BACTERIAL POPULATIONS IN DUTCH CRICKET AND MORIO WORM CULTURE: A PILOT STUDY

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*Professor **INVIS** at **HAS University of Applied Sciences**, Den Bosch, NL &

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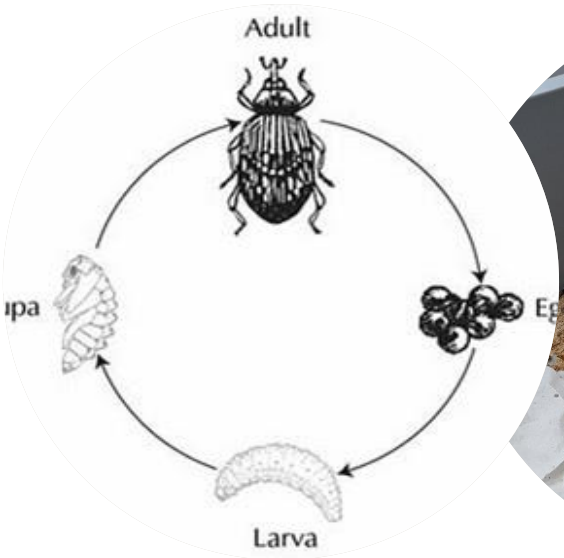

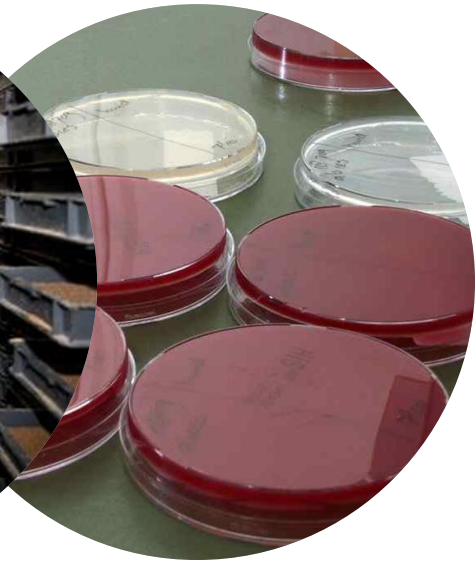

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Intro: Insect culture for:

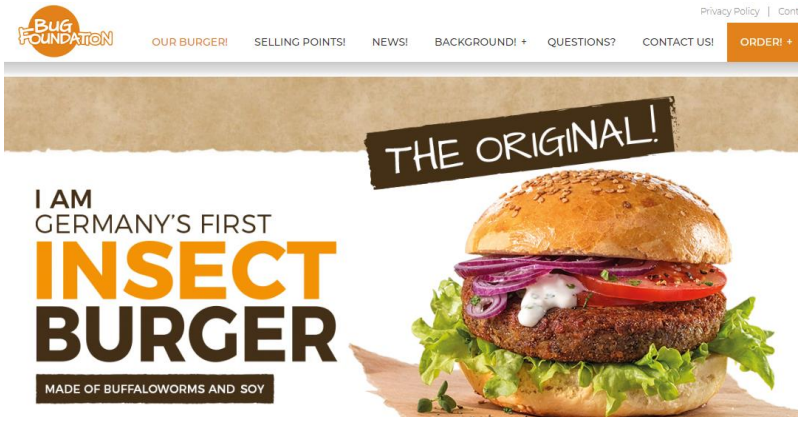
- Feed:

A view of a salmon farm near Rolla and Andorja Island in Norway.

PHOTOGRAPH BY ARTUR WIDAK, NURPHOTO, GETTY

Why Salmon Eating Insects Instead of Fish Is Better for Environment

- Food:



Insect species for culture

For feed

Black soldier fly

Hermetia illucens

2,5 cm

House and band crickets

Acheta domesticus en Gryllodes sigillatus

5 cm

Migratory grasshopper

Locusta migratoria

10 cm

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Insect species for culture

Lesser Mealworm
Alphitobius diaperinus
0,7 cm

Mealworm
Tenebrio molitor
1-2 cm

Morioworm
Zophobas morio
2,5-3cm

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INVIS: Analysis : the chain

...Veterinary, contact zoonotic, food safety risks?

Leftover streams

feed

Insect-larvae

Insect meal in feed

Poultry Swine a.o.

Safe food

food

aquaculture

Fishfarmingexpert.com

food

"Oer-egg", Pet food, angling

Crickets, mealworms

Foodinaction.com

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Fast growing insect industry...

Report Dutch Council on Animal Affairs, 2018 (Van Huis *et al.)*

Lectureship healthy and safe insect culture

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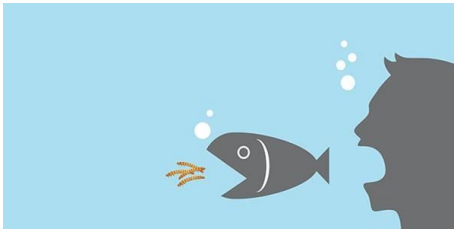
- At **HAS University of Applied Sciences**
- Since Jan 2018, for 4 years
- Aim: healthy and contact safe (bact) insect culture for (fish) feed and food, and BSc education

www.has.nl



INVIS

HAS University of Applied Sciences, Den Bosch:

INVIS core team

Dr. Arjan Borghuis (HAS), Dr. Olga Haenen (WBVR),
Dr. Ellen Weerman (HAS)

Project at Insect farm Kreca BV, Ermelo, NL

Photo's: Kreca BV

kreca® Ento-Food BV

Two cricket species and morio mealworm

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Monitoring at Kreca BV by Babette Weller, BSc student, Materials & Methods:

Autumn 2018,

Crickets : week 0, 3 and 6 (insect, water, box)
Morio worms: week 0, 3, 6, 9 (insect, substrate)

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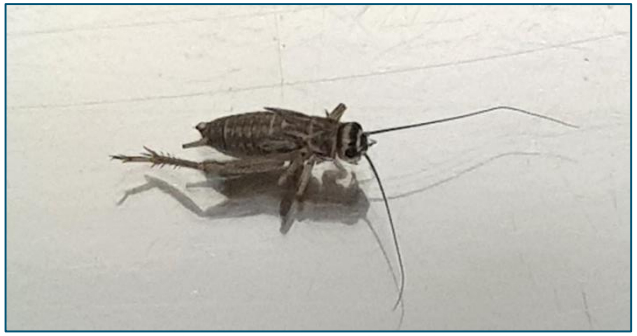
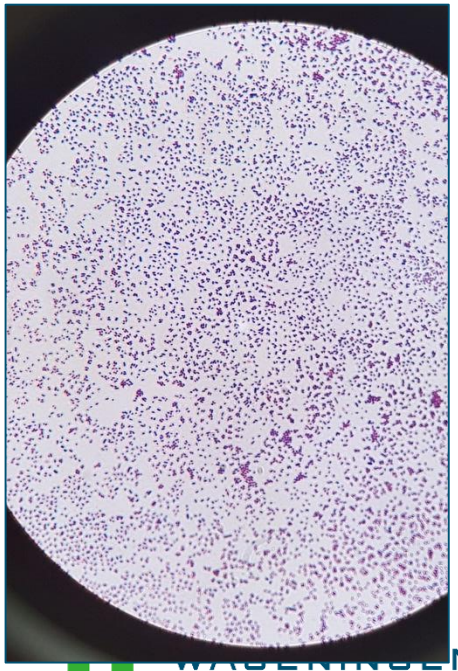
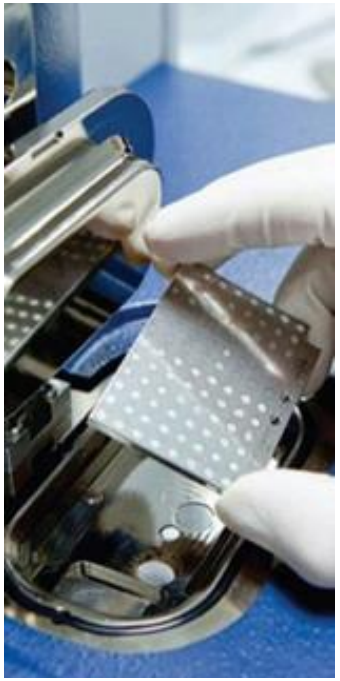
Bacteriology of crickets and mealworms:
Isolation, biochemistry and MALDI-TOF analysis







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MALDI-TOF to identify bacterial colonies:

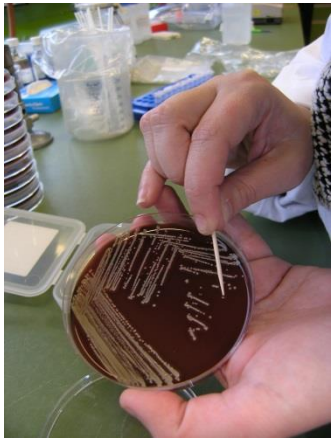
Matrix-assisted laser desorption/ionization – Time of Flight : 3 steps

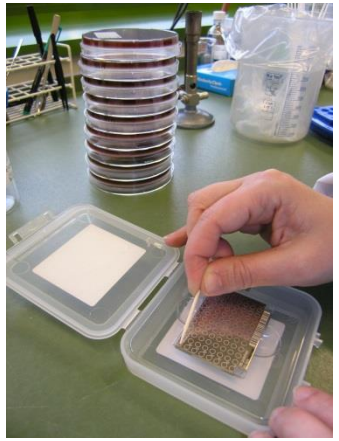
1) sample applied to a metal plate and covered with a suitable matrix

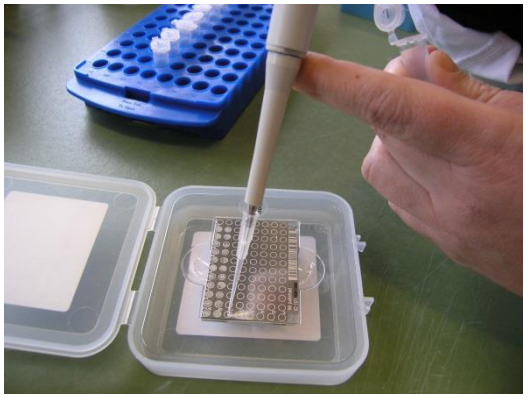
2) a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material.

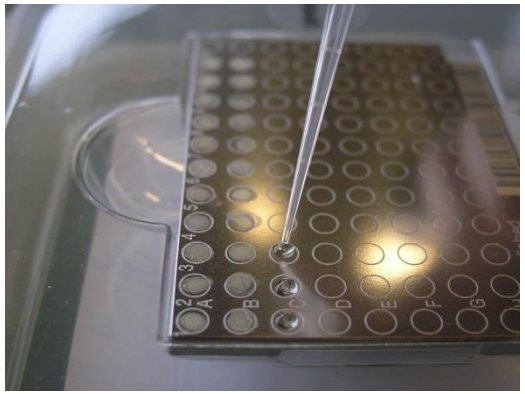
3) the analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into whichever mass spectrometer is used to analyse them.

MALDI-TOF in practice at our lab









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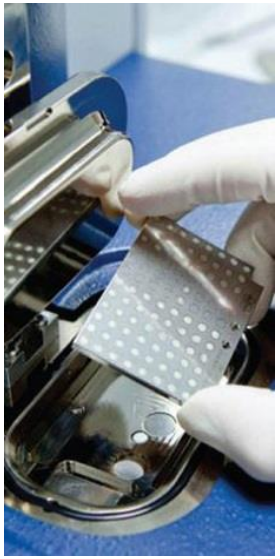
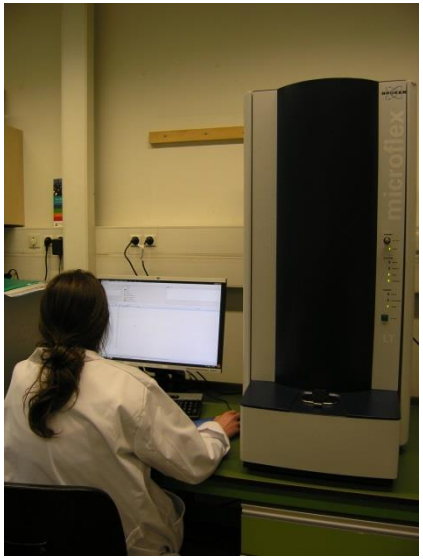
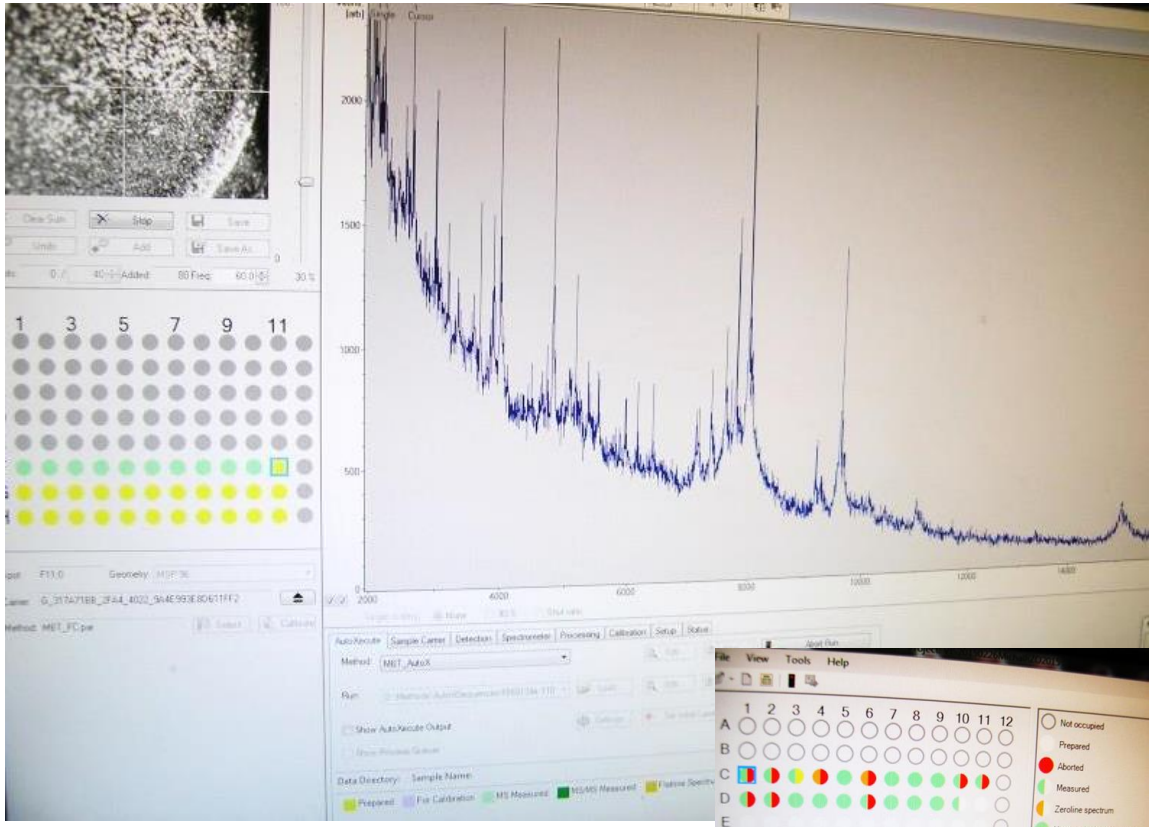
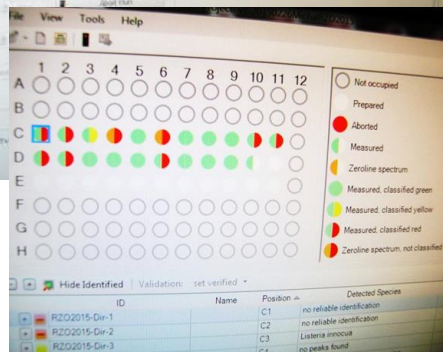
MALDI-TOF in practice at our lab





Direct method: In 15 minutes plate is read for possibly 92 species identifications of bacteria...
Whole process takes 1 hour.





RESULTS:

Score Based Pattern Matching:

2.0-3.00 Species identification (green)

1.7-1.99 Genus identification (yellow)

0.0- 1.69 Unreliable identification (red)

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Results

- Various species of bacteria were detected, mostly commensals to humans and animals
- Regarding **insect pathogenic bacteria**:

➢ In the morio worms: *Bacillus pumilus* (fungicide), *Enterobacter cloacae* (opport. human pathogen) & *E. kobei,* and *Klebsiella pneumoniae* (opportunistic human pathogen)

➢ In the house cricket: *Lysinibacillus sphaericus* was detected in its drinking water (pest control bacterium against *Aedes aegypti*)

- Some of the commensal bacteria may turn zoonotic in rare cases, only when humans are strongly immunocompromised.

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Literature search, scarce on **insect diseases (**other than from viruses): **some bacteria:** pathogenic?

- *Aeromonas* spp.
- *Serratia liquefaciens*
- *Serratia marcescens*
- *Acinetobacter baumannii*
- *Lactobacillus antri*
- *Lactococcus formosensis*
- *Staphylococcus arlettae*
- *Buttiauxella agrestis*
- *Pseudomonas aeruginosa*
- ***Rickettsiola* spp.**

Klebsiella, *Enterococcus*, *Providencia*, *Alcaligenes*, *Cirrobacter*, *Pseudomonas*, *Bacillus* *Sphingobacterium*, *Morganella*, *Ochrobactrum*, *Acinetobacter*, *Paenalcaligenes*, *Miniimonas*, *Paenochrobactrum*, *Cronobacter*, *Verrucomicrobia*

Pseudomonas aeruginosa
Bacilus thuringiensis
Bacilus amyloliquefaciens
Bacillus cereus
Bacillus laterosporus
Bacillus licheniformis
Bacillus megaterium
Bacillus pumilus
Bacillus subtilis

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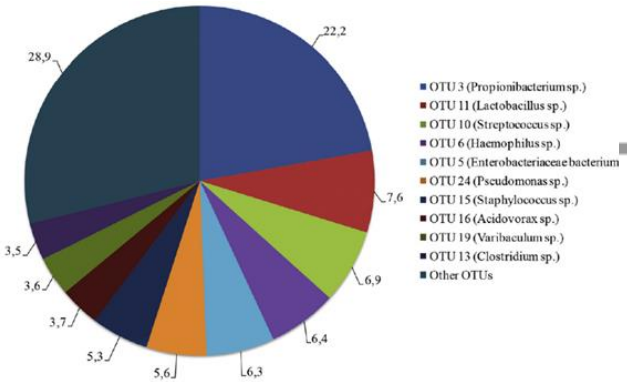
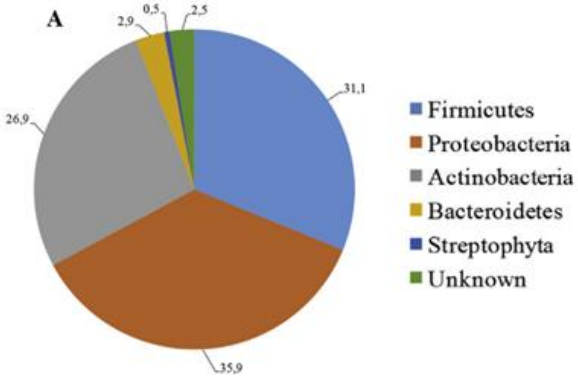
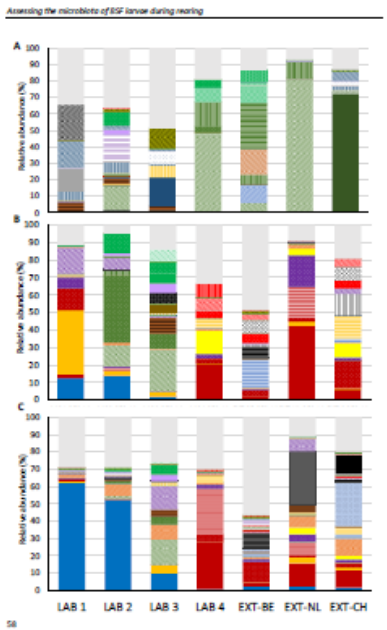
→ No primary fish pathogens seen

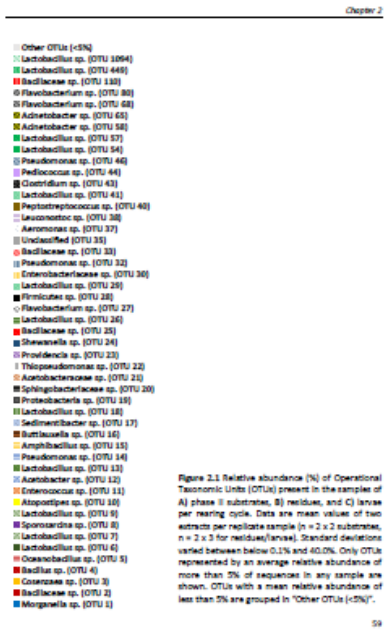
Group KU Louvain Microbial community in mealworms for **food**:



Stoops *et al.*, 2016







PhD defence **Dr. Enya Wynants**,
KU Louvain, Belgium,
16 May 2019 **(group Van Campenhout)**

High throughput gene amplicon sequencing:

Relative abundance of
Operational Taxonomic Units (OTUs)
of >5% (thesis), **mainly *Morganella***

Conclusions

- No alarming results regarding bacteria in this pilot study at this farm, given the fact, that standard hygiene measures are practiced to prevent for infections
- However, further testing is needed on presence of veterinary and potential contact zoonotic bacteria: in time and at various farms, like advizes by the RDA
- Still much to discover in many small new farms, >31 in NL
- We aim further (inter)national cooperation on bacteriology of insects.

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I like to thank the insect farm Kreca BV for their cooperation, and NWO for funding this KIEM project.

Thank you!

Olga Haenen
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