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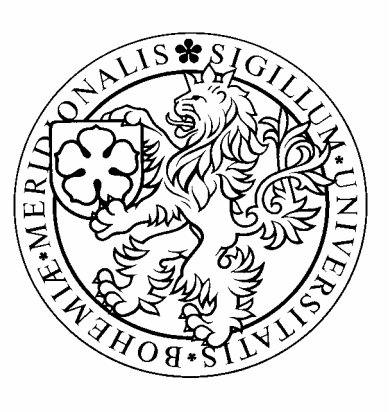
Department of Molecular Biology and Biochemistry



RNDr. thesis

Tick immune response to the infection with *Chryseobacterium indologenes*

Mgr. Veronika Burešová

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Annotation

Ticks belong to the most important disease vectors. The soft tick *Ornithodoros moubata* and the hard tick *Ixodes ricinus* were used for the research of tick immune response to the infection with Gram-negative bacterium *C. indologenes*. While *O. moubata* ticks were sensitive to the infection with *C. indologenes*, the ticks *I. ricinus* showed resistance to this bacterium. Hemocytes of both tick species showed phagocytic response after *C. indologenes* overcoming midgut barrier or upon bacteria intrahemocoelic inoculation.

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Manuscript title: **A comparison of *Chryseobacterium indologenes* pathogenicity to the soft tick *Ornithodoros moubata* and hard tick *Ixodes ricinus***

Authors: **Veronika Burešová, Zdeněk Franta, Petr Kopáček**

We, the undersigned, confirm that Veronika Burešová contributed substantially to this publication.

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Mgr. Zdeněk Franta

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RNDr. Petr Kopáček, CSc.

Abstrakt

Gram negativní bakterie *Chryseobacterium indologenes* byla izolována ze střevního obsahu klíšťáka *Ornithodoros moubata*, jako infekční agens mající za následek vysokou mortalitu klíšťáků po jejich sání. Tato bakterie je citlivá k antibiotiku Cotrimoxazol a po jeho přidání do krve při umělém sání byla významně snížena mortalita těchto klíšťat. Experimentální infekce zdravých klíšťat během umělého sání způsobila 100% mortalitu při koncentraci bakterií vyšší než 10^6b/ml. Pozoruhodně, infekce klíšťat *Ixodes ricinus* stejnou dávkou bakterie *C. indologenes* během kapilárního sání nebyla pro klíšťata letální. Vyšetřením střevního obsahu po experimentální infekci bylo zjištěno mnohonásobné pomnožení bakterií ve střevě klíšťáků *O. moubata*, zatímco ze střeva klíšťat *I. ricinus* byla bakterie zcela eliminována. U obou klíšťat byla prokázána penetrace bakterií ze střeva do hemocoelu. Po inokulaci *C. indologenes* do hemocoelu klíšťat byla bakterie fagocytována hemocyty oběma druhů klíšťat. Zatímco pro všechny klíšťáky *O. moubata* je infekce *C. indologenes* injikací do hemolymfy do tří dnů letální, pro klíšťata *I. ricinus* nikoli. Z těchto výsledků usuzujeme, že imunitní odpověď *I. ricinus* v boji s bakterií *C. indologenes* je mnohem efektivnější než je tomu u *O. moubata*.

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A comparison of *Chryseobacterium indologenes* pathogenicity to the soft tick *Ornithodoros moubata* and hard tick *Ixodes ricinus*

Veronika Burešová [a], Zdeněk Franta [a], Petr Kopáček [b,*]

[a] Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic
[b] Institute of Parasitology, Biological Centre Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

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Abstract

A yellow-pigmented Gram-negative bacterium, *Chryseobacterium indologenes*, was found in the gut contents of about 65% of soft ticks *Ornithodoros moubata* from a perishing laboratory colony. The isolated putative pathogen, *C. indologenes*, was susceptible to cotrimoxazol and addition of this antibiotic (Biseptol 480) to the blood meal significantly decreased the tick mortality rate. The artificial infection of healthy *O. moubata* by membrane feeding on blood contaminated with *C. indologenes* was lethal to all ticks at concentrations $\geqslant 10^6$ bacteria/ml. On the contrary, a similar infection dose applied to the hard tick *Ixodes ricinus* by capillary feeding did not cause significant mortality. Examination of guts dissected from infected *O. moubata* and *I. ricinus* revealed that *C. indologenes* was exponentially multiplied in the soft tick but were completely cleared from the gut of the hard ticks within 1 day. In both tick species, *C. indologenes* were found to penetrate from the gut into the hemocoel. The phagocytic activity of hemocytes from both tick species was tested by intrahaemocoelic microinjection of *C. indologenes* and evaluated by indirect fluorescent microscopy using antibodies raised against whole bacteria. Hemocytes from both tick species displayed significant phagocytic activity against *C. indologenes*. All *O. moubata* injected with *C. indologenes* died within 3 days, whereas the increase of the mortality rate of *I. ricinus* was insignificant. Our results indicate that hard ticks possess much more efficient defense system against infection with *C. indologenes* than the soft ticks. Thus, *C. indologenes* infection has the potential to be a relevant comparative model for the study of tick immune reactions to transmitted pathogens.

Keywords: Tick; Pathogen; *Chryseobacterium indologenes*; *Ornithodoros moubata*; *Ixodes ricinus*; Blood-feeding; Innate immunity

1. Introduction

The research on tick pathogens, parasitoids or predators, which may be used as a reasonable alternative to chemicals acaricides in the regulation of tick population, lags far behind the use of a similar strategy for the biological control of plant pests (Samish and Řeháček, 1999). Moreover, better understanding of how pathogens avoid the innate defense mechanisms in the tick can markedly improve knowledge about tick–pathogen interaction in general.

The soft tick, *Ornithodoros moubata* (Murray, 1877), is a serious disease vector since it transmits the spirochetes *Borrelia duttonii*, causing African tick-borne relapsing fever in man (Varma, 1956), and the African swine fever virus, having a fatal impact on the raising of domestic pigs in tropical areas of sub-Saharan Africa (Wardley et al., 1983). *Ornithodoros moubata* has also been recently shown to be a reservoir and vector for the West-Nile virus (Lawrie et al., 2004). Noda et al. (1997) described two groups of *Francisella*-like endosymbionts in *O. moubata*, originally identified as *Francisella tularensis* and *Wolbachia persica*. The latter bacterium has been later re-classified as *Francisella arboreus* by Scoles (2004). In the last decade, *O. moubata* has become a useful laboratory model because of its size and the relative simplicity of artificial feeding using a ParafilmM® membrane (Schwan et al., 1991). However, artificially fed ticks have the permanent risk of being infected with pathogens that are rarely if ever met in

* Corresponding author. Fax: +420 385 310 388.
E-mail address: kopajz@paru.cas.cz (P. Kopáček).

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