

## Biomass and blastospore production in *Beauveria bassiana* (Bals.) Vuill. as influenced by media components

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**ABSTRACT:** *Beauveria bassiana*, an entomopathogenic fungus, was grown under submerged condition using six different media. When different supplements were studied, 2 percent polyethylene glycol (PEG) favoured both higher biomass (102mg/ml) and blastospore ( $14 \times 10^8$ ) yield, while chitin (0.2%), favoured only higher biomass (101mg/ml) turn over.

**KEY WORDS:** *Beauveria bassiana*, blastospores, polyethylene glycol

*Beauveria bassiana*, an entomopathogenic fungus, produces blastospores under submerged culture conditions (Samsinakova, 1966; Bidochka *et al.*, 1987). The quality of blastospores produced under such conditions is dependent on the components of the culture medium. Apart from C and N sources, the water activity and chitin also play an important role in determining the quality of the blastospores in terms of spore germination, growth and infection. So, the present study was undertaken to find out the influence of an inert solute PEG, a known osmoticum and chitin on the production of biomass and blastospores. This work was carried out in Vector Control Research Centre, Pondicherry, during 1996 – 1997.

*Beauveria bassiana* was cultured in the following media: (1) glucose (4%), peptone (1%) (SDB); (2) glucose (4%), peptone (1%), chitin (0.2%) (SDB + CP); (3) glucose (4%), peptone (1%), polyethylene glycol (1%) (SDB + PEG 1%); (4) glucose (4%), peptone (1%), polyethylene glycol (2%) (SDB + PEG 2%); (5) glucose (4%), peptone (1%), polyethylene glycol (3%) (SDB + PEG 3%); (6) glucose (4%), peptone (1%),

polyethylene glycol (4%) (SDB + PEG 4%). These media were prepared in quantities of 500ml in 2 litre flasks.

*Beauveria bassiana* obtained from the culture collection of Vector Control Research Centre was grown on Sabouraud Dextrose Agar (SDA) glucose 4gm; peptone 1gm; distilled water 100ml; pH 5.5] plates for 20 days at 25° C. After sporulation the conidia were collected in Triton X 100 (0.03%) and the optical density (OD) of the suspension was adjusted to 1.0 at 540nm (Woods and Grula, 1984). One ml of this suspension containing  $10^8$  conidia was inoculated to 50ml of SDB, incubated on a rotary shaker at 100rpm at 25°C for three days, then transferred to 200ml SDB, and incubated for 4 days. The culture was inoculated to 2 litre flasks containing 500ml (5% inoculum) of different media and incubated on rotary shaker for 15 days at the above-mentioned conditions. At the time of harvest blastospores present in the culture were counted in duplicates using haemocytometer (Booth, 1971). Then the culture was filtered through Whatman

No. 1 filter paper and the biomass was weighed.

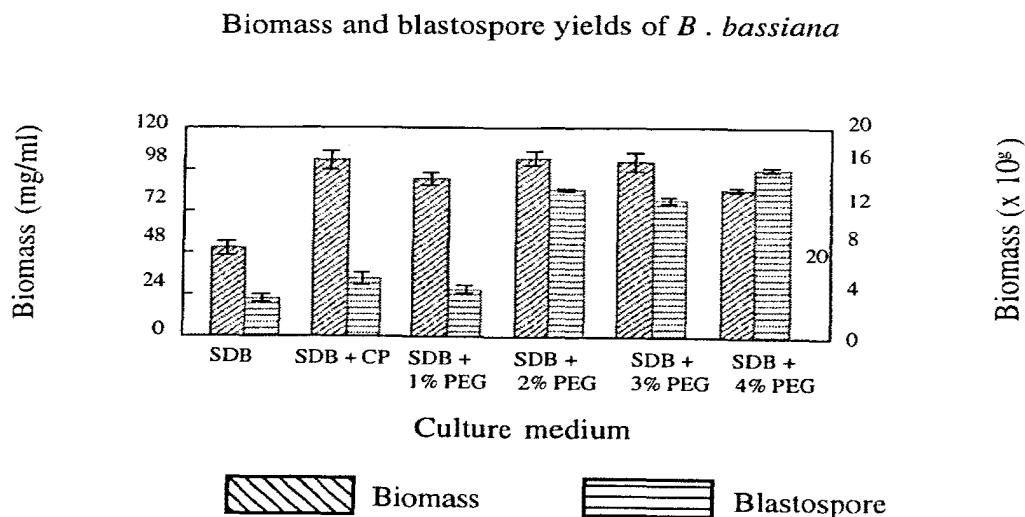
Figure 1 shows the weight of biomass and the blastospore yield in different media. Among different media, the one containing PEG (2%) was found to favour higher biomass (102mg/ml) and blastospore yield ( $14 \times 10^8$ ). In general, fungi can thrive well in culture condition with low water activity (Beech *et al.*, 1979). Effect of water activity on the morphology, growth and blastospore production of entomopathogenic fungi was studied by Humphreys *et al.* (1989) and they reported that in the case of *B. bassiana* the yield of blastospore increased with reduced water activity of nutrients with the incorporation of 2.4M PEG. Reducing the available water below optimum has been reported to increase lag phase, consequently decreasing the growth rate (Beech *et al.*, 1979). Further, when cells from an exponentially growing culture in a rich medium are transferred to a fresh identical medium there is no lag in resuming growth (Davis *et al.*, 1973). In the present study SDB was used for both inoculum development as well as production. It is, therefore, inferred that PEG at 2 per cent (0.003M) concentration, prolonged the growth at a steady state and increased the biomass yield by controlling the

water activity. Apart from increased blastospore production, reduced water activity can lead to the accumulation of trehalose in spores, which has been reported to extend the survival of conidia (Hallsworth and Magan, 1994).

The target site of *B. bassiana* in the insect is the cuticle and the main components of insect cuticle are protein, chitin and lipid (Parry, 1995). Sterilization of chitin by autoclaving or boiling causes release of D-glucosamine and N-acetyl glucosamine from the macromolecule and it was reported that *B. bassiana* could utilize these compounds and release chitinases (Smith and Grula, 1983). As expected, although the presence of chitin in the production medium increased the biomass yield (101mg/ml) the blastospore yield was significantly low ( $5.45 \times 10^8$ ). The present study, thus, shows that SDB, supplemented with 2 per cent polyethylene glycol yields higher biomass as well as blastospores whereas SDB supplemented with chitin favours only higher biomass yield.

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## REFERENCES

- Beech, F. W., Linton, A. H. and Madelin, M. F. 1979. Microbiology of food and beverages, pp. 340–353. In: Hawker, L. E. and Linton, A. H. (Eds.). *Microorganisms: function, form and environment*. Edward Arnold Publishers Ltd., London.
- Bidochka, J. M., Pfeifer, A. T. and Khachatourians, G. 1987. Development of the entomopathogenic fungus *Beauveria bassiana* in liquid culture. *Mycopathologia*, **99**: 77–83.
- Booth, C. 1971. Introduction to general methods, pp. 2–45. In: *Methods in Microbiology*. Academic Press, London.
- Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., Wood, W. B. and McCarty, M. Jr. 1973. Bacterial nutrition and growth, pp. 90–104. In: *Microbiology*. Harper and Row Publishers Inc., London.
- Humphreys, A. M., Matwele, P., Trinci, A. P. J. and Gillespie, A. T. 1989. Effects of water activity on morphology, growth and blastospore production of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces farinosus* in batch and fed batch culture. *Mycological research*, **92**: 257–264.
- Hallsworth, J. E. and Magan, N. 1994. Improved biological control by changing polyols/ trehalose in conidia of entomopathogens. In: *Brighton Crop Protection Conference- Pests and Diseases*, **8D**: 1091–1096.
- Parry, M. 1995. A review of mycochemical and insect interactions. *Biocontrol News and Information*, **16**: 27–33.
- Samsinakova, A. 1966. Growth and sporulation of submerged cultures of the fungus *Beauveria bassiana* in various media. *Journal of Invertebrate Pathology*, **8**: 395–400.
- Smith, R. J. and Grula, E. A. 1983. Chitinase is an inducible enzyme in *Beauveria bassiana*. *Journal of Invertebrate Pathology*, **42**: 319–326.