

Effect of the entomopathogenic fungus *Beauveria bassiana* on the humoral immune response of *Galleria mellonella* larvae (Lepidoptera: Pyralidae)

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Abstract. In this study the entomopathogen *Beauveria bassiana* caused no humoral immune response in *Galleria mellonella* when it entered the host through the integument in a natural manner. Initiation of mycosis by injection of living or heat-inactivated blastospores elicited the release of antimicrobial proteins such as lysozyme and cecropins within the hemolymph. Maximum levels were reached 24 h after treatment. Afterwards, these levels declined faster in larvae with established mycosis. Subsequent studies documented the ability of *B. bassiana* to suppress increases in antibacterial activity within the hemolymph of *G. mellonella* larvae that were pretreated with injections of microbial provokers. The latter induced non-specific de novo synthesis and subsequent release of several proteins within the hemolymph. The protein synthesis of diseased larvae was not generally affected, even when the larvae were moribund. Among the proteins synthesised during the humoral response, only certain proteins such as lysozyme were suppressed, as detected by SDS-PAGE and autoradiography. The effects of *B. bassiana* upon the humoral immune response of *G. mellonella* are discussed in regards to other parasites such as nematodes or Hymenoptera, which reportedly suppress the synthesis of endogenous host proteins.

INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* occurs worldwide and is known to infect a wide range of insect species. Among the imperfect fungi, this species infects a greater number of host species than most, thus it is used to control several insect pests (Tanada & Kaya, 1993). This entomopathogen is able to penetrate the host integument which completely encloses the host body within a chitinous cuticle, and develop within the hemocoel. Successful invasion and propagation within a host is determined by the ability of the fungus to enter the hemocoel and to suppress or avoid the host's immune responses (Götz, 1991). It has been demonstrated that cellular antifungal reactions such as phagocytosis and multicellular encapsulation are suppressed during the development of fungal diseases (Vey & Götz, 1986; Vilcinskas et al., 1997a). Although several studies have been endeavoured to evaluate the effects of fungal pathogens on cellular reactions, at present no data is available concerning modulation of humoral immune response during mycosis.

Several insect proteins that exhibit antimicrobial activity have been isolated and characterised (Hoffmann, 1995). Among the proteins involved in insect humoral immunity, lysozyme and cecropins have been shown to exhibit antibacterial as well as antifungal activities (Collins & Pappagianis, 1974; Jaynes, 1989; Vilcinskas, 1994). A remarkable increase in lysozyme activity within the hemolymph has been reported for many tested insect species after injection of soluble or particular microbial provokers (Götz &

Trenczek, 1991). In this study, lysozyme and cecropin-like activity within the cell-free hemolymph were used to monitor humoral immune response in *G. mellonella* larvae. To distinguish between these antimicrobial activities, we used lytic zone assay with freeze dried *Micrococcus luteus* to measure the extent of lysozyme activity and inhibition zone assays against living *Escherichia coli* to detect cecropin-like activity. The latter is known to be predominantly carried out by cecropins (Hoffman et al., 1981).

In order to study the effects of *B. bassiana* infection on the humoral immune response of *G. mellonella*, we compared lysozyme activity and anti-*E. coli* activity within the cell-free hemolymph of both untreated and diseased larvae 24 h after injection of bacterial (LPS or living *Enterobacter cloacae*) or fungal provokers (zymosan or living yeast cells). Additionally, using SDS-PAGE and autoradiography, we examined de novo synthesis of hemolymph proteins in both untreated and infected larvae after injections of bacterial and fungal provokers.

MATERIAL AND METHODS

Insects

Last instar larvae, each weighing between 250–350 mg, were used in the infection experiments and as a source of hemolymph samples. The larvae were reared on an artificial diet (22% maize meal, 22% wheat germ, 11% dry yeast, 17.5% bee wax, 11% honey and 11% glycerine) at 31°C in darkness. The larvae were infected with *B. bassiana* either by external contamination with conidia that were harvested from stationary cultures or injection of blastospores that were propagated within submerged cultures.

Injection and bleeding

Soluble (zymosan and LPS) or cellular (yeast cells, bacteria) provokers were dissolved or suspended within sterile physiological saline (172 mM KCl, 68 mM NaCl, 5 mM NaHCO₃, pH 6.1, 420 mosm) which was also used for control injections. Provoker solutions or suspensions and *B. bassiana* blastospore suspensions were injected dorsolaterally into the hemocoel of *G. mellonella* larvae by use of 1 ml disposable syringes and 0.4 × 20 needles (Luer) mounted on a microapplicator. The injected volume was always 10 µl. A total of 10 larvae were injected per provoker, and each treatment was replicated twice. Hemolymph samples were obtained as follows: last instar larvae were pre-cooled at 4°C for 15 min, one of each larvae's prolegs was pierced with a needle and hemolymph outflow was collected in sterile glass capillaries that were prepared with a few crystals of phenylthiourea to prevent melanization of the samples. Prior to use assays for antibacterial activity and SDS-gelelectrophoresis, hemolymph was rendered cell-free by centrifugation (2 × 10,000 g, 5 min, 4°C).

Preparation of LPS and zymosan

Lipopolysaccharide (LPS) from bacterial cell walls is a known soluble provoker, that induces high levels of antibacterial proteins in insects (De Verno et al., 1984). 1 mg LPS was dissolved in 1 ml saline, incubated for 30 min at 55°C and ultrasonicated (2 s). The soluble contents (B 1-3 glucans) of a zymosan suspension (preparation of freeze dried yeast cells) was used to provoke a humoral immune response. 20 mg of zymosan A (Sigma) were suspended in 1 ml sterile saline, homogenised with a whirlmixer and centrifuged (10,000 g, 5 min). The supernatant that resulted from a second centrifugation was injected. The injected volume of both solutions was always 10 µl per larva.

Fungi and bacteria

Fresh baker's yeast *Saccharomyces cerevisiae* was used as a non-pathogenic fungal provoker of humoral immune response. Yeast was grown overnight in submerged cultures at 37°C. The liquid medium consisted of 0.2% glucose, 0.1% yeast extract and 0.05% peptone dissolved in 0.1 M phosphate buffer, pH 7. For injection purposes, yeast cells were harvested from the medium by centrifugation (10,000 g, 10 min) and were then resuspended within sterile saline. This suspension was subsequently adjusted to the required cell concentration (10⁵ per larva) by use of counting chamber (type Bürker 0.1 mm, Brand).

B. bassiana strain M227 was obtained from the Institute of Entomology, Academy of Sciences of the Czech Republic. The fungus was cultivated at room temperature on Sabouraud agar with 2% glucose.

Conidia from stationary cultures were used to inoculate nutrient broth (25 g Standard I, Merck, and 20 g glucose dissolved in 1 l distilled water and autoclaved) upon a gyrotary shaker (200 rpm) and produce submerged cultures. Blastospores were harvested after 3 growth phase days by filtering the culture medium through sterile Miracloth (Calbiochem) which removed mycelia. The filtrate was centrifuged (10,000 g, 10 min) and the pellet containing the blastospores was resuspended and washed three times with sterile saline. After the final wash, counting chambers (Bürker) were used to adjust the concentration of blastospores. To distinguish different humoral immune response caused by injection of living or dead *B. bassiana* blastospores, a portion of the blastospores was heat-inactivated by incubation of the suspension within a water bath at 70°C for 30 min.

Enterobacter cloacae strain B12 was used to provoke a humoral immune response by injection of living bacteria. Bacteria were propagated in overnight cultures within nutrient broth (Standard I, Merck), harvested by centrifugation and resuspended in sterile saline. The concentration of the suspension was adjusted using Bürker counting chambers. The injected dose was always 2×10^5 bacteria per larva.

Assaying of antibacterial activity

Lysozyme-activity was monitored with a lytic zone assay against freeze-dried *Micrococcus luteus* (Sigma) in accordance with Mohrig & Messner (1968). 5 ml of sterile *M. luteus* agar (1% Agar L 11, Oxoid; 5 mg/ml freeze-dried *M. luteus*, Sigma; 0.1 mg/ml streptomycin sulfate, Serva and 67 mM potassium phosphate buffer, pH 6.4) was placed into Petri dishes (9 cm). Holes with a diameter of 3 mm were punched into the agar and filled with 4 µl of cell-free hemolymph. The diameters of the lytic zones were measured after 24 h of incubation in the Petri dishes maintained at 37°C. Hen egg white lysozyme (Sigma) was used for standardisation.

Activity against *Escherichia coli* was measured by inhibition zone assay with a lipopolysaccharide-defective, streptomycin- and ampicillin-resistant mutant of *E. coli* K12 strain D31 (Boman et al., 1974) in accordance with Faye & Wyatt (1980). Petri dishes (9 cm) were filled with 5 ml agar suspension (2.5% nutrient broth I, Merck; 1% Agar L11, Oxoid and 0.1 mg/ml streptomycin sulphate) containing 2×10^4 viable bacteria. Incubation of the agar plates and measurement of the diameters of the inhibition zones around the 3 mm holes filled with 4 µl cell-free hemolymph were performed as described above. Gentamycin (Serva) was used for standardisation.

SDS-PAGE and autoradiography

The protein patterns of the cell free hemolymph samples were compared using SDS-PAGE according to Laemmli (1970) with minor modifications. Gels containing 10% acrylamide (pH 8.8) and 5% stacking gels (pH 6.6) were used. Staining was performed with Coomassie Blue (R250, Serva). Synthesised proteins released into the hemolymph of *G. mellonella* larvae during humoral immune response were detected by radioactive labelling, and was enabled by injections of ^{14}C labelled protein hydrolysate (100 µl dissolved 900 µl saline = 900 MBq/ml) 4 h before bleeding. Radioactivity within the hemolymph was measured using a liquid scintillator (Liquid scintillator SLD-31, Chemopetrol Neratovice) and β-ray-counter (2050 CA Tri carb liquid scintillation analyser, Packard Instruments Company). To visualise labelled proteins, the gel was first soaked in Amplify (Amersham) for 30 min, subsequently dried and analysed by fluorography (Hyperfilm-β max, Amersham). The molecular weight was estimated according to Rainbow markers.

RESULTS

Humoral reactions within the hemolymph in response to infection with *B. bassiana*

We observed no increased levels of lysozyme-activity (Fig. 1) or detectable anti-*E. coli*-activity (data not shown) in *G. mellonella* larvae infected with the conidia of *B. bassiana* that had germinated on their chitinous integument and entered the hemocoel in a natural manner. In confirmation, no differences between the protein patterns of cell-free hemolymph samples from untreated and naturally infected larvae were detected after separation of hemolymph proteins using SDS-polyacrylamide gel electrophoresis (Fig. 2). Hyphal bodies occurred within the hemolymph three days after infection (data not shown).

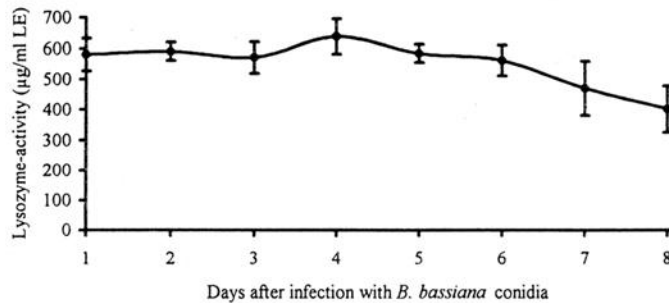


Fig. 1. Lysozyme activity ($\mu\text{g/ml}$ hen-egg-white lysozyme-equivalents) within the cell-free hemolymph of *G. mellonella* larvae after infection with *B. bassiana* conidia. No significant changes in lysozyme activity were detected during mycosis. Data are presented as means \pm standard deviations from 10 larvae.

Strongly enhanced levels of lysozyme and anti-*E. coli*-activity in the cell-free hemolymph of *G. mellonella* larvae were detected after injection of bacterial provokers (LPS or living *E. cloacae*) or fungal provokers such as zymosan, living yeast cells and living or heat-inactivated blastospores of *B. bassiana*. Maximum levels observed 24 h after treatment were equivalent to approximately 10 mg/ml hen-egg-white lysozyme or 6 mg/ml gentamycin and were determined by lytic zone assay against freeze dried *M. luteus* and

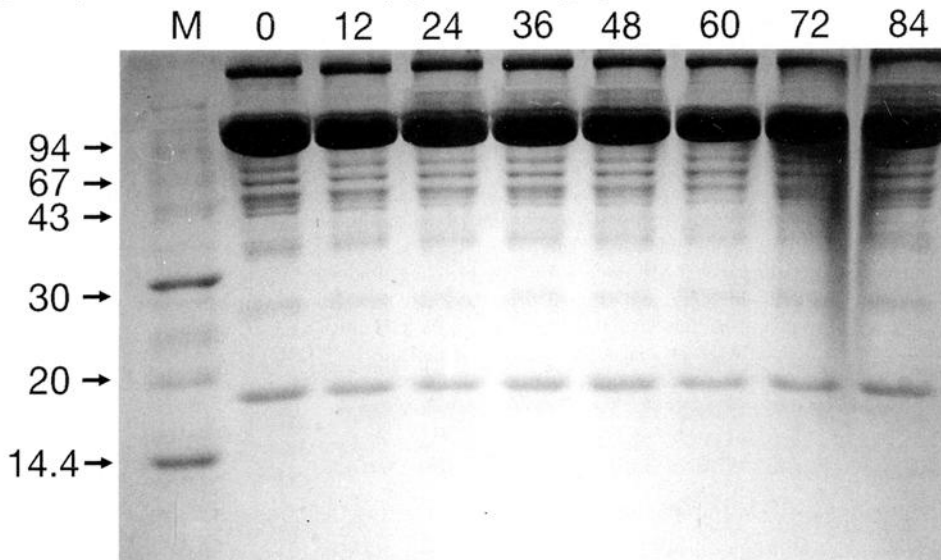


Fig. 2. Electrophoretic patterns of cell-free hemolymph samples from *G. mellonella* larvae analysed in SDS-PAGE gel and stained with Coomassie blue R250. Hemolymph samples were obtained from untreated *G. mellonella* larvae and larvae that were infected with *B. bassiana* conidia so that mycosis was initiated in a natural manner. No changes in hemolymph protein patterns or enhanced levels of lysozyme (15 kDa) were detected during mycosis. M – marker proteins; 0 – untreated larvae; hemolymph samples were obtained 12, 24, 36, 48, 60, 72 and 84 h after infection with *B. bassiana* conidia. Left lane: molecular weight of marker proteins.

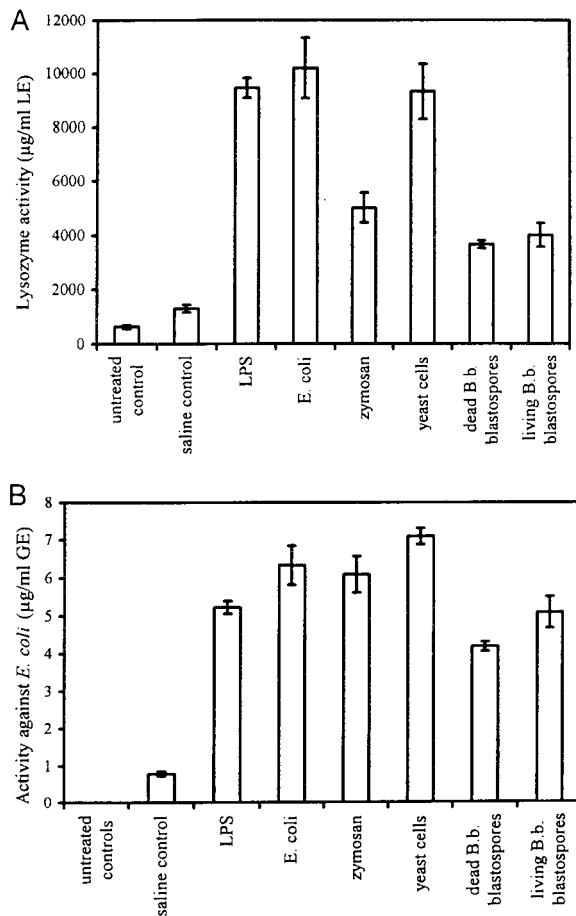


Fig. 3A–B. A – lysozyme activity ($\mu\text{g/ml}$ hen-egg-white-lysozyme-equivalents); B – antibacterial activity against *E. coli* ($\mu\text{g/ml}$ gentamycin-equivalents) within the cell-free hemolymph of *G. mellonella* 24 h after injection of LPS, living *E. coli* (10^5 per larva), zymosan, yeast cells (10^5 per larva) and dead or living blastospores of *B. bassiana* (10^5 per larva). Lysozyme activity and anti-*E. coli* activity were significantly enhanced as compared with untreated or saline injected larvae. Data are presented as mean \pm standard deviations from 10 larvae.

an equivalent dose of heat-inactivated blastospores. Both types of antibacterial activity declined faster when mycosis was initiated by living blastospores. During a later stage of mycosis, lysozyme-activity within diseased larvae was lower in untreated larvae (Fig. 5). Larvae, that had not been used as a source of hemolymph survived the injection of heat-inactivated blastospores, whereas those infected with living blastospores died within 4 days. Infection with *B. bassiana* conidia caused mycosis which killed the larvae within one week.

inhibition zone assay using living *E. coli*, respectively. Although yeast cells and *B. bassiana* blastospores were injected in the same concentrations, the latter induced a weaker immune response (Fig. 3). These results were confirmed by electrophoretic analysis of cell-free hemolymph samples. The protein pattern of hemolymph samples obtained from injected larvae exhibited a stronger lysozyme band than those from untreated larvae. Among the proteins induced during humoral immune response, the lysozyme band around 15 kDa was more prominent after injection of both bacterial and fungal provocators, suggesting its non-specific induction (Fig. 4).

Humoral immune response of *B. bassiana*-infected *G. mellonella* larvae

A significant increase in antibacterial activity within the hemolymph was observed in response to injection of either heat-inactivated or living *B. bassiana* blastospores, and maximum levels were recorded 24 h after treatment. Injection of living *B. bassiana* blastospores resulted in higher hemolymph lysozyme and anti-*E. coli*-activity than that caused by

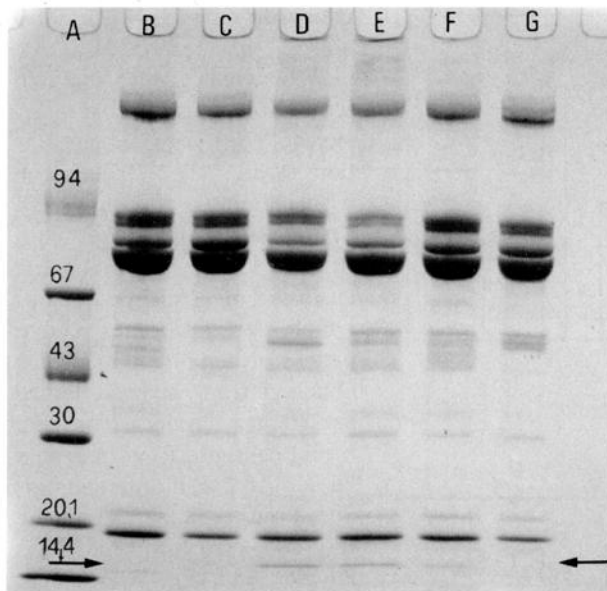


Fig. 4. Protein patterns from *G. mellonella* cell-free hemolymph samples analysed in SDS-PAGE and stained with Coomassie blue R250: A – marker proteins (94, 67, 43, 30, 20.1 and 14.4 kDa), untreated larvae (B), larvae injected with saline (C), *E. coli* (D), zymosan (E), heat-inactivated yeast cells (F) or heat-inactivated *B. bassiana* blastospores (G). The lysozyme band around 15 kDa was more pronounced (arrows) in the hemolymph of larvae that had been injected 24 h before hemolymph collection. The intensity of the lysozyme band corresponds to lysozyme activity measured in hemolymph samples by lytic-zone assay (see Fig. 3).

The relative increases in lysozyme and anti-*E. coli*-activity observed in the hemolymph of *G. mellonella* larvae 24 h after injection of bacterial or fungal provocators were significantly lower than in *B. bassiana*-infected larvae. Injection of living blastospores (10^4 per larva) 48 h before the introduction of microbial provocators resulted in weakened humoral responses, whereas no suppression of humoral immune response was observed after injection of heat-inactivated *B. bassiana* blastospores (Fig. 6). In comparison to untreated larvae, the injection of living yeast cells into *B. bassiana*-infected larvae induced significantly lower levels of lysozyme activity and anti-*E. coli*-activity within the cell free hemolymph, but this was dependent upon the amount of time between the injection of the elicitor and the timing of blastospore infection. Little or no inhibition was observed at

all when the yeast cells were injected immediately or within one day after introduction of living *B. bassiana* blastospores, whereas injection that took place between two and three days after infection produced a weaker humoral immune response in comparison with untreated larvae (Fig. 7). Larvae that had not been used as a source of hemolymph samples died within 4 days after infection with equal doses of blastospores.

We also studied the induction of lysozyme and anti-*E. coli*-activity after previous injection of bacterial or fungal provocators in larvae, that were infected with *B. bassiana* conidia and thus exposed to mycosis in a natural manner. The results confirmed, in principle, our findings mentioned above, but when the experiment was replicated, significant suppression of the humoral immune response was detected at different times after initiation of mycosis. It is difficult to obtain reproducible data when mycosis is initiated with conidia that infect the host in a natural manner because the number of germinating conidia per larvae and the time required for germination and subsequent penetration of host's cuticle cannot be sufficiently controlled. To address this problem, we infected *G. mellonella* with both living or heat-inactivated *B. bassiana* blastospores in the same concentration to

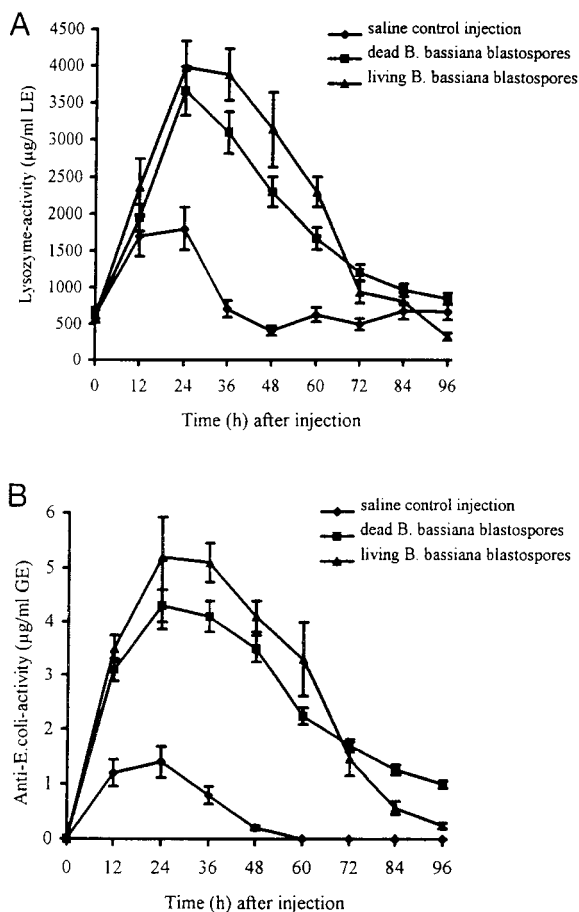


Fig. 5A–B. A – lysozyme activity ($\mu\text{g/ml}$ hen-egg-white-lysozyme-equivalents); B – antibacterial activity against *E. coli* ($\mu\text{g/ml}$ gentamycin-equivalents) within the cell-free hemolymph of *G. mellonella* after injection of dead or living blastospores of *B. bassiana*. Hemolymph samples were obtained in 12 h intervals. Lysozyme activity and anti-*E. coli* activity were enhanced after injection, reaching maximum levels after 24 h and then declining faster in infected larvae (injected with living blastospores). Data are presented as means \pm standard deviations from 10 larvae.

antimicrobial compounds are synthesised in response to cuticular abrasion (Brey et al., 1993), it could be expected that invading fungal structures could also elicit a humoral response. It is possible that lesions caused by invading hyphae are insufficient to provoke a detectable humoral reaction within the hemolymph. Successful penetration of the integument following treatment with conidia resulted in subsequent formation of hyphal bodies within the hemolymph. Although their development was not accompanied with detectable

discriminate between potential effects induced by the injection itself and those caused by development of the fungus.

The use of SDS-PAGE and radioactive labelling of proteins that were synthesised during the humoral immune response showed that protein synthesis within *B. bassiana*-infected larvae was generally not suppressed as compared with untreated larvae. Numerous new or enhanced bands were visible after autoradiography analysis of hemolymph samples collected from larvae that had been injected with various provocators 24 h before bleeding. At least one band was weak or undetectable in hemolymph samples from untreated larvae. According to the molecular weight of induced proteins, the lysozyme band was less prominent in diseased larvae, thus confirming the results from the lytic zone and inhibition zone assays (Fig. 8).

DISCUSSION

The entomopathogen *B. bassiana* did not provoke any detectable humoral reactions within *G. mellonella* larvae when mycosis was initiated with conidia that infected them in a natural manner (Figs 1, 2).

Due to previous reports that anti-

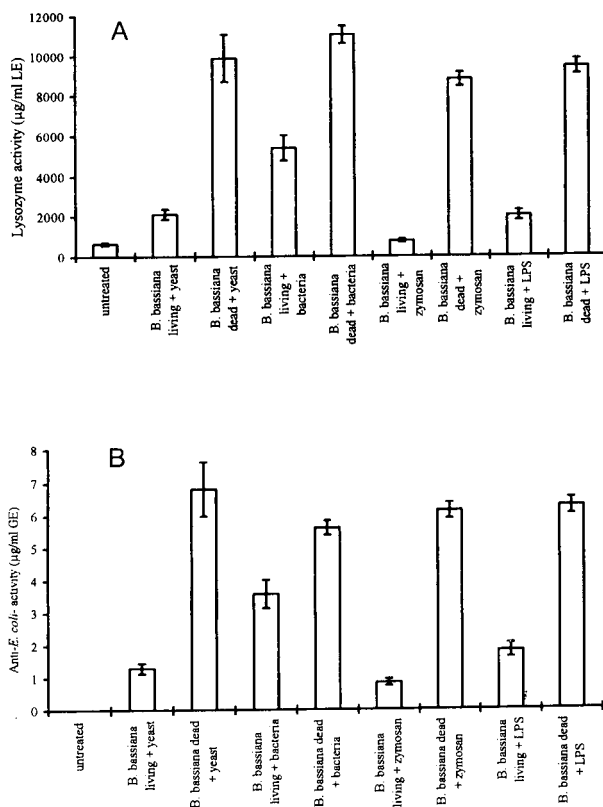


Fig. 6A–B. A – lysozyme activity (µg/ml hen-egg-white-lysozyme-equivalents); B – antibacterial activity against *E. coli* (µg/ml gentamycin-equivalents) within the cell-free hemolymph of *G. mellonella* 24 h after injection of yeast cells (10^5 per larva), living *E. coli* (10^5 per larva), LPS or zymosan in larvae which have been preinjected 48 h before with dead or living blastospores of *B. bassiana* (10^5 per larva). The enhanced levels of lysozyme activity and anti-*E. coli* activity were lower in larvae that were infected by injection of living *B. bassiana* blastospores. Data are presented as mean \pm standard deviations from 10 larvae.

humoral immune reaction, injection of blastospores did elicit antimicrobial activity within the hemolymph. The absence of humoral defense reactions during natural mycosis development could either be explained by the formation of hyphal bodies that lack β -1-3 glucans on the cell surface, which is a substance required by plasmacytes for fungal recognition (Mařha et al., 1990), or the release of fungal secondary metabolites that reportedly incapacitate the hosts' cellular immune reactions (Gillespie & Claydon, 1989; Tanada & Kaya, 1993; Khachatourians, 1996; Vilcinskas et al., 1997a,b). However, the decline of enhanced lysozyme activity and anti-*E. coli* activity within the hemolymph after injection of living blastospores was more accelerated than in control larvae that were injected with an equal dose of dead blastospores. This was the first indication that the humoral immune response of *G. mellonella* in diseased larvae was influenced. This observation was confirmed by comparative studies with injections of different microbial elicitors in naturally infected and untreated larvae.

The capacity to release certain immune proteins after injection of bacterial or fungal provokers was significantly lower in larvae that were infected two or three days before with *B. bassiana* by injection of blastospores. Larvae injected with heat inactivated blastospores exhibited no weakened humoral response. From this information, we conclude that the de novo synthesis of antibacterial proteins and their release into the hemolymph is affected by development of the fungus. Suppression of the humoral response against injected yeast cells was obvious two or three days after initiation of mycosis, as shown by lytic zone and inhibition zone assays used to monitor lysozyme and cecropin-like activity.

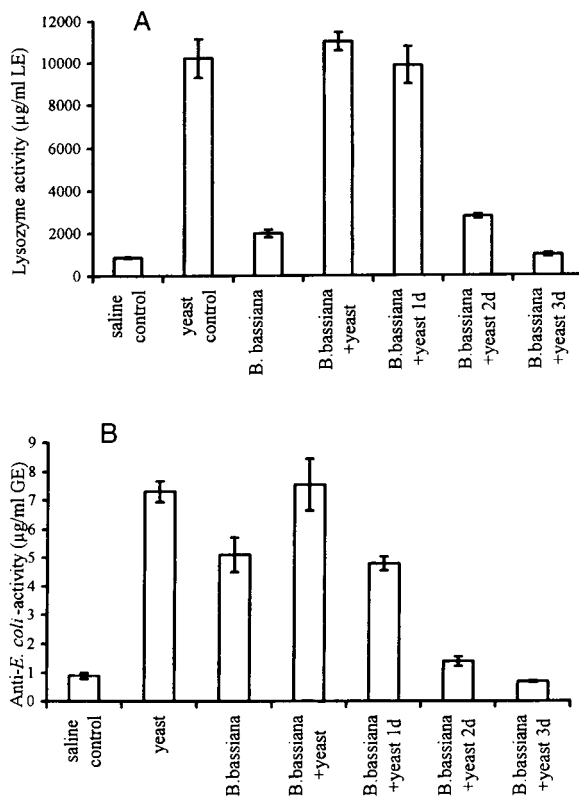


Fig. 7A-B. A - lysozyme activity ($\mu\text{g/ml}$ hen-egg-white-lysozyme-equivalents); B - antibacterial activity against *E. coli* ($\mu\text{g/ml}$ gentamycin-equivalents) within the cell-free hemolymph of *G. mellonella* 24 h after injection of yeast cells (10^5 per larva) in larvae which were preinjected with living *B. bassiana* blastospores (10^5 per larva) 1, 24, 48 or 72 h before. Controls: Lysozyme and anti-*E. coli* activity 24 h after injection of saline, yeast cells or *B. bassiana* blastospores alone. Data are presented as mean \pm standard deviations from 10 larvae.

results revealed from the lytic zone and inhibition zone assays. These data exclude both the possibility that proteases released by the fungus in infected larvae non-specifically digested hemolymph proteins and the possibility that a damping effect on host protein synthesis was due to impaired metabolism.

Although numerous studies have been undertaken to evaluate the suppression of host cellular immune reactions through entomopathogenic fungi (Khachatourians, 1996), as yet we still have only fragmentary knowledge concerning the effects of fungi upon host humoral immunity. The results presented herein are the first to support the theory that entomopathogenic fungi are able to suppress humoral immune reactions of host insects. However, there are several groups of pathogens, parasites and parasitoids that have been

Although we have no evidence that these proteins affect the development of *B. bassiana* in *G. mellonella* larvae, we determined suppression of their synthesis by the fungus.

However, the limited increase of antimicrobial proteins within the hemolymph of diseased larvae does not alone verify specific suppression of the humoral response by the fungus. The induced levels could be lower due to non-specific disturbances of the host's metabolism caused by the development of the entomopathogen. Subsequent studies using radioactive labelling indicate that proteins synthesised during the humoral immune response of infected larvae, even in a late stage of mycosis, were not suppressed in general. The vast majority of synthesised proteins were not affected by the fungus.

Comparison of the electrophoretic profiles of radioactive-labelled proteins within hemolymph samples showed that at least one band, according to the molecular weight lysozyme was less abundant in the hemolymph of larvae infected with *B. bassiana*, confirming the re-

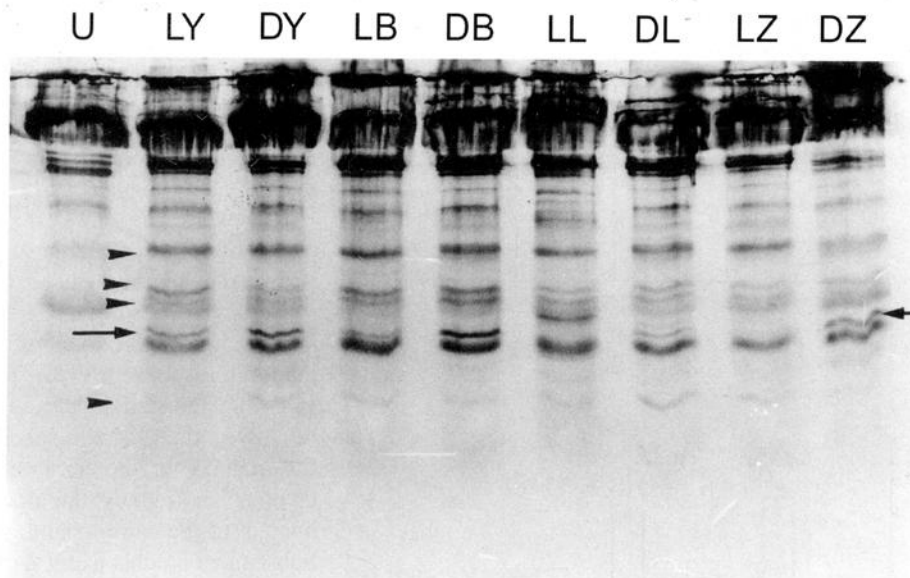


Fig. 8. Electrophoretic pattern of proteins synthesised during humoral immune response and labelled with injected ^{14}C protein lysate (4 h before bleeding). Cell-free hemolymph was obtained from *G. mellonella* larvae that were previously injected with dead (D) or living (L) blastospores of *B. bassiana* and 48 h later with yeast cells (10^5 per larva) (Y), living *E. coli* (10^5 per larva) (B), LPS (L) or zymosan (Z). Larvae were bled 24 h after the second injection. Untreated larvae were used as controls (U). (→) denotes new or enhanced bands of protein synthesised during humoral immune response provoked by injections; (←) denotes synthesised proteins that occurred weaker or were not detectable in larvae infected with living blastospores.

reported to suppress humoral immune reactions in insect hosts. For example, the bacterial entomopathogen *Bacillus thuringiensis* was shown to release inhibitors which interfere with the humoral defence system, therefore contributing to its insecticidal nature (Edlund et al., 1976). A parasitic nematode has also been reported to impair humoral immune reactions of insect host, thereby protecting its symbiotic bacteria as well (Götz et al., 1981). Among parasitoids, several Hymenoptera are known to suppress other insect's humoral defense reactions (Vinson, 1993). Similarly, entomopathogenic fungi also develop in the hemocoel of their hosts, where they are exposed to humoral and cellular defense reactions. More than 50 cationic peptides or polypeptides with antibacterial properties have been isolated from immunised insects (Cociancich et al., 1994). Only a limited number of these purified proteins exhibited antifungal activity in vitro (Fehlbaum et al., 1995; Iijima et al., 1993). In addition to their antibacterial activity, lysozyme and cecropins have also been shown to impair the growth of fungi and are widely distributed throughout the Lepidoptera and Diptera (Collins & Pappagianis, 1974; Jaynes, 1989; Vilcinskas, 1994; Vilcinskas & Matha, 1997). With regards to the high quantities of such proteins released and determined within the hemolymph of *G. mellonella* larvae after injection of bacterial or fungal provokers, the suppression of their synthesis during mycosis may enable development of the fungus within the hemolymph.

From present knowledge, it is difficult to evaluate how *B. bassiana* influences only the synthesis of antibacterial proteins during the humoral immune response in *G. mellonella*. The induction of immune proteins in insects is probably controlled by different mechanisms. Lemaitre et al. (1995) reported the existence of two different pathways leading to the expression of two types of target genes, that encode either antibacterial peptides or the antifungal peptide drosomycin in *Drosophila*. In our study the injection of both bacterial and fungal elicitors enhanced the lysozyme and anti-*E. coli* activity in the hemolymph by comparable levels, suggesting non-specific induction during humoral immune response of *G. mellonella*. No obvious differences were observed among proteins induced after injection of fungal or bacterial provocators and autoradiography of hemolymph proteins separated by SDS-PAGE. We have no evidence to indicate different pathways for the induction of either antibacterial or antifungal peptides in *G. mellonella* until proteins which exclusively act against fungi are discovered in the species. The synthesis of antimicrobial proteins in insects during humoral immune response is predominantly localised in hemocytes and in fat body. The partially suppressed induction of lysozyme and cecropins is probably a side effect of impaired cellular defense reactions observed in infected *G. mellonella*. Entomogenous fungi inhibit the phagocytic activity, attachment and spreading of host plasmatocytes during mycosis by liberated secondary metabolites such as destruxins and cytochalasins (Vilcinskas et al., 1997a,b). Plasmatocytes represent the major phagocytic cell type in *G. mellonella* and normally release factors during immune reactions that trigger the synthesis of antibacterial proteins in fat body (Faye & Wyatt, 1980; Hoffman, 1995; Ratcliffe, 1993). The inhibition of plasmatocyte activity during mycosis could influence their capacity to stimulate the synthesis of antimicrobial proteins. Further investigations concerning the mechanism of the suppression of host immune reactions through entomopathogenic fungi are in progress.

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