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Efficient production of phialoconidia of Verticillium lecanii for biocontrol of cucumber powdery mildew, Sphaerotheca fuliginea

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Abstract

A method is described that yields over 3×10^9 phialoconidia/ml of *Verticillium lecanii*.

Additional keyword: mycoparasites.

Spore production of mycoparasitic fungi may vary considerably and mass production of fungal conidia for biological control often meets with problems. Some mycoparasites do not produce sufficient inoculum for small-scale experiments (Hijwegen, 1988). Usually, special studies on cultivation methods are required as demonstrated for *Ampelomyces quisqualis* by Schmitz-Elsherif (1990).

Verticillium lecanii (Zimm.) Viégas strain Fyto 88.1, isolated from *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll. (Hijwegen, 1988), can easily be grown and sporulates well on most solid media such as agars containing potato-dextrose broth, malt extract plus mycological peptone, oat meal, chitin or skimmed milk. In this way productions of $1\text{-}4 \times 10^7$ conidia/cm^2 ($0.5\text{–}2 \times 10^9$ conidia/petridish) can be obtained. When, however, 8×10^{10} conidia were required every week for a greenhouse experiment, (Verhaar and Hijwegen, unpublished) surface grown cultures were inadequate and other methods of cultivation were investigated. *V. lecanii* was grown at 20 °C and 135 rpm in liquid media containing milled, autoclaved oat meal, skimmed milk or malt extract plus mycological peptone. Yields of conidia were rather low. Conidium production was enhanced by raising the incubation temperature to 25 °C. Yeast cell walls and malt extract plus mycological peptone gave a good yield of conidia. These media were, however, surpassed by far by growing *V. lecanii* in autoclaved oat meal suspension.

V. lecanii has been reported to produce only blastoconidia in liquid culture (Latgé et al., 1986). After consulting Dr W. Gams, CBS, Baarn this was investigated using our strain Fyto 88.1. In our experiments submerged mycelium, producing micro-verticils with micro-phialides carrying normal phialoconidia, was formed in liquid culture. Budding was not observed. Conidia produced in liquid culture could not be distinguished microscopically in size or shape from conidia produced on solid oat meal medium. In 1% oat meal more mycelium was formed than in 3% oat meal. This raises the question whether oat meal contains substances that influence the mycelium–conidia ratio.

To investigate conidium production quantitatively, 0.8 cm^2 pieces of agar with mycelium from the margin of a growing culture of *V. lecanii* were added to 300-ml Erlenmeyer flasks containing 100 ml of 1, 2, or 3% milled and autoclaved oat meal (Quaker HO

Naturel) in distilled water. These flasks were incubated in the dark at 25 °C in a rotary shaker at 135 rpm. Other flasks containing 2% autoclaved oat meal were inoculated with *V. lecanii* and incubated in the same way at 20 °C or 30 °C. For every treatment two flasks were used. The experiment was repeated twice. Ten-ml samples were removed under sterile conditions after 3, 5, 7 and 10 days. The suspensions were diluted 10-fold or, if necessary, 100-fold and the conidia were counted in a hemocytometer. A production of over 3×10^9 conidia/ml could be obtained at 25 °C in 3% oat meal (Fig. 1).

Germination was assessed at 3, 5, 7 and 10 days by placing 20 µl droplets containing 10^6 conidia/ml on water agar and counting germinated and non-germinated conidia on the next day (Fig. 2).

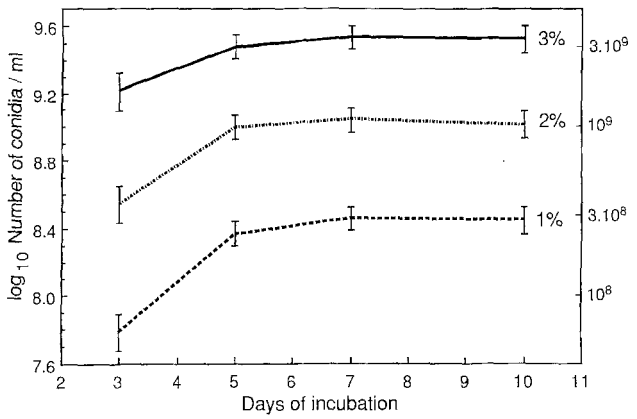

Fig. 1A. Effect of oat meal concentrations (1%, 2% or 3%) on the number of *Verticillium lecanii* conidia produced in liquid media incubated at 25 °C. The graph gives the mean number of conidia/ml in four replications. Data were log transformed (y-axis) and back-transformed (right y-axis). The standard errors of the mean are given in the graphs by bars.

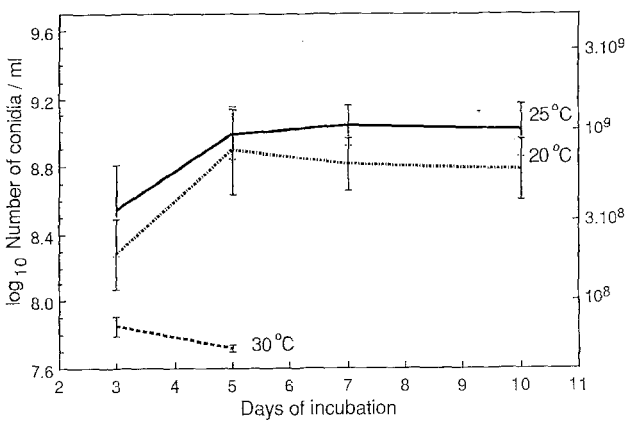

Fig. 1B. Effect of incubation temperatures of 20, 25 or 30 °C on the number of *Verticillium lecanii* conidia produced in 2% liquid oat meal medium. The graph gives the mean number of conidia/ml in four replications. Data were log transformed (y-axis) and back transformed (right y-axis). The standard errors of the mean are given in the graphs by bars.

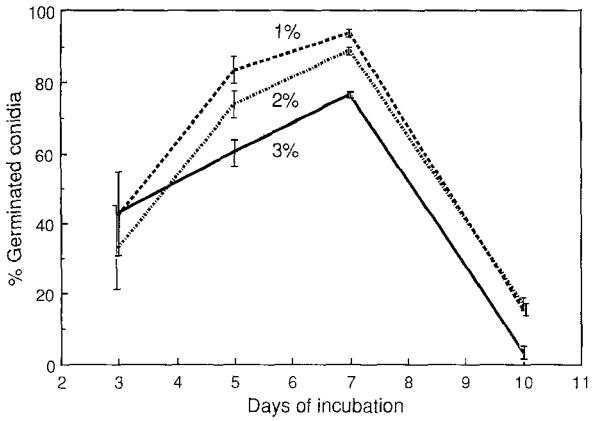

Fig. 2. Germination of conidia of *Verticillium lecanii* produced in liquid media containing 1%, 2% or 3% oat meal, incubated at 25 °C, after 3, 5, 7 or 10 days incubation. Drops of 20 µl containing 2×10^4 spores were placed on water agar. Germination was assessed after incubation for 24 h at 20 °C. The data are means of two replicates. The bars indicate the standard errors of the mean.

Incubation at 30 °C resulted in a limited production of conidia, which germinated poorly. Poor germination of conidia produced at temperatures above the optimum for growth does not seem to be uncommon with mycoparasitic fungi (Van Eynatten and Verhaar, unpublished). After 7 days of cultivation lysis of mycelium and conidia had occurred and the cultures had to be discarded.

As a routine procedure *V. lecanii* was cultured 7 days at 25 °C and 135 rpm in a 3% oat meal suspension every week during 6 subsequent weeks. One flask per week, containing more than 10^9 conidia/ml, was always sufficient for the 8×10^{10} conidia needed for the weekly applications on cucumber powdery mildew, *S. fuliginea*, during 6 subsequent weeks, indicating that the method is reliable.

In a later experiment $2\text{-}4 \times 10^{10}$ conidia were required weekly. As with 3% oat meal suspensions the pores of the filter became plugged sometimes, it was decided to grow *V. lecanii* in a 1% suspension. The method proved to be reliable during the production of eight consecutive batches and 1 or 2 flasks were always sufficient for each of the weekly applications in the greenhouse experiment.

In conclusion, a high production of phialoconidia with high germinability was obtained by growing *V. lecanii* in 1–3% milled oat meal during 7 days at 25 °C in the dark at 135 rpm.

References

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