

# Expression of a prtX-like protease by *Xenorhabdus nematophila* and *Photorhabdus luminescens*

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# Introduction

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*Xenorhabdus nematophila* Breton strain

*Photorhabdus luminescens* Az29 strain

- Both are highly pathogenic to insects;
- Both release prtX-like metalloproteases in the growth medium.
- prtX-like are known to be virulent factors in a large number of pathogenic bacteria;
- In this work we study the expression of prtX in these both bacteria.

# Materials and Methods

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## ▪ **Bacterial strains and culture conditions**

*Xenorhabdus nematophila* strain Breton and *Photorhabdus luminescens* sp Az29 were maintained in NBTA plates at 10°C and subcultured weekly.

## ▪ **Isolation of *prtA* gene**

Degenerated primers were designed to amplify fragments of genes codifying for PrtA homologous proteins.

Amplified fragments were sequenced and used to design specific primers to screen genomic libraries and to synthesize a probe for Northern blots. Inserts from selected clones were sequenced by primer-walking.

# Materials and Methods

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- **Bacterial strains and culture conditions**

- **Isolation of *prtA* gene**

- ***In vivo* assay in *X. nematophila***

*Galleria mellonella* larvae were injected with  $10^3$  cells of *X. nematophila*.

For expression studies the whole internal content of the larvae was homogenated at 6, 12, 18, 24, 30 and 36 hours post-injection.

For histological studies larvae were dissected and mid-gut excised.

# Materials and Methods

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## ▪ RNA isolation

Total RNA for all the assays, was extracted of the several bacterial cultures with Trizol (Roche) and purified with RNeasy Micro Kit (Quiagen).

## ▪ Northern-blot analysis in *Xenorhabdus nematophila*:

Probe labelled with digoxigenin-dUTP ( DIG Prime DNA Labelling Kit, Roche).

Hybridization and stringency washes were done at 50°C. Chemiluminescence detection according to the DIG High Prime DNA Labelling Kit (Roche Diagnostics, GmbH).

# Materials and Methods

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## ▪ Quantification of the expression of *prtA* in *Xenorhabdus nematophila* and *Photorhabdus luminescens*

Expression of *prtA* was quantified relatively to expression of 16S rRNA gene. Primers used were as follow:

### ***P. luminescens***

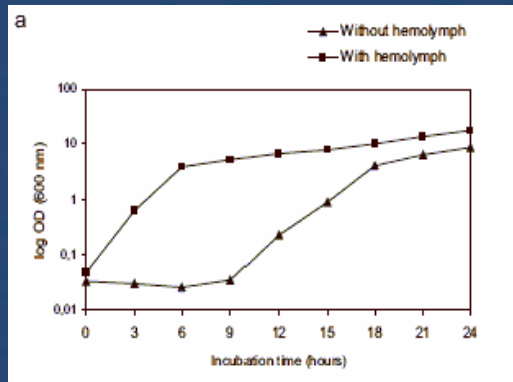
Specific primers for *prtA* (PPrtAR and PPrtaF) and Universal primers for 16S rRNA gene (16S-For and 16S-Rev)

### ***X. nematophila***

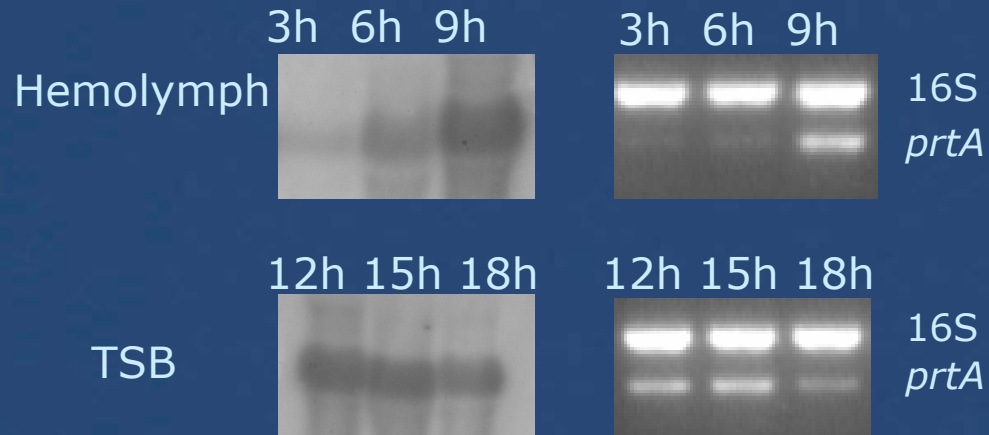
Specific primers for *prtA* (Xn-Probe-R and Xn-Probe-F). Specific primers (Xn16S-F57 and Xn16S-R474) were designed from 16S rRNA gene, witch was amplified and sequenced *de novo* in our laboratory (GenBank Accession N° DQ282116).

# Expression of *prtA* in *X. nematophila*

## Bacterial growth



## Northerh-blot RT-PCR

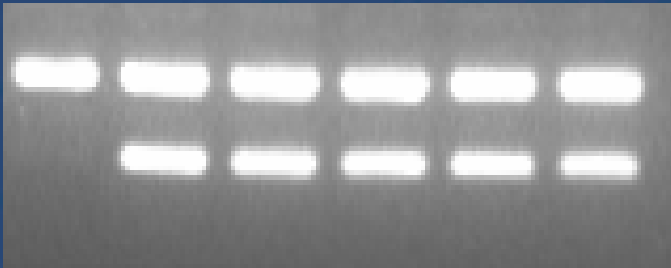


- In bacteria growing in a medium supplemented with hemolymph, *prtA* expression starts at 3 hours, at the beginning of the exponential phase. A maximum is reached at 9 hours.
- In bacteria growing in TSB, *prtA* expression occurs at 12 hours, also in the beginning of the growth phase. A maximum is reached at 15 hours

# Expression of *prtA* in infected *Galleria*

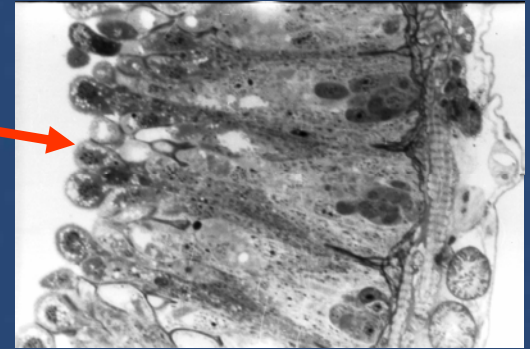
## RT-PCR

6h 12h 18h 24h 30h 36h



## Midgut histology

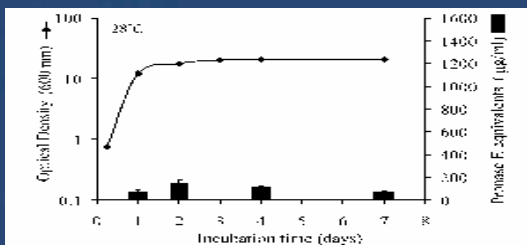
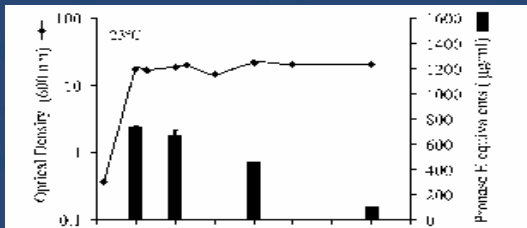
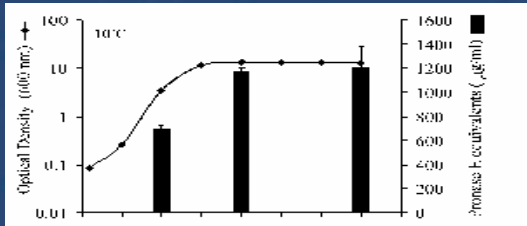
Bulks



- *In vivo*, *prtA* expression was detected 12 hours post-injection when the larvae were still alive. It was not possible to visualize any increment of the expression.
- Light microscope observation showed cytopathological changes on midgut epithelial cells 12 hours post-injection

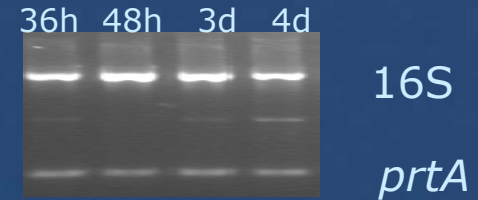
# Expression of *prtA* in *P. luminescens*

## Proteolytic activity

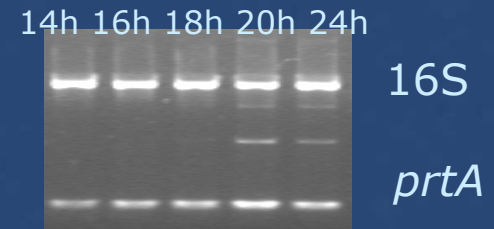


## RT-PCR

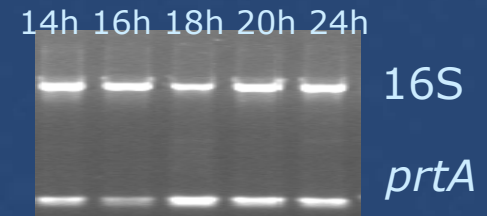
10°C



23°C



28°C



*P. luminescens* growing at different temperatures also expressed *prtA* in the beginning of the exponential phase, depending on the optimum of growth temperature. At 28°C and 23°C it is expressed much early than at 10°C.

# Discussion

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- Both bacteria express *prtA* at the beginning of growth phase.
- In infected insects, *prtA* is expressed a few hours post-injection. The infected insects evidence histolysis with the formation of bulks, the same signs described for prtX-like metalloproteases that are considered virulence factors in others organisms.
- It seems evident that *prtA* has an important role in the pathogenicity of entomopathogenic bacteria... at least in our own bacteria!

**This work was elaborated by part of our  
Team!!**



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