Impact and persistence of the opportunistic bacteria Serratia marcescens in the feed and in Tenebrio molitor larvae under mass rearing conditions

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Introduction

Industrial insect rearing aims to produce large quantities of high quality insects, following safety and secure sanitary conditions. Diseases in insect rearing caused by pathogens can compromise insect quality and production. Therefore, it is important to gain knowledge on pathogens characteristics such as prevalence, virulence, means for detection and persistence. In addition, knowledge on the behavior of human opportunistic pathogens in insect rearing is of importance for sanitary risk issues. It is also important to assess the influence of biotic and abiotic rearing conditions on insect's sensitivity to pathogens. This study focuses on the opportunistic pathogen *Serratia marcescens* on the industrial farming of *Tenebrio molitor*.

Objectives : evaluate the impact and persistence of *Serratia marcescens (Sm)* **in** *Tenebrio molitor (Tm)* **rearing by:**

- Evaluating the impact of biotic/abiotic stresses on *Tm*
- Assessing the performance of methods for *Sm* detection
- Characterizing the persistence of *Sm* in *Tm* larvae and feed

Serratia marcescens

Serratia marscecens is a red-pigmented Gram negative enterobacterium. This bacteria is a common opportunistic pathogen of insect and humans.

→ **Detection and persistence ?**

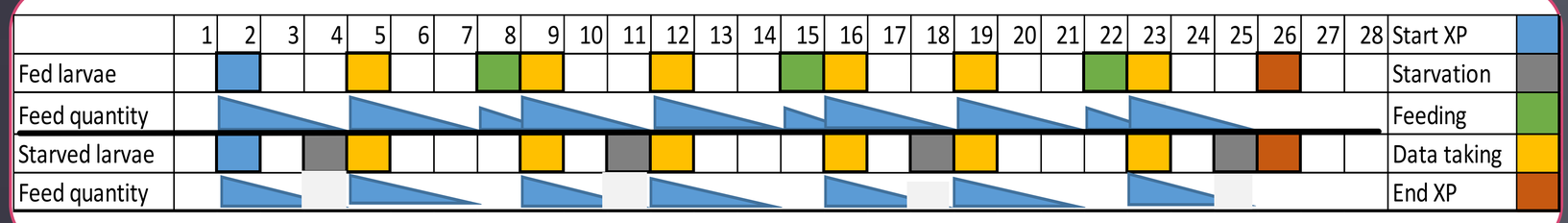
Tenebrio molitor

Tenebrio molitor is a beetle of the family Tenebrionidae. As all holometabolous insects this species grows through four life stages: egg, larva, pupa, and adult. The total life cycle from egg to adult is approximately 90 days.

→ **Study of stress factors**

Stress factors impact on *Tm* rearing with *Sm*

Protocol for the applied combined stress



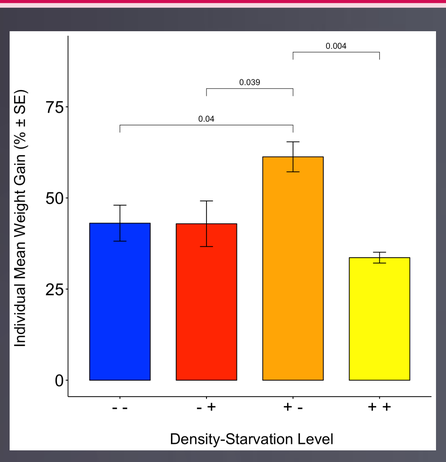
A 2-level factorial experiment was designed to apply the following stress factors:

- Starvation **(+)** /continuous feeding **(-)**
- Automatic sieving **(+)** / handmade sieving **(-)**
- High density (0,8g/cm²) **(+)** / low density (0,4g/cm²) **(-)**
- *Serratia* exposure **(+)** / no exposure to *Serratia* **(-)**

Larval containers were provided with humid (30%) wheat bran. Starvation was brought by the removal of feed from the container the day prior to data recording while the continuous fed larvae received supplementary feeding in the event that they had finished their feed.

The figure on the left shows the relative quantity of feed provided and the frequency of data recording .

Impact of starvation and larval density on Individual Mean Weight Gain



One Key Performance Indicator (KPI) used in insect rearing is the Individual Mean Weight Percentage Gain (IMWG%.) This indicator shows the relative growth of larvae in regards to their initial weight.

The figure on the left shows IMWG% according to the combined stress factors: density(-/+) and starvation (-/+).

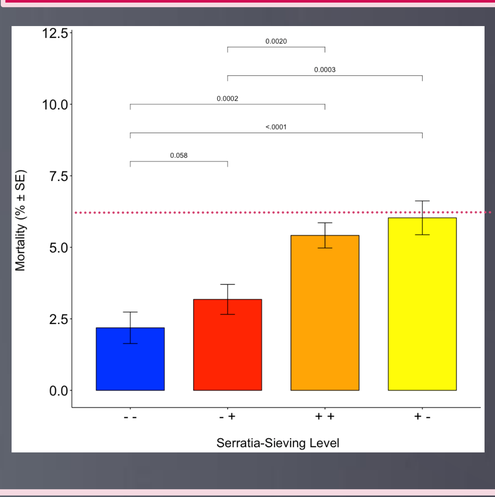
An analysis of variance (ANOVA) indicated the presence of an interaction between density and starvation on IMWG%.

The highest IMWG% was observed for the larvae reared at high density and continuous feeding. A 31% decrease in IMWG% was observed between the high-density groups if the starvation stress was applied.

CONCLUSION

At low density conditions starvation seemed not to have an impact on IMWG%. At high densities however, starvation presented a large negative impact on IMWG%.

Impact of *Serratia* exposure and sieving on mortality



The figure on the left shows the mortality rate in percentage according to the the combined stress factors: *Serratia* (-/+) and Sieving (-/+).

Larvae exposed to *Serratia* presented a higher mortality than larvae that were not exposed to the pathogen. The mortality was approximately twice as high in the *Serratia* exposed larvae.

CONCLUSION

Serratia has an impact on mortality of Tenebrio molitor but not enough to risk a collapse of the larval production.

Quantification and persistence of *Serratia marcescens* in *Tenebrio molitor*

Detection of *Serratia marcescens* by qPCR

Tenebrio molitor larvae were fed with *Serratia* contaminated feed during 17 hours with 9 .10^5 bacteria/5 larvae

ADN extraction from gut or feces or larvae
(Kit Mo Bio ®)

qPCR
kit qPCR test *Serratia marcescens* (PCR Max ®)

Quantification of bacteria

Samples	Without Serratia	With consumption of Serratia (25µL of cultureon bran)		qPCR detection
5 larvae	0	218	(+/- 169)	Not optimal
5 guts	0	582	(+/- 604)	OK but not easy
Feces of 5 larvae during 24H	0	2143	(+/- 1690)	OK and easy

CONCLUSION

- The q-PCR allows *Serratia* detection at a very low threshold.
- Q-PCR is precise for quantification only in samples of easy DNA extraction.
- Large variation was observed among replicates.
- qPCR technique is too expensive for persistence analysis.
- Need another method = **specific Erythritol growth medium**.

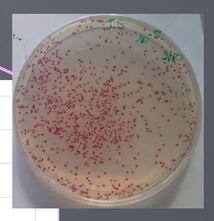
(ref. Irving J. and al.(1972). Applied Microbiology)

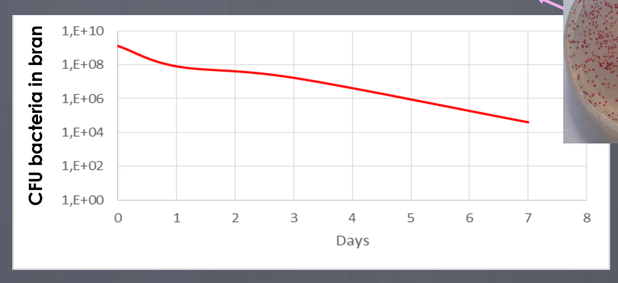
Persistence of *Serratia* in feed (wheat bran) and in feces of *Tenebrio molitor*

Control of specific medium : Bacterial counts in 50 mg samples

Bacteria growth medium	Feed (Bran)	Feces of *Tm*	*Tenebrio*
BHI (all)	700	10 ^{4}/larva	1 400
Erythritol *(Sm)*	0	0	0

Serratia CFU on erythritol medium





Quantity of *Serratia* in bran : detected on erythritol plate from bran soaked with culture (6.10^9 CFU) and incubated at 30°C

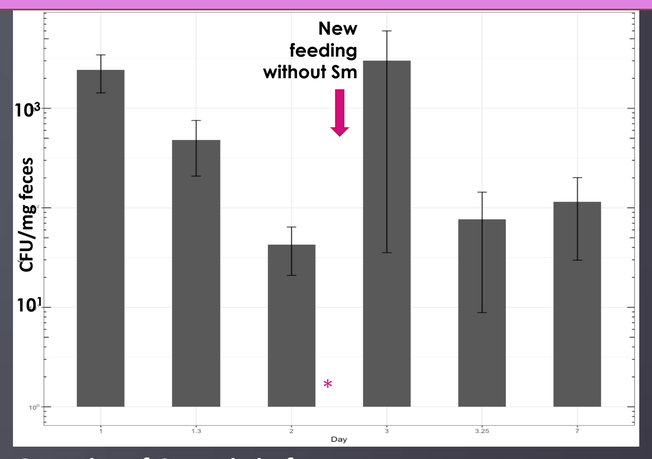
CONCLUSION

- No *Serratia* was found in *Tm* and feed from the rearing
- Low Persistence of *Serratia* in contaminated wheat bran after 7 days with a 5 log reduction (100.000 fold less)
- CFU counting on selective medium is less precise in quantity than qPCR but indicates viability not only presence

First feeding with 6.10^8 Serratia/5 larvae

Feces from 5 larvae are collected at different timepoints. *Serratia* is detected from diluted feces on erythritol agar.

0H-24H | 24H-27H | 30H-32H | 48H | 50H-56H | 56H-72H | 75H-77H | 78H at Day7



* No more feces

Quantity of *Serratia* in feces : mean of 4 batches plus SEM

CONCLUSION

Serratia persists in low quantities in *Tenebrio* over time but a large variation was present among replicates: After 24H of ad d libitum ingestion of bran soaked with *Sm*, the amount of *Sm* found, in the larva is about 5.103 bacteria (decrease of 5 log or 10,000 times more than in the bran at 24 H) *Sm* can persist in the gut and reappears when the larvae feed again.

Conclusions

- *Tenebrio molitor* is only weakly affected by the presence of *Serratia marcescens* even under stressed conditions.
- *Serratia marcescens* can survive in small quantities in the feed substrate or in the larva (gut and feces.)
- Sanitation of the final insect product is necessary to remove the presence of *Serratia marcescens.*
- This pathogen could be considered as a potential marker in the assessment of infectious pressure.

Perspectives

Ynsect is developing innovative techniques to prevent infectious risks by sampling and monitoring the rearing production through the use of embedded sensors and molecular methods for the detection of pathogens. The development of knowledge and techniques in insect health for this new industry is essential. The future is built today through collaborative projects between companies and academic laboratories such as the Micalis team of INRA.

This study belongs to a research project funded by the INRA and CIRAD GloFoods metaprogram based on a specific collaboration between Ynsect and INRA Micalis teams. This project is intended to extend to other partners.