indicates that Rice chaff (Variety: Devamallige), which is cheap and abundant, can be used as the substrate in solid state fermentation for the production of value added products such as fibrinolytic enzyme.

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REFERENCES

Astrup T and Mullertz S. 1952. ArchsBiochem. Biophys 40:346.

Cadroy Y, Haarkeer LA. Thrombosis. In: Antonaccio MJ, editor, Cardio vascular pharmacology, 3rd Ed. New York: Raven Press; 1990. p. 515.

Collen D. and Lijnen H.R. (1991), Blood 78, 3114–3124.

El-Aassar SA. 1990. Appl Microbial Biotechnol 33:26-30

Gopinath SM, Suneetha TB, Ashwinipatil GM. Journal of research in Biology (2011) 4: 242-245

Haber E., Quertermous T., Matsueda G.R. and Runge M.S. (1989), Science 243, 51–56.

Lijnen H.R. and Collen D. (1993), Biotech. Adv. 11, 115–30.

Lonsane B.K., Castaneda G.S., Raimbault M., Roussos S., Gonzalez G.V., Ghildyal N.P., Ramakrishna M. and Krishnaiah M.M. (1992), Proc. Biochem. 27, 259–273.

Muniswaran P.K.A. and Charyuln N.C.L.N. (1994), Enzyme Microb. Technol. 16, 436–440.

Ohno A., Ano T. and Shoda M. (1993), Journal of Fermentation and Bioengineering 75, 23–27.

Pandey A. (1992), Proc. Biochem. 27, 109–117.

Reddy DS. Newer thrombolytic drugs for acute myocardial infarction. Indian J ExpBiol1998; 36 : 1-15.

SumiH,Hamada H, Tsushima H, Mihara H and Muraki H. 1987. BirkhauserVerlag, CH-4010. Experentia 43.

Sun Tao Li Peng, Liu Beihui, Liu Deming and Li Zouhu. 1997. Biotechnology Letters, Li Peng, Liu Beihui, Liu Deming and Li Zouhu. Vol 19(5):465-467.

Sargantanis J., Karim M.N., Murphy V.G. and Ryoo D. (1993), Biotech. Bioeng. 42, 149–158.