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A modified test for chitinase and cellulase activity in soil mites

Jaroslav Smrž

Department of Zoology, Charles University, Vinična 7, Praha 2, 128 44, Czech Republic

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Summary

The histochemical method of Jaspar-Versali et al. (1982) was modified for the detection of enzyme activity in homogenate of soil mites.

Key words: Tissue homogenate, oribatid and acarid mites, enzyme, activity

Introduction

Chitin and cellulose form the substantial bulk of the food of saprophagous soil mites. Enzyme production in soil animals (frequently including chitinase and cellulases) have been tested especially by application of colorimetry (Siepel & Ruiter-Dijkman 1993; Urbášek & Starý 1994), due to no-reducing substances for polysaccharides (Fehlings method see Marcuzi & Lafisca 1978) or by Aminoff's method for chitinproducts (op.cit.), by radioisotope labelling (Zinkler et al. 1986), pure chemical methods (Luxton 1972) or electrophoresis (Wauthy et al. 1985; Ziegler & Wauthy 1987; Ziegler et al. 1990). Cellulose digestion has been reviewed by Martin (1983). Jaspar-Versali et al. (1982) detected chitinase and cellulse secretion within the alimentary tract of the carabid beetle *Abax ater*. They used the thin film of the substrate (chitin or cellulase) underlying frozen histological sections of gut. After incubation, toluidin blue stained all the film except the area adjoining the enzymatically active gut cells. This histochemical method is here somewhat modified for simple qualitative enzyme detection as the first step in the study of the nutritional biology of saprophagous soil mites.

e-mail: smrz@mbox.cesnet.cz

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Materials, Methods and Results

The microslides were coated as described in the original method: Cellulose film consisting of a solution (3 %) of carboxymethyl cellulose (Sigma). Chitin (Sigma) had to be dissolved (3 %) by the process of Trujillo (1968) resulting in carboxymethylchitin. Both dissolved substrates were dropped onto clear microslides, which were orientated into the nearly vertical position to form the thin chitin layer. After drying in this position at the laboratory temperature (22–25°C), the coated microslides were fixed as described by Jaspar-Versali et al. (1982).

Mites were sterilized before the homogenization to remove microorganisms from the body surface. Ethyl alcohol (96 %)was applied for 2 minutes (see Smrž et al. 1991) followed by 10 % detergent solution (common laboratory cleaning detergent i.e. Czech commercial products "Jar" or "Pur") in distilled water (2 min) and finally distilled water only (2 min). The control plating of the last supernatant was microbiologically negative on 2 % MPA (pH 7) as well as on malt agar (2 % MPA + malt extract, pH 6) in all tests. The sterilized mites were homogenized on sterilized microslides in a drop (approx. 0.5 ml) of sterilized distilled water (pH 6.3) using a glass rod with a roughened end.

The homogenate on the microslide was then placed for into a closed plastic box at 100 % humidity and incubated 24 hours at laboratory temperature (22–25°C). After incubation, microslides were dried under laboratory temperature and stained with 2 % basic fuchsin (Gurr) for 30 minutes and then rinsed with distilled water. Chitinase activity was disclosed by staining on the hotplate at approximately 35°C. Enzyme activity digestion ot the substrate – of the drop of homogenate was indicated by the unstained area within the stained microslide coat as a whole.

The number of mites used depended on their size and grazing activity. Approximately, 1–3 mites about 1 mm in length (e.g. *Damaeus onustus*) can be used for significant detection. The number of tested mites, of course, increased with their decreasing size. Ten individuals of *Tyrophagus putrescentiae* (size approx. 0.3 mm) were enough for the significant test. Other oribatid mites (*Scutovertex minutus, Trichoribates trimaculatus, Steganacarus magnus*) were also examined.

Discussion

The histoenzymological test of Jaspar-Versali et al.(1982) is simple and sufficient for qualitative purposes. The modification described above was prompted by the somewhat different requirements of this in our laboratory. The concentration of substrates appeared to be the crucial point. Their film had to be thin on microslides in order to be digested during the relatively short time and under low temperature of the incubation. The 4 % solution of cellulose or chitin formed too a thick layer for these purposes. The staining contrast, however, appeared to be sufficient at this concentration. The lower concentration of substrate (2 %) formed a very thin film, but it was very poorly stained. In the case of the low enzyme production, the intensity of colour was only decreased in the digested area (not quite blank). Hence, it was difficult to distinguish it from the other (undigested, but poorly stained) film. This was especially the case with chitin, whereas cellulose was stained more intensively. Therefore, a 3 % solution was applied for both substrates.

The major modification was represented by the dye used. The original one – toluidin blue –was substituted by basic fuchsin, which yielded a more contrasting staining, especially of chitin, in this application. Moreover, the pH values are of minor importance here in comparison with the toluidin blue. The colour intensity increased on the hot plate during staining in chitin, whereas cellulose was stained sufficiently under laboratory temperature.

This method exhibited relatively high sensitivity. The homogenate of one individual *Damaeus onustus* or *Steganacarus magnus* resulted in a clear unstained area, whereas as ten individuals of *Tyrophagus putrescentiae* were requested (see above). Of course, the secretion of enzymes depend also on consumption activity, type and palatability of food or abiotic environmental factors (Smrž 1996; Smrž & Catská 1987, 1989; Smrž et al. 1991). This test, however, seems to be suitable for the first step of study i.e. simple detection of chitinase or cellulases.

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