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ZDZISŁAW GLIŃSKI, MARIA SWOBODA-MAZUREK

Bacteriolytic activity of hemolymph of the hibernated queens of Bombus agrorum – a preliminary study

Aktywność bakteriolityczna hemolimfy matek *Bombus agrorum* w okresie hibernacji – wstępne obserwacje

K e y w o r d s: bumble bee, hibernation, bacteriolytic activity, lysozyme

S ł o w a k l u c z o w e: trzmiel, hibernacja, aktywność bakteriolityczna, lizozym

The hibernated bumble bees are exposed in the environment to attacks of a great number of parasites, bacterial and viral pests. Most of them cannot trespass the anatomical and physiological barriers of the insect body cavity and infect the hemocoel. Although the efficacy of anatomical and physiological barriers protecting insects against infection is very high, bacteria can penetrate into hemocoel via wounds, cuticle and gut abrasions.

The *Apoidea*, like other species of insects, are able to resist the development of disease by immune phenomena even after the microorganisms, notable bacteria, have entered the body cavity. The immune phenomena in the honey bee are realized by recognition of self from non-self, expelling or rendering it not dangerous to the bee host. Haemocytic and cell-free immune responses mediate these key defense mechanisms of immunity.The cellular and humoral immunity are interrelated (1, 4).

The experiments in insects showed that non-immune hemolymph has a bactericidal effect on a variety of bacterial species, mainly against Gram-positive saprophytic bacteria. This antibacterial activity of a naive hemolymph is associated mainly with the presence of lysozyme (1, 7). In holometabolous insects both lysozyme and phagocytosis together play a key role in antibacterial defense in the early phase of the body cavity infection (5).

The objective of the preliminary studies was to measure the level of bactericidal activity of hemolymph lysozyme in the hibernating queens of *Bombus agrorum*.

MATERIAL AND METHODS

Bumble bees. Twenty-seven hibernating *B. agrorum* queens were used as a source of blood lysozyme. The bumbles were preserved in a refrigerator at 4°C for 4 months. Samples of haemolyph were taken by the use of microsyringe (5 µL/insect) from a dorsal vessel into the Eppendorf tube on ice-bath and mixed with 10 µL of insect physiological saline.

Bioassay of blood lysozyme activity. The concentration of lysozyme (EC.4.3.3.3) in an individual hemolymph sample was determined by a cup-diffusion assay technique. An assay plate was composed of 10 ml 0.2 M Sorensen buffer (pH 6.4); 750 mg of lyophilized cells of *Micrococcus luteus* : 300 mg oxytetracycline sulphate and 1.0% agarose. The wells in one assay plate were filled with the sampled hemolymph, 10 µL per well; however, wells in the other set of plates were filled with the known concentrations of egg white lysozyme (EWL), serving as a standard. Diameter of the zone of lysis around the wells was measured after 24 h of incubation at 28°C. Concentration of hemolymph lysozyme was calculated from a regression line of 250, 125, 62.5, 12.62, 7.81, 3.9, 1.5, 0.75 and 0.35 µg of the standard/ml, multiplied by a dilution factor in terms of EWL activity (µg/ml) (2).

RESULTS

Blood of hibernating bumble bees characterized a presence of bacteriolytic activity against *M. luteus*, the indicator bacterium for the presence of lysozyme action. The level lysozyme in *B. agrorum* hemolyph varied from 2.5 to 5.3 µg EWL/ml of blood. The mean value was 4.9 µg EWL/ml of blood

DISCUSSION

It is evident that antibacterial activity of lysozyme in hibernating bumble bee, *Bombus agrorum* is low. Like in other insect species, the antibacterial spectrum of this enzyme is narrow. It acts bactericidally only on a few Gram-positive bacteria. Insect lysozyme primarily attacks such bacterial species as *M. luteus, Sarcina lutea, Bacillus subtilis*. Only exceptionally lysozyme can attack Gram-positive bacteria (3).

It may be suggested that the innate level of blood lysozyme in hibernating bumble bees is sufficient to begin killing of bacterial invaders in hemocoel just after their penetration from the intestines or cuticle. Most probably in the bumble bees, lysozyme together with phagocytic cells constitute the second line of defense against invasion of potentially pathogenic organisms (3, 6).

CONCLUSIONS

1. Bacteriolytic activity of lysozyme is involved in the immune phenomena in the hibernating bumble bees.

2. It is necessary to examine phagocytic activity of hemocytes of hibernating bumble bees and then determine if lysozyme together with phagocytosis can clear hemocoel of the hibernating bumble bee queens from bacterial invaders.

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STRESZCZENIE

Trzmiele przeżywają okres hibernacji w środowisku, w którym występuje wiele drobnoustrojów. Zakażenie więc nie rozwija się zawsze w następstwie obecności zarazków na powłokach ciała trzmieli. Poziom lizozymu w hemolimfie zimujących matek *Bombus agrorum* jest niski i waha się w granicach 2,5–5,3 μg EWL/ml. Przypuszcza się, że aktywność bakteriolityczna lizozymu łącznie z fagocytozą we wczesnej fazie zakażenia niszczą skutecznie bakterie, które wniknęły do jamy ciała owada.