Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Cytotoxic Activity in Culture Filtrates from the Entomopathogenic Fungus Beauveria sulfurescens

P. Mollier,* J. Lagnel,* J. M. Quiot,+ A. Aioun,* and G. Riba*

*Institut National de la Recherche Agronomique, Station de Recherche de Lutte Biologique, La Minière 78285 Guyancourt Cedex, France; and +Station de Recherche de Pathologie Comparée, INRA-CNRS, 30380 Saint-Christol-lez-Ales, France

Received November 16, 1993; accepted May 25, 1994

The hyphomycete fungus Beauveria sulfurescens secretes into the culture medium several components toxic for Galleria mellonella larvae. Concentrated and dialyzed (cutoff 12,000) B. sulfurescens culture filtrate also contains a high-molecular-weight compound cytotoxic for the Mamestra brassicae cell line. A fractionation in two chromatographic steps separated a cytotoxic fraction (IC50 = 1.15 ± 0.05 µg/ml), which was poorly toxic to larvae (LC50 = 150 ± 10 µg/ml) and an insecticidal fraction (LC50 = 2.1 ± 0.1 µg/ml) devoid of detectable cytotoxicity. The two kinds of activity, i.e., cytotoxicity in vitro and toxicity in vivo are thus independent. The cytotoxic activity, initially detected on a M. brassicae cell line, also affected two other insect cell lines (G. mellonella and Spodoptera frugiperda). The cytotoxic fraction provoked not only the inhibition of cell proliferation, but also cell death. Adhesive mammalian cell lines were not sensitive. Nonadhesive mammalian cell lines of murine myeloma and hybridomas were poorly sensitive.

© 1994 Academic Press, Inc.

KEY WORDS: Beauveria sulfurescens; insect; entomopathogen; toxicity; cell lines; cytotoxicity.

MATERIALS AND METHODS

Fungal Strain and Cultivation

The strain used in these experiments (from the La Minière culture collection) was originally isolated from soil in France and designated as B. sulfurescens (ATCC 7159). The strain was maintained in 90-mm petri plates at 25°C on complete agar medium (Ravallec et al., 1989). The fungus was grown in a submerged culture in a M2M medium containing 5 g/liter casein hydrolysate N3 PEM, 30 g/liter glucose, 4 drops/liter Berthelot solution, L-amino acids (in g/liter: alanine 0.13, arginine 0.18, asparagine 0.2, aspartic acid 0.1, glutamine 2.0, glycine 0.16, histidine 0.8, lysine 0.8, proline 2.0), vitamins (in mg/liter: p-aminobenzoic acid 2, biotin 0.02, calcium panthenolate 20, nicotinic acid 4, pyridoxin 4, riboflavin 2, thiamin 4), salts (in g/liter: KCl 0.8, NaCl 0.1, CaCl2 · 2H2O 0.1, MgSO4 · 7H2O 0.5, KH2PO4 1). An inoculum was prepared by washing the conidia on the surface of a sporulating aerial mycelial mass and then diluting it to a final concentration of 109 conidia/ml.
Beauveria sulfurescens CYTOTOXIC ACTIVITY

Lial mat with M₂M and collecting the liquid. This conidia suspension (5 × 10⁶ total conidia per milliliter of M₂M) was cultured with shaking (80 rpm) on a reciprocal shaker for 4 days at 25°C. Fifty-milliliter aliquots of this culture were used to inoculate 1 liter of M₂M in 5-liter Erlenmeyer flasks. Subcultures were agitated for 3 days at 25°C.

**Fractionation of Toxic Activity**

The protocol for fractionation of toxic activity is shown in Fig. 1.

**Step 1. Extraction and precipitation with ammonium sulfate.** Fungal cultures were centrifuged at 1500g for 15 min and the supernatant (containing the toxic activity) was collected. The supernatant was filtered through No. 2 Whatman paper and then through 1.2-, 0.6-, and 0.22-μm pore size filters (Millipore, Bedford, MA). Crystalline ammonium sulfate was added to the culture filtrate to 90% saturation (600 g/liter) and the sample was incubated at 4°C for 1 hr, then centrifuged at 7200g for 30 min. The pellet was resuspended in a minimal volume of distilled water, dialyzed (membrane cutoff 12,000) against three changes of water at 4°C for 16 hr, and sterilized by filtration.

**Step 2. Precipitation with ethanol.** Ethanol (pre-cooled to −80°C) was added to the ammonium sulfate-concentrated filtrate (ethanol: 4.5, filtrate: 1, v/v) under continuous magnetic stirring. The mixture was allowed to stand for 30 min in ice to equilibrate and then centrifuged at 7200g for 15 min in a precooled centrifuge. The protein pellet was then vacuum-dried in a Speed-Vac apparatus (Savant, Farmingdale, NY), resuspended in a minimal volume of distilled water, and dialyzed (membrane cutoff 12,000) against three changes of water at 4°C for 16 hr.

**Step 3. Affinity chromatography on immobilized lectin: concanavalin A.** Following ethanol precipitation, the concentrated sample was made up to a final concentration of 0.02 mM phosphate buffer–0.2 mM NaCl–1 mM CaCl₂, pH 7, sterilized by filtration, and loaded on a concanavalin A (ConA)-Ultrogel (IBF, Villeneuve-la-Garenne, France) column (2.6 × 7 cm) equilibrated with the above buffer. The ConA-unreactive form was removed by washing the column with the equilibration buffer. The ConA-reactive form was eluted stepwise with the above buffer containing 10 mM methyl α-D-glucopyranoside and then 250 mM methyl α-D-mannopyranoside. The flow rate was 0.24 ml/min during sample loading and 0.8 ml/min during the washing and elution steps. Fractions of 10 ml were collected.

**Step 4. Hydroxyapatite chromatography.** The fraction eluted from Con A with methyl α-D-mannopyranoside was dialyzed at 4°C against three changes of water and then made up to 1 mM NaCl and applied to a hydroxyapatite column (BDH Chemicals, Ltd., Poole, UK) (1.2 × 13 cm), previously equilibrated with 1 mM NaCl. The column was washed with 1 mM NaCl and eluted stepwise with 1 mM MgCl₂ and 1 mM MgCl₂. Then the column was washed with 1 mM sodium phosphate, pH 7, and an elution gradient (1 to 150 mM sodium phosphate, pH 7, in 25 ml) was applied. The flow rate was 1 ml/min and the fraction size was 5 ml.

**Determination of Toxic Activity**

The median lethal concentration (LC₅₀) of the B. sulfurescens crude supernatant and chromatographic fractions were calculated by the graphic method of probit analysis (Finney, 1952). Each fraction tested was first dialyzed against three changes of water, vacuum-dried in a Speed-Vac apparatus, and resuspended in sterile water at the convenient concentration. Several dilutions were made. The dilutions were sterilized by filtration and injected (20 μl) into the hemocoel of G. mellonella sixth-instar larvae (20 larvae per dose, mean wt. = 205 ± 10 mg). The LC₅₀ values were scored 7 days after the injection. Protein concentrations in samples were determined by the Bradford method (Bradford, 1976).

**Cell Lines**

The M. brassicae (SPCMb92) and G. mellonella (SPCGm17) cell lines were obtained from Dr. J. M. Quiot. SPCMb92, SPCGm17, and the Spodoptera frugiperda line (S9, ATCC CRL 1711) were grown in

---

**FIG. 1.** Fractionation procedure of the B. sulfurescens culture filtrate insecticidal activity.
Hink's medium (Gibco BRL, Paisley, UK) supplemented with 10% fetal bovine serum (Gibco) at 26°C. The porcine fetal testis cell line ST (ATCC CRL 1746) and the dog kidney epithelial cell line MDCK (Misfeldt et al., 1976) were grown in MEM (Gibco) supplemented with 10% fetal bovine serum, at 37°C. The human carcinoma cell line CaCO2 (Fogh et al., 1977) was grown at 37°C in DMEM supplemented with 2 mM glutamine, 1% nonessential amino acid (Gibco) and 20% fetal bovine serum.

The myeloma cell line SP20 (Shulman et al., 1978), and the hybridoma G43 (Delmas et al., 1992) and 70K5C11 (produced using Barley yellow mosaic virus, obtained from T. Delaunay, Jouy-en-Josas) were grown at 37°C in RPMI 1640 supplemented with 2 mM glutamine, 2 mM sodium pyruvate, 3 mM 2-mercaptoethanol (for hybridomas), and 10% fetal bovine serum.

**Kenacid Blue Method for Protein (KBP) Cytotoxicity Assays**

Each fraction to be tested was dialyzed at 4°C against three changes of water for 16 hr, vacuum-dried, reuspended at various concentrations in the appropriate cell media, and sterilized by filtration. Cells were plated in appropriate media in 96-well plates at a density of 10^5/ml (100 µl per well). After a resting time of 1 hr (SPCMb92, SPCGm17, Sf9, SP20, G43, and 70K5C11), 24 hr (ST, MDCK), or 72 hr (CaCO2), the medium was removed and replaced with the medium containing the fractions to be tested for toxicity (3 wells per concentration, with 12 untreated cell control wells). The cells were incubated for a further 72 hr and total cellular protein content was determined as described by Knox et al. (1986).

**Tetrazolium Salt (MTT) Assay of Cytotoxicity**

SPCMb92 cells were treated as described for the KBP assay. Then, the MTT method was applied as described by Hansen et al. (1989).

**Statistical Analysis**

Cytotoxicity tests were carried out in triplicate for each concentration. The results for tested samples are expressed as percentages of untreated control cells value ± SEM. The IC_{50} values (i.e., the concentration of sample that reduced total cellular protein to 50% of that of untreated cell control wells) are the mean ± SEM of the values from at least two independent experiments.

**RESULTS**

**Fractionation of B. sulfurescens Culture Filtrate Insecticidal Activity**

The insecticidal activity in B. sulfurescens crude filtrate was fractionated by a four-step fractionation procedure: precipitation of most proteins with 90% ammonium sulfate, ethanol precipitation (4.5 v/v), affinity chromatography on immobilized ConA, and hydroxyapatite chromatography (Table 1).

Chromatography on ConA gave three fractions: (i) a ConA-unreactive fraction (Fraction A), (ii) a ConA-reactive fraction, eluted with a low concentration (10 mM) of methyl α-D-glucopyranoside (Fraction B), and (iii) a ConA-reactive fraction, eluted with a high concentration (250 mM) of methyl α-D-mannopyranoside (Fraction C). These fractions contained 38.5, 5.4, and 4.2% of the applied protein, respectively. Fraction C was the most toxic for insects (cf. Table 2). This fraction contained 3% of the ammonium sulfate-precipitated protein.

Hydroxyapatite chromatography also gave three fractions: (i) a fraction retained and eluted by 1 M MgC12 (Fraction D), (ii) a fraction retained and eluted by 1 M MgC12 (Fraction E), and (iii) a fraction retained and eluted by a phosphate gradient (Fraction F). The insecticidal activity was recovered in fraction F, which contained 7.4% of the protein applied to the column and 0.2% of the ammonium sulfate-precipitated protein.

**TABLE 1**

| Step                          | Fraction | Volume (ml) | Protein (µg/ml) | Total amount of protein (mg) | Recovery of protein (%) |
|------------------------------|----------|-------------|-----------------|-----------------------------|-------------------------|
| Ammonium sulfate precipitation | Concentrated filtrate | 85          | 367.6           | 31.25                       | 100.00                  |
| Ethanol precipitation        | Concentrated filtrate | 22          | 1377.8          | 31.00                       | 99.2                    |
| Chromatography on ConA*      | A: Not retained | 140         | 71.4            | 10.00                       | 38.5                    |
|                              | B: Glucose-eluted | 18          | 77.8            | 1.40                        | 5.4                     |
|                              | C: Mannose-eluted | 27          | 40.7            | 1.10                        | 4.2                     |
| Chromatography on hydroxyapatiteb | D: Not retained | 20          | 0.4             | 0.008                       | 0.9                     |
|                              | E: 1 M MgCl2-eluted | 20          | 3.2             | 0.064                       | 7.5                     |
|                              | F: Phosphate gradient-eluted | 15          | 4.2             | 0.063                       | 7.4                     |

* 26 mg (19 ml) of the ethanol-concentrated filtrate was loaded onto the ConA column.

b 850 µg (21 ml) of the mannose-eluted fraction from ConA chromatography was applied to the hydroxyapatite column.
**Beauveria sulfurescens CYTOTOXIC ACTIVITY**

**TABLE 2**

*In Vivo Toxicity on G. mellonella Larvae and in Vitro Cytotoxicity on M. brassicae Cell Line of the Partially Purified Fractions from the B. sulfurescens Culture Filtrate*

| Step               | Fraction                          | LC50c (µg/ml) | IC50d (µg/ml) | LC50/IC50 |
|--------------------|-----------------------------------|---------------|---------------|-----------|
| Ethanol precipitation | Concentrated filtrate          | 40 ± 10       | 4.2 ± 0.1     | 9.5       |
| ConA               | A: Not retained                   | 150 ± 10      | 1.15 ± 0.05   | 116.6     |
|                    | B: Glucose-eluted                 | >225          | >225          |           |
|                    | C: Mannose-eluted                 | 19 ± 1        | 19 ± 1        | 1.0       |
| Hydroxyapatite     | D: Not retained                   | >0.4          | >2            |           |
|                    | E: 1 M MgCl2 eluted               | >3.2          | >10.6         |           |
|                    | F: Phosphate gradient-eluted      | 2.1 ± 0.1     | >4.2          | <0.5      |

* LC50 is the median lethal concentration.
* IC50 is the concentration of the tested fraction that reduces the final cellular protein content to 50% of that of control well.

**In Vivo Toxicity on Insect Larvae and in Vitro Cytotoxicity of Partially Purified Fractions of B. sulfurescens Culture Filtrate**

Each partially purified fraction was tested for toxicity on larvae and for cytotoxicity on the *M. brassicae* (SPCMb92) cell line. The toxicity for insect larvae is expressed as LC50, scored 7 days after injection. The cytotoxicity was quantified by the KBP method (Knox et al., 1986). This method evaluates cell proliferation by measuring total cellular protein after 72 hr, and the results are expressed as the IC50. For each tested fraction, the ratio of the LC50 to the IC50 was then calculated. The results are shown in Table 2.

The LC50 of the starting fraction (*B. sulfurescens* culture filtrate concentrated with ammonium sulfate and ethanol) was 40 ± 10 µg/ml and the IC50 was 4.2 ± 0.1 µg/ml. The ratio of LC50 to IC50 was thus 9.5.

The LC50 and IC50 of each of the fractions from ConA chromatography were determined. Fraction A had an LC50 of 150 ± 10 µg/ml and an IC50 of 1.15 ± 0.05 µg/ml. For this fraction, the insecticidal activity was 3.75-fold lower, whereas the cytotoxicity was 3.6-fold higher, than that of the starting fraction. The ratio of LC50 to IC50 for Fraction A was 116.6. Fraction B was not toxic either to larvae or to cells, even when concentrated up to 225 µg/ml. Fraction C had an LC50 2.1-fold lower and an IC50 4.75-fold higher than that of the starting fraction. Thus, Fraction C was enriched in insecticidal activity but was less cytotoxic, compared to the starting fraction. The ratio of LC50 to IC50 for Fraction C was 1.

Fraction C was further fractionated by hydroxyapatite chromatography. The fraction toxic for larvae obtained from this step (Fraction F) had an LC50 of 2.1 ± 0.1 µg/ml. Fraction F displayed no detectable cytotoxic activity at concentrations up to 4.2 µg/ml. The ratio of LC50 to IC50 was thus below 0.5.

**Cytotoxicity of Fraction A against Various Cell Lines**

The cytotoxicity of Fraction A for various cell lines, including insect lines and adhesive and nonadhesive mammalian lines, was assayed by the KBP method. Fraction A was cytotoxic to the insect cell lines (Fig. 2) with IC50 values of 0.70 ± 0.05, 1.15 ± 0.05, and 36 ± 2 µg/ml for cell lines from *G. mellonella* (SPCGm17), *M. brassicae* (SPCMb92), and *S. frugiperda* (Sf9), respectively. In contrast, 108 µg/ml of Fraction A was not cytotoxic for adhesive mammals cell lines from porcine fetal testis (ST), dog kidney (MDCK), or human carcinoma (CaCO2) (not shown). It weakly inhibited cell proliferation of the nonadhesive mammal cell lines SP2O (murine myeloma) and two murine hybridomas (Fig. 3).

Even at concentrations of Fraction A that produced the maximal cytotoxic effect, the cellular protein was not reduced to zero (Fig. 2). This could be explained either by the presence of cellular debris, which was not eliminated by washing, or by the presence of remaining living cells.

**Cytotoxicity of Fraction A for the M. brassicae Cell Line**

The MTT method is based on the reduction of the soluble yellow tetrazolium salt to a blue insoluble formazan product by mitochondrial succinic dehydrogenase and thus indicates both cell proliferation and cell

![FIG. 2. Cytotoxicity, measured by the KBP assay, of the ConA-unreactive fraction (Fraction A) on cell lines from *M. brassicae* SPCMb92 (●), *G. mellonella* SPCGm17 (△), and *S. frugiperda* Sf9 (○). Values are means ± SEM.](image-url)
The samples thus contained no living cells. SPCMb92 cells did not produce any formazan (Fig. 4). Survival in the presence of 144 μg/ml of Fraction A, SPCMb92 cells did not produce any formazan (Fig. 4). The samples thus contained no living cells.

**DISCUSSION**

In this work, *B. sulfurescens* was cultivated on M_2M medium. The composition of M_2M was empirically defined to mimic the composition of *G. melo*llena hemocoel (Grenier et al., 1974). It has been shown that the secretion of toxins is highest in the presence of complex organic nitrogen sources such as ground maize, skim milk, yeast extract, or *G. melo*llena homogenate (Kucera, 1971, 1981). In a previous study, we cultivated the fungal strain on a medium containing yeast extract (MY20) (Mollier et al., submitted for publication). Because of the high viscosity of MY20, sterile filtration was difficult. Moreover, MY20 contained pigments that affected the chromatography columns used for fractionation. We therefore used M_2M in this study.

Affinity chromatography on ConA and hydroxyapatite chromatography were used to fractionate the insecticidal activity in culture filtrate concentrated with ammonium, sulfate and ethanol. As previously observed (Mollier et al., submitted for publication) the ConA-reactive, methyl α-D-mannopyranoside-eluted fraction (Fraction C) displayed the highest insecticidal activity. This activity was further purified by hydroxyapatite chromatography. The procedure included two elution steps: with 1 M MgCl_2, to elute proteins with isoelectric points theoretically ranging from 5 to 8, and then with a phosphate gradient to elute acidic proteins (Gorbonoff, 1990). The insecticidal activity was eluted from the hydroxyapatite column by the phosphate gradient, indicating that the toxin has an acidic isoelectric point. The insecticidal fraction finally obtained from successive ConA and hydroxyapatite chromatographies (Fraction F) was 0.2% of the starting protein and possessed an LC_50 value of 2.1 ± 0.1 μg/ml (Table 2).

The concentrated and dialyzed culture filtrate of *B. sulfurescens* was shown to be cytotoxic for a cell line from *M. brassicaceae* ovary (SPCMb92).

In order to determine if this cytotoxicity was due to the same molecule(s) as the in *vivo* insecticidal activity, each partially purified fraction was tested for cytotoxicity against the SPCMb92 cell line. This line was highly sensitive to *B. sulfurescens* concentrated filtrate and easy to cultivate. The KBP assay used (Knox et al., 1986) measures cell proliferation. The rationale for this choice is that cells in continuous culture proliferate at a known rate, and this rate would be reduced by products that affect one or more essential cytological functions (DNA or protein synthesis, or mitochondrial or membrane properties).

The ratio of LC_50 to IC_50 of the insecticidal fraction decreased with each step of the fractionation. Thus, the fractions enriched in larval toxicity were less cytotoxic. At the end of the fractionation procedure, the insecticidal fraction (Fraction F) displayed no detectable cytotoxicity at the tested concentrations (Table 2). In the previous study (Mollier et al., submitted for publication), the insecticidal activity was fractionated by a different procedure: culture filtrate concentrated by tangential ultrafiltration was submitted to anion-exchange chromatography and ConA chromatography. The insecticidal fraction obtained possessed no detectable cytotoxicity at a concentration equal to 17-fold the LC_50 (not shown). These results indicate that the *in vivo* toxicity and the *in vitro* cytotoxicity are unrelated activities and presumably due to different agents. In consequence, the *in vitro* assay could not be used to replace the assay using insect larvae to follow the purification of the insecticidal product.

The cytotoxic activity of the ConA-unreactive fraction (Fraction A) has not previously been described. It is unlikely to be due to putative cyclodepsipeptides which were eliminated by dialysis of the culture filtrate: the active compound is presumably a higher-molecular-weight species.

This high-molecular-weight cytotoxic species was not equally active against all cell types. In the same concentration range, Fraction A was cytotoxic for in-
sect cell lines (Fig. 2), but not adhesive mammalian cell lines (not shown). A slight inhibition of nonadhesive mammalian cell proliferation was observed with murine myeloma and hybridomas (Fig. 3). The KBP assay measures cell proliferation. The tetrazolium assay (MTT) measures both cell proliferation and survival (Mosmann, 1983). MTT assays showed that Fraction A not only inhibited cell proliferation but also killed cells (Fig. 4). Cell lysis was confirmed by optical microscopy (results not shown).

ACKNOWLEDGMENTS

This research was partially supported by ZENECA Agrochemicals (Jealott's Hill Research Station, Bracknell, Berkshire RG 12 6EY, UK). We are grateful to Drs. J. Grosclaude and T. Delaunay for having suggested cytotoxic assays and offering the appropriate laboratory facilities in VIM (Virologie et Immunologie Moléculaire) INRA, Jouy-en-Josas, France. We thank Mrs. S. Labiau, L. Abinne-Molza, S. de Vaureix, and A. Charpilienne for their expert technical assistance. We are grateful to Dr. S. Chwetzoff for his critical review of the manuscript and to Dr. A. Vey for his constructive suggestions. We thank Mrs. C. Cormenier for typing the manuscript.

REFERENCES

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Delmas, B., Gelfi, J., L’Haridon, R., Vogel, L. K., Sjöstrom, H., Noren, O., and Laude, H. 1992. Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. Nature 357, 417–419.

Finney, D. J. 1952. Probit analysis. Cambridge Univ. Press, Cambridge.

Fogh, J., Fogh, J. M., and Orfio, T. 1977. One hundred and twenty seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 56, 221–226.

Grenier, S., Delobel, B., Bonnot, G., and Laviolette, P. 1974. Persistence des activités enzymatiques du corps adipeux de Galleria mellonella L. (Lepidoptera) en milieux définis. C.R. Acad. Sci. Paris 278, 2545–2548.

Gorbunoff, M. J. 1990. Protein chromatography on hydroxyapatite columns. In “Methods in Enzymology, Vol. 182, Guide to Protein purification” (M. P. Deutscher, Ed.), pp. 329–339. Academic Press, New York.

Hamil, R. L., Higgins, C. E., Booz, H. E., and Gorman, M. 1969. The structure of beauvericin: A new cyclodepsipeptide antibiotic toxic to Artemia salina. Tetrahedron Lett. 49, 4255–4258.

Hansen, M. B., Nielsen, S. E., and Berg, K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods 119, 203–210.

Kleinhauf, H., and von Döhren, H. 1986. Peptide Antibiotics. In “Biotechnology” (H. Pape and H.-J. Rehm, Eds.), pp. 283–306. V.C.H. Verlagsgesellschaft, Federal Republic of Germany.

Knox, P., Uphill, P. F., Fry, J. R., Benford, J., and Balls, M. 1986. The frame multicentre project on in vitro cytotoxicity. Fed. Chem. Tox. 24, 457–463.

Kucera, M. 1971. Toxins of the entomogenous fungus Beauveria bassiana. II. Effect of nitrogen sources on formation of the toxic protease in submerged culture. J. Invertebr. Pathol. 17, 211–215.

Kucera, M. 1980. Proteases from the fungus Metarhizium anisopliae toxic for Galleria mellonella larvae. J. Invertebr. Pathol. 35, 304–310.

Kucera, M. 1981. The production of toxic protease by the entomopathogenous fungus Metarhizium anisopliae in submerged culture. J. Invertebr. Pathol. 38, 33–38.

Kucera, M., and Samsinakova, A. 1968. Toxins of the entomopathogous fungus Beauveria bassiana. J. Invertebr. Pathol. 12, 316–320.

Lysenko, O., and Kucera, M. 1971. Microorganisms as sources of new insecticidal chemicals: Toxins. In “Microbial Control of Insects and Mites” (H. D. Burges and N. W. Hussey, Eds.), pp. 205–227. Academic Press, New York.

Misfeldt, D. S., Hamamoto, S. T., and Pitelka, D. R. 1978. Transepithelial transport in cell culture. Proc. Natl. Acad. Sci. USA 75, 1212–1216.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Quiot, J. M., Vey, A., and Vago, C. 1985. Effects of mycotoxins on invertebrate cells in vitro. Adv. Cell. Cult. 4, 199–212.

Ravallec, M., Vey, A., and Riba, G. 1989. Infection of Aedes albopictus by Toxoplasma gondii. J. Invertebr. Pathol. 53, 7–11.

Roberts, D. W. 1981. Toxins of entomopathogenic fungi. In “Microbial Control of Pests and Plant Diseases, 1970–1980” (H. D. Burges, Ed.), pp. 441–465. Academic Press, London.

Shulman, M., Wilde, C. D., and Kohler, G. 1978. A better cell line for making hybridomas secreting specific antibodies. Nature 276, 269–270.

Suzuki, A., Kanaoka, M., Isogai, A., Murakoshi, S., Ichinoe, M., and Tamura, S. 1977. Sassianolide: A new insecticidal cyclodepsipeptide from Beauveria bassiana and Verticillium lecanii. Tetrahedron Lett. 22, 2167–2170.

Vey, A., Quiot, J. M., and Pais, M. 1986. Toxémie d’origine fungique chez les invertébrés et ses conséquences cytotoxiques: Etude sur l’infection à Metarhizium anisopliae (Hyphomycète: Moniliinales) chez les Lépidoptères et les Coléoptères. C.R. Soc. Biol. 180, 105–112.

Vey, A., and Riba, G. 1989. Toxicité insecticide issues de champignons entomopathogènes [Insecticidal toxins produced by entomogenous fungi]. C.R. Acad. Agric. Fr. 75, 149–149.