NOTES

Production of Resting Spores of *Entomophthora thaxteriana*


A prerequisite for any application of Entomophthoraceae is the large-scale production of resting spores. Experiments were done with *Entomophthora thaxteriana*, a species which forms conidia and resting spores in culture. The isolate used was obtained in 1965 from J. Weiser (Praha) and cultured in Darmstadt. E. Müller-Kögler gave this isolate to the American Type Culture Collection (Registration No. ATCC 26006). This fungus was propagated in submerged cultures because this method is the most efficient one for mass production. Cultivation was in 200-ml Erlenmeyer flasks (twice for each solution) and with 50 ml nutrient solution. Each flask was inoculated with a small piece of mycelium from 2 to 3 wk old agar slant cultures, incubation was on a rotary shaker at 28°C and 150 rpm.

The resting spores were harvested after 14 days. The content of each flask (mycelium in culture medium) was homogenized separately and then centrifuged at 5000 rpm. Each sediment was washed three times with distilled water and resuspended in 5 ml water. The number of resting spores per milliliter nutrient solution was estimated in a counting chamber (Thoma) using phase-contrast microscope. Only complete, thick-walled resting spores were counted, because only these spores are of practical value.

In medium consisting of 3 g corn syrup and 0.5 g peptone (meat, peptic digested; Witte, Frankfurt/M. Germany) per 100 ml distilled water, *E. thaxteriana* formed 1000 resting spores/ml. With tap water, however, 5000 spores/ml were obtained. Accordingly, the influence of different ions on the production of thick-walled resting spores was investigated by adding 2 mM cation/100 ml nutrient solution in distilled water. The results are shown in Figure 1.

A medium containing glucose (4.0%), peptone (1.0%), and yeast extract (0.5%) which allowed an excellent vegetative growth of *E. thaxteriana* in surface cultures was also tested for resting spores production: the effectiveness of different kinds of N-sources: papain-digested soyabean (Merck, Darmstadt, Germany), peptic- (Witte) and tryptic-digested (Merck) meat, and tryptic-digested casein (Merck); also two kinds of yeast extract were used: preparations manufactured by Merck and Baltimore Biological Laboratories, respectively.

Figure 2 shows that the amount of resting spores was highly dependent on the kind of peptone and, to a lesser degree, also on the type of yeast extract. It is remarkable that adding of 2 mM KCl to 100 ml of the medium rich in nutritive substance reduced the number of resting spores somewhat. This re-
FIG. 1. Number of resting spores of *Entomophthora thaxteriana* in corn syrup (3.0%)–peptone (0.5%)–solution with different salts of alkali and alkali earth (2 mM cation/100 ml).

FIG. 2. Number of resting spores of *Entomophthora thaxteriana* in glucose (4.0%)–peptone (1.0%)–yeast extract (0.5%)–solution with different preparations of peptone and yeast extract (0.148% KCl).

result contrasts with that obtained with the corn syrup–peptone–medium, poor in nutritive substance, because there KCl had a remarkable enhancing effect. Perhaps, the relation between carbon and nitrogen in the medium is important for the response to K-ions.

In addition, experiments to mass produce *E. thaxteriana* in a 20-liter fermenter were successful.

Using a rich medium (with 5.5% ingredients), it was possible to obtain 500,000 resting spores/ml nutrient solution in a period of 14 days (Fig. 2).

I am grateful to Dr. Müller-Kögler for his advise and critical comments during my work.

**ALBRECHT GRÖNER**

*Biologische Bundesanstalt für Land-und Forstwirtschaft*

*Istitut für biologische Schädlingsbekämpfung*,

*Darmstadt, Germany*

*Received July 15, 1974*