# Bioluminescence determination of antibacterial activity of *Bombyx mori* and *Galleria mellonella* haemolymph


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## Introduction

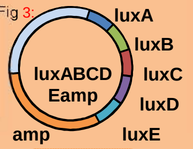
Bioluminescence is the production and emission of light by living organisms. Genus ***Photorhabdus*** includes terrestrial Gram negative bacteria, which are mainly found in association with **entomopathogenic nematodes** *Heterorhabditis* spp. Upon entering an insect host nematodes release bacterial cells from their intestinal tract which quickly establish a lethal septicaemia in the host (ffrench-Constant *et al.*, 2003). Similarly to *P. luminescens*, **transformed *Escherichia coli* K12** is capable of light production. Insect immunity involves both humoral and cellular aspects. Cellular activities in the insect rely on haemocytes which perform phagocytosis, encapsulation and nodulation. Humoral factors include especially highly potent antimicrobial peptides (AMPs) and the enzyme phenoloxidase. The aim of this study was to analyse **antibacterial activity** of insect haemolymph using direct real time measurement of changes in **bioluminescence** produced by *P. luminescens* or *E. coli* K12.

## *Photorhabdus luminescens*

*P. luminescens* (Fig. 1) is the only **terrestrial bacteria** capable of bioluminescence. It is G- bacteria which can produce light because of the expression of bacterial **luciferase** (Lux) and its substrate. This unique enzyme catalyses the oxidation of long-chain aldehyde (substrate) and reduced flavin mononucleotid ($FMNH_2$) produced only by living cells followed by the emission of **light** (Hakkila *et al.*, 2002).

Fig 1: *P. l.* culture under phase contrast.

Fig 3:





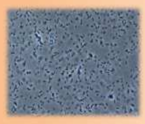


Fig 2: *E. coli* culture under phase contrast.

$$FMNH_2 + RCHO + O_2 \xrightarrow{Lux} FMN + RCO_2H + H_2O + \text{light}$$

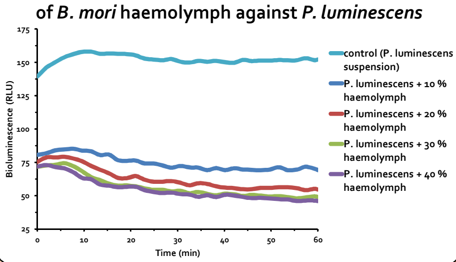
## *Escherichia coli* K12

As well as *P. luminescens, E. coli* K12 (Fig. 2) is G- bacteria. It was **geneticaly transformed** with **luxABCDEamp operon** (Fig. 3). Genes luxA and B codes two subunits of bacterial luciferase; luxC, D and E codes the fatty acid reductase complex needed for aldehyde synthesis. This operon is originaly from genus *Photorhabdus* thus the principle of light emition is the same for both species (Atosuo *et al.*, 2012).

## Insect antimicrobial peptides

Insects do not have a complement system as vertebrates thus mostly AMPs are likely to be responsible for bactericidal effect. Most of the AMPs detectable in the haemolymph upon microbial infection are produced within a few hours by the fat body, haemocytes and other specific tissues (Lemaitre & Hoffmann, 2007). Apart from induced AMPs synthesis there is also constitutive level of AMPs present in haemolymph. These peptides are synthesised by either the **haemocytes** or the **fat body**. In Lepidoptera linear α-helical (cecropins and moricins), cysteine-stabilized (defensins), proline-rich and glycine-rich inducible AMPs have been identified; moreover the peptides cooperate with lysozyme which is naturally occurring in haemolymph. AMPs are **attracted by** electrostatic forces to negatively charged groups on the surface of bacteria e.g. **lipopolysaccharide** or teichoic acid. After attachment to bacterial membrane AMPs interact with phospholipids double layer which usually leads to pore creation and **cell lysis**.

**Fig 4: Antibacterial activity of different concentrations of *B. mori* haemolymph against *P. luminescens***





***P. luminescens***

Concentrations of haemolymph ranging from 10 % to 40 % were tested against both *E. coli* (Fig 5) and *P. luminescens* (Fig 4). For subsequent experiments 20 % was selected as an optimal dilution of haemolymph. This concentration suppresses approximately 50 % of bacteria in 30 minutes.

***E. coli***

**Fig 5: Antibacterial activity of different concentrations of *B. mori* haemolymph against *E. coli***

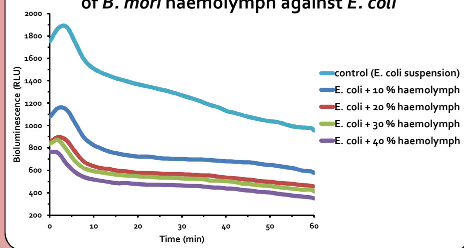
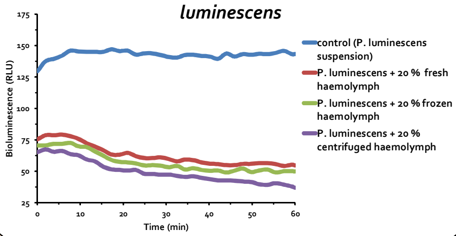




**Fig 6: Antibacterial activity of fresh, frozen and centrifuged *B. mori* haemolymph against *P. luminescens***





***P. luminescens***

Freshly collected haemolymph was compared to haemocyte-free haemolymph and haemolymph stored at –20 °C for 30 days. All tested samples showed comparable antibacterial activity against *E. coli* (Fig 7) and *P. luminescens* (Fig 6) →antibacterial activity is caused by humoral factors.

***E. coli***

**Fig 7: Antibacterial activity of fresh, frozen and centrifuged *B. mori* haemolymph against *E. coli***

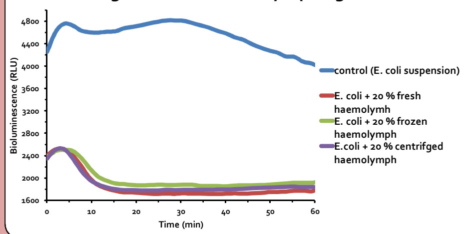
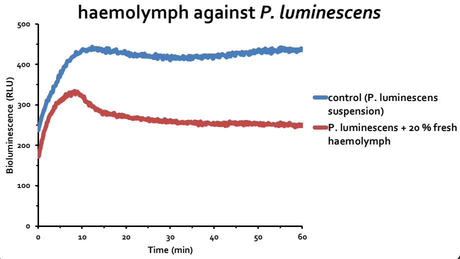




**Fig 8: Antibacterial activity of 20 % *G. mellonella* haemolymph against *P. luminescens***



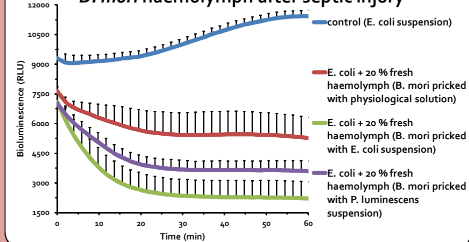


***P. luminescens***

Similarly to *B. mori*, haemolymph from *G. mellonella* showed strong antibacterial activity against both *E. coli* and *P. luminescens*, approximately 50 % decrease in bacterial bioluminescence signal in 30 minutes was observed (Fig 8).

***E. coli***

**Fig 9: Antibacterial activity against E. coli of 20 % *B. mori* haemolymph after septic injury**





*B. mori* larvae pricked by *E. coli* or *P. luminescens* showed higher antibacterial activity five hours after pricking (Fig 9). The increase of antibacterial activity was reflected in decreased *E. coli* bioluminescence, significantly different compared to untreated control or larvae pricked with insect saline. Insects treated with *E. coli* had stronger antibacterial response than larvae treated by natural insect pathogen *P. luminescens*.

## Conclusion

Bioluminescent bacteria assay can be used as a new, fast and real-time method for assessment of insect haemolymph antibacterial activity. Dose dependence in antibacterial activity was found in both *B. mori* and *G. mellonella*. Results with haemocyte free haemolymph and haemolymph stored in -20°C confirmed that antibacterial activity is of humoral origin. Measurements done on pricked larvae showed that constitutive level of AMPs which are present in haemolymph can be increased by their induction with both *E. coli* and *P. luminescens*.


**Refferences:**

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