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PROTEOLYTIC AND LIPOLYTIC ENZYMES FROM LARVAE OF OEDEMAGENA TARANDI (L.)

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AURSTAD, KJELL and ALV HELGE SKEIE: *Proteolytic and lipolytic enzymes from larvae of Oedemagena tarandi (L.).* Acta vet. scand. 1973, *14*, 168—175. — Some biological properties of proteolytic and lipolytic enzymes of the larval stages of Oedemagena tarandi (L.) were examined. While the proteinase titres seemed to vary only slightly from one larval stage to another, the lipase titres of the first larval stage seemed to be much greater than those of the second and third larval stages. The zymogram technique used showed only one proteinase, which was inhibited by all the biological inhibitors tested. Bacteriological examinations of the external surfaces and internal organs of the different larval stages showed only a very sparse flora.

Oedemagena tarandi (L.); proteolytic enzyme; lipolytic enzyme; biological inhibitors.

Intensive studies on the biology and the economic importance of Oedemagena tarandi (L.) have been made by *Hadwen* (1926), *Breev & Karazeeva* (1952, 1953) and *Breev & Savelev* (1954).

Although *Thorsell & Nordquist* (1962) described some hydrolyzing enzymes in larvae of O. tarandi, little is known about the biological properties of the enzymes of the larval stages of this insect. Knowledge of the enzymes secreted by the immature, and adult, stages of insects may be of value in the search for new insecticides (*Thorsell & Nordquist*). Furthermore, such enzymological studies may be of significance for meat spoilage prevention, and in taxonomic and phylogenetic work (*Dahle et al.* 1971, *Hilali et al.* 1972).

The aim of the present work was to elucidate some of the biological properties of the proteinases and lipases of the im-

mature stages of O. tarandi. Furthermore, it was considered worthwhile to determine the normal bacterial flora of the larvae of this insect.

MATERIALS AND METHODS

Collection of larvae

First, second and third stage larvae of O. tarandi were collected from subcutaneous tissue of freshly slaughtered reindeer in the northern part of Norway. The material was delivered to the laboratory no later than 35 hrs. after slaughtering, and then stored at —20°C until used.

The identification of the larvae was performed according to *Zumpt* (1965).

Enzymological examinations

The material to be examined was macerated and centrifuged. The supernatants were removed by suction and stored at —20°C until used. The supernatants were examined for proteinases according to the casein precipitating method of *Sandvik* (1962), and for lipases according to the agar gel method, incorporating tributyrin as substrate (*Ellinghausen & Sandvik* 1965).

Titrations of the proteinase activities were carried out by transferring aliquots of 25 µl from serial 2-fold dilutions of the solutions to wells of 7 mm diameter in sodium caseinate containing agar, followed by incubation at 37°C for 16 hrs. The estimation of diffusion units was based on the diameters of the precipitation zones which occurred (*Dahle* 1969).

The effects of naturally occurring proteinase inhibitors of various animal sera on the proteinases of the larval stages of O. tarandi were tested by the crosswise casein precipitation inhibition test (CPI-test) of *Fossum* (1970).

Zymograms of the proteinases of the larvae were prepared in agar gels by the electrophoretical procedure described by *Dahle* (1970).

Bacteriological examinations

The external surfaces and internal organs of the larvae were examined according to general bacteriological procedures. Incubations were performed aerobically, anaerobically, and in an atmosphere of 10 % CO_2 at 30°C and 37°C, and the organisms

were identified on the basis of cultural and physiological properties (*Breed et al.* 1957).

RESULTS

The extracts of the macerated larvae of different stages were examined for proteinases and lipases by two separate agar diffusion methods. Fig. 1a shows precipitation zones of casein in agar gel caused by proteinases extracted from the third stage larvae, while Fig. 1b shows transparent zones of hydrolyzed tributyrin caused by lipases from the same sample. The figure

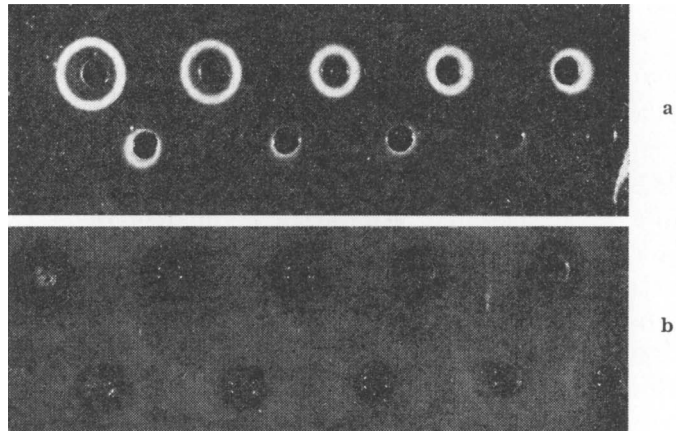

Figure 1. Titration of casein precipitating enzymes in sodium caseinate-agar gel (a), and lipolytic enzymes in tributyrin-agar gel (b). The titres of casein precipitating enzymes are determined using standard curves obtained when the diameters of the zones, caused by the enzymes in serial 2-fold dilutions, are plotted against the logarithm of the degree of dilution.

also shows the decreasing diameters of the zones throughout the dilution series, and this was used as the basis for the estimation of the concentrations of the enzymes in the various extracts.

Using the procedure for the estimation of diffusion units described by *Sandvik* (1962), and modified by *Dahle* (1969), the proteinase concentration in the particular enzyme solution prepared was of the order of ca. 16,000 diffusion units per ml. The

proteinase concentrations seemed to vary only slightly from one larval stage to another.

The tributyrinase titres for the different larval stages are listed in Table 1. The first larval stage seemed to possess much greater tributyrinase activity than the third stage larvae.

Table 1. Tributyrinase titres of different larval stages of Oedemagena tarandi (L).

Samples	Titres
First stage larva	1:4096
Second stage larva	1:2048
Third stage larva	1: 512

In order to investigate whether more than one proteinase was present in the extracts, zymograms were prepared in agar gels. However, these revealed only one proteinase, with cathodic migration, at pH 6.2.

In contrast to the larvae of Calliphora erythrocephala, no external lipolytic or proteolytic enzymes could be demonstrated in any of the larval stages of O. tarandi (*Dahle et al.* 1971).

The crosswise CPI-test was used to examine for resistance against naturally occurring proteinase inhibitors, and inhibitors from various blood sera were tested. The effects of some of these inhibitors are shown in Fig. 2, where it can be seen that the proteinases are inhibited by all the inhibitors studied.

The external surface and internal organs of the larvae were examined bacteriologically. By the methods used, only a very sparse flora consisting of micrococci and enterobacteria was isolated from the different larval stages of O. tarandi.

DISCUSSION

Proteinases and lipases from insects have been thoroughly described by several authors (*Thomsen* 1952, 1954, *Thomsen & Møller* 1959, 1963, *Dahle et al.* 1971 and *Hilali et al.* 1972). Among the food spoiling enzymes, proteinases and lipases are of particular interest (*Dahle* 1971b), and they can be conveniently demonstrated by the agar diffusion methods used in the present study.

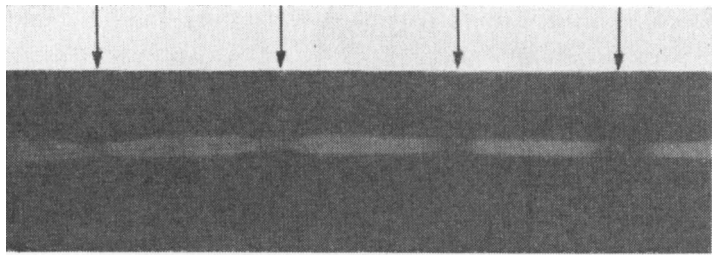

Figure 2. Demonstration of proteinase inhibitors by the crosswise CPI-test. Narrow filter paper strips moistened with solutions of inhibitors were placed on sodium caseinate-agar plates, and incubated for 3 hrs. at 37°C. After removal of these strips, a similar strip, moistened with the proteinase solution, was applied to the surface of the agar gel at right-angles to the direction of application of the inhibitors, and the plate incubated for a further 16 hours at 37°C. Proteolytic activity is indicated by the greyish-white zones in the transparent agar, while inhibition is indicated by interruptions in the precipitation zones. The inhibitors are, from left to right as marked by arrows: reindeer-serum, cow-serum, horse-serum and sheep-serum.

Although the determinations of the enzymes are semiquantitative, and do not represent the exact amounts of enzymes present in the materials, the casein precipitating activity, and the lipolytic activity of the solutions indicated that the larvae of O. tarandi possessed very high biocatalytic activities (Fig. 1 and Table 1). This is also in accordance with the findings of *Dahle et al.* and *Hilali et al.* for the larvae of Calliphora erythrocephala, and larvae and imagos of Dermestes landarius. While there was no distinct difference in the proteolytic activities of the three larval stages of O. tarandi, the first larval stage seemed to have a much higher lipolytic activity than the other ones (Table 1). The problem of induced enzymes in animals and insects has not been thoroughly analysed as in certain types of yeasts and bacteria (*Rose* 1968, *Dahle* 1971a). Some reports from entomologists, however, indicate that the problem is of importance for insects. Thus *Thomsen & Møller* (1963) found that the proteolytic activity of the adult female of Calliphora erythrocephala is highly influenced by the diet during the first 5 days after emergence.

The high lipolytic activity of the first larval stage of O. tarandi may be connected with the migration of this larva in the

connective tissue and the muscles towards the rump and spine of the host.

The existence of more than one proteinase fraction in proteinase producing organisms is a basic problem in nature which has been discussed for some bacterial proteinase systems by *Dahle* (1971b). *Hilali et al.* demonstrated three proteolytic fractions from macerated larvae and imagos of Dermestes lardarius using the zymogram technique. However, not more than one proteinase fraction could be demonstrated from macerated larvae of O. tarandi using the same technique. At present, the zymogram technique for lipases has not been finally standardized, but the number of lipase fractions and their mutual relationships are also of interest, together with the proteinases, for possible application in taxonomical and phylogenetic studies (*Hilali et al.*).

In contrast to inhibitors against proteinases, naturally occurring inhibitors against lipolytic enzymes have not been demonstrated in animal blood sera. It is difficult to give a biological interpretation of this phenomenon although the problems could be of significance for developing control systems for insects.

Bacteriological examinations were carried out in order to elucidate wether transmission of putrifying microorganisms through the larval stages in the life cycle of the warble-fly occurred. The results indicate, however, that only a sparse flora is to be found in the larvae. The microorganisms found may be due to contamination arising during the transport to the laboratory of the material for the investigations.

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SAMMENDRAG

Proteolytiske og lipolytiske enzymer fra larver af Oedemagena tarandi (L.).

Biologiske egenskaper hos proteolytiske og lipolytiske enzymer fra larvestadier hos Oedemagena tarandi (L.) ble undersøkt. Mens proteinasetitret fra det ene larvestadiet til det andre varierte bare ubetydelig, ble lipase-titret hos det første larvestadiet funnet å være mye større enn hos det andre og tredje larvestadiet. Med hjelp av zymogram-teknikk kunne det bare påvises en proteinase, og denne ble inhibert av alle de biologiske inhibitorene som ble prøvd. Bakteriologiske undersøkelser av overflate og indre organer av de forskjellige larvestadier viste bare en meget sparsom flora.

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