Effect of post-harvest starvation and rinsing on microbial numbers in mealworm larvae

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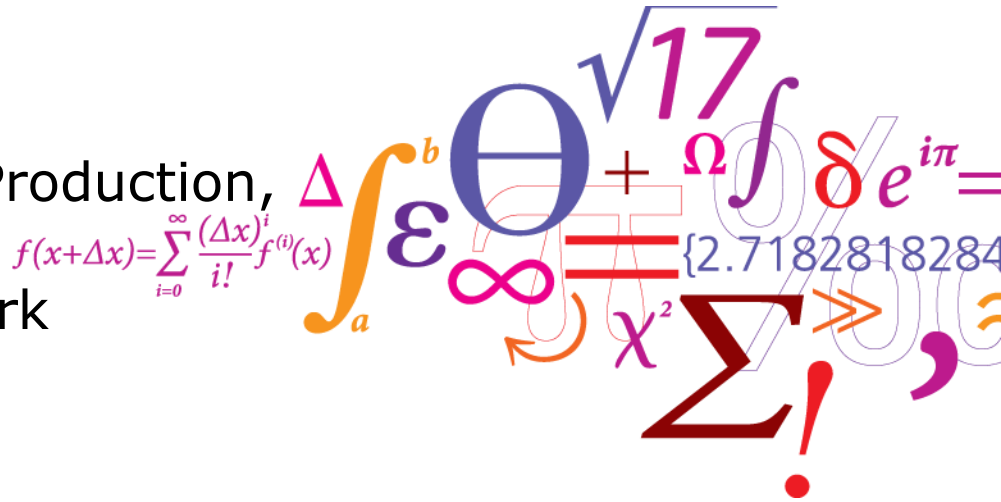

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DTU Food
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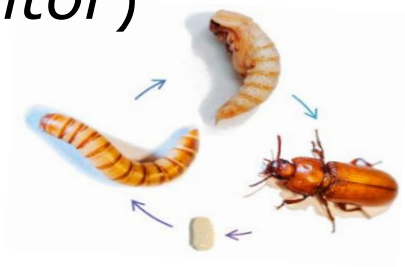
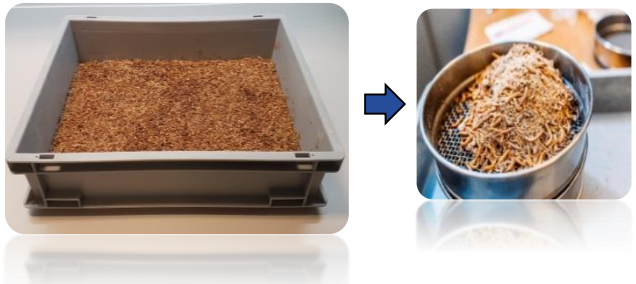
Background: mealworm production

Eggs from mealworm bettle (*Tenebrio molitor*) are grown to pre-pupae larvae stage



At harvest, larvae are separated from the remaining substrate (frass)



Starvation 1-2 days
- to empty the gut i.e.
  - remaining substrate
  - gut bacteria

→ Killing of larvae
- freezing
- heating or
- chopping

Aim:

To test the microbial numbers in mealworm larvae at harvest after

- Starvation 1-2 days
- Rinsing with tap water
- Feeding with sterile substrate 1-2 days

Experimental set-up - I:

Day 0

Separation

- No wash
- Rinsed, 200 ml water, 1 min

Day 1

24 h Starvation

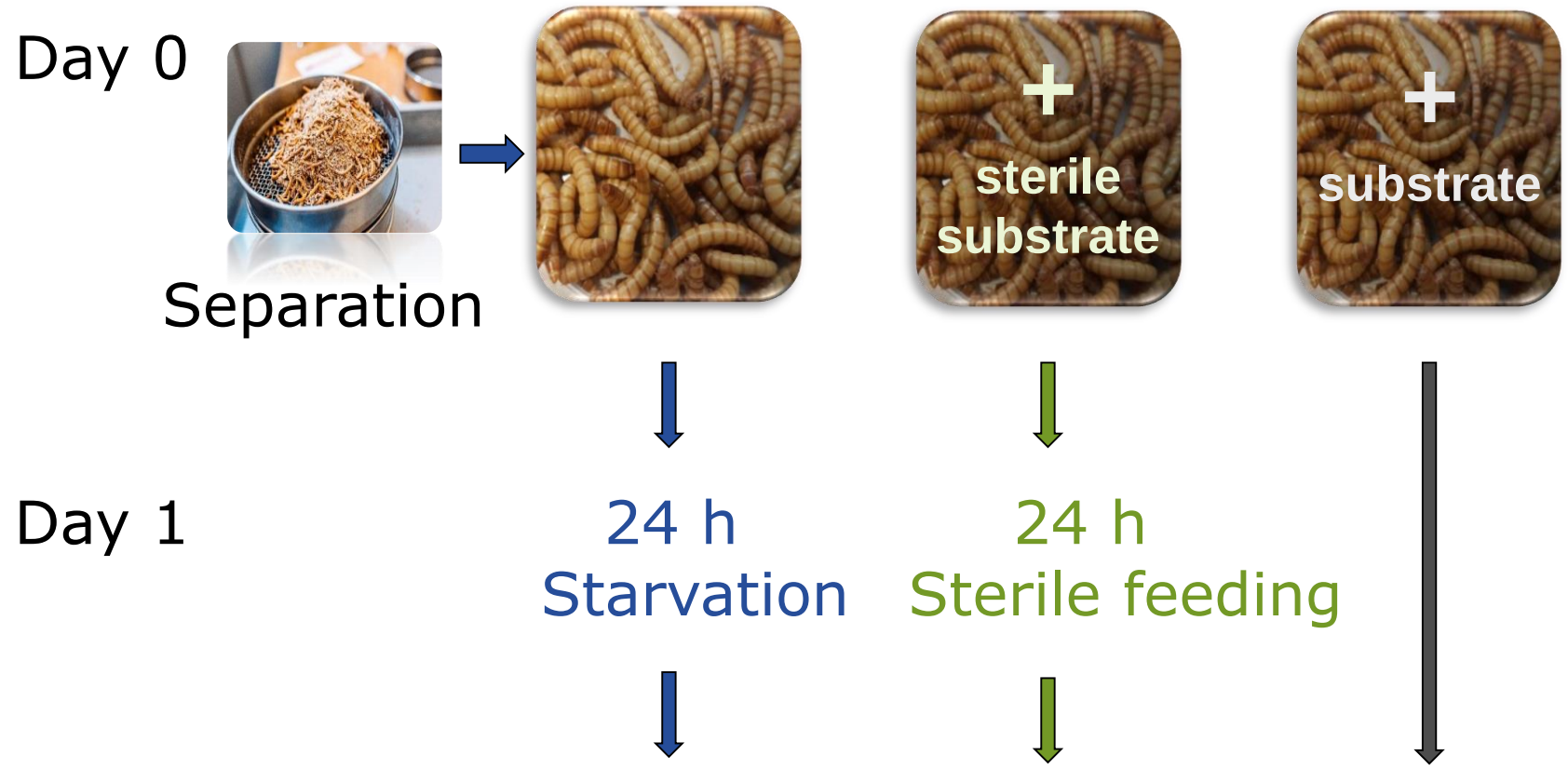
- No wash
- Rinsed, 200 ml water, 1 min

Day 2

48 h Starvation

- No wash
- Rinsed, 200 ml water, 1 min

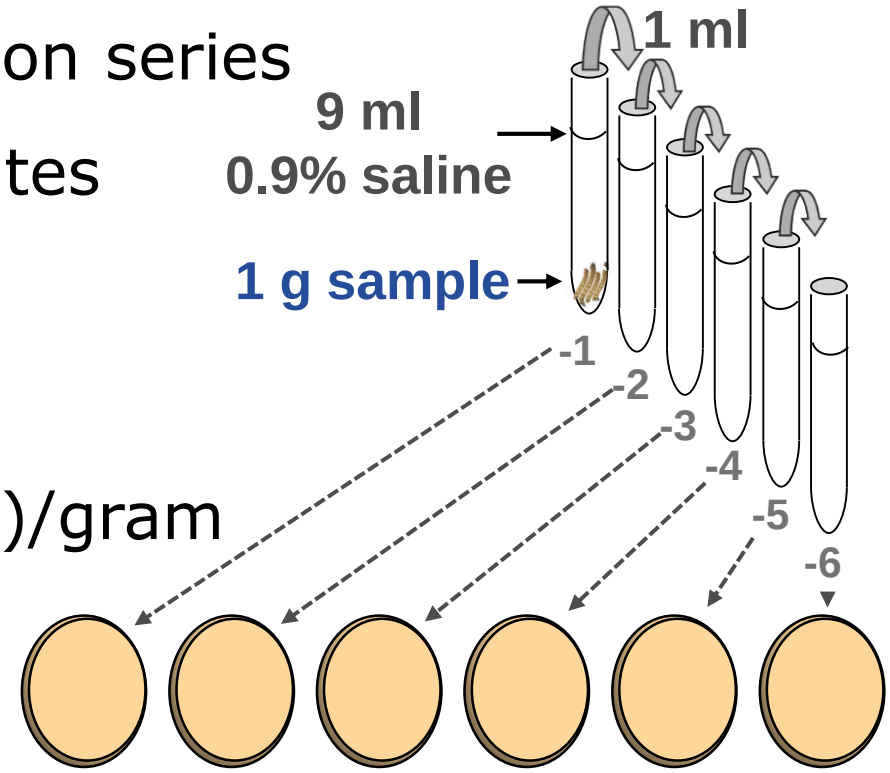
Experimental set-up - II:



Method: Enumeration of bacteria in mealworms (& substrate)

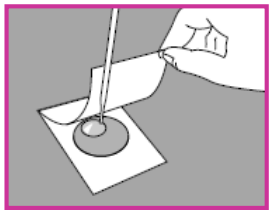
- Homogenization of 1 g sample with a mortar pestle
- Preparation of 10-fold dilution series
- Plate-spreading on agar plates
- Incubation
- Counting of colonies

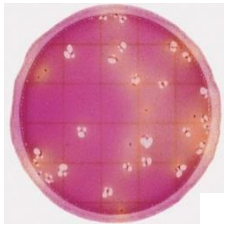
→ Colony forming units (CFU)/gram



Method: Bacterial groups enumerated

- Total aerobic count
  Plate count agar (PCA) at 37°C, 24h
- 'Psychrotrophs' **(II)**
  PCA at 6.5°C, 10 days
- *Enterobacteriaceae*
  Enterobacteriaceae count plate (ECP, Petrifilm, 3M) at 37°C for 24h
- Lactic acid bacteria (LAB) **(II)**
  de Man-Rogosa-Sharpe agar (MRS) at 30°C (anaerobic) for 48h
- Bacterial endospores **(II)**
  100°C for 5 min → PCA at 30°C, 3 days





Results:
Starvation of larvae – rinsing I

Sampling:	Total aerobic count (I) Log CFU/g No rinse	Rinse	Mean
Start	8,36	8,20	8,32±0,08
	8,36	8,35	
Starvation 24 h	7,64	7,85	7,85±0,29
	7,65	8,26	
Starvation 48 h	7,78	7,92	7,91±0,10
	7,93	8,02	

No significant effect of rinsing

Some effect of starvation 0.4 log ~ 60% reduction

Results:
Starvation of larvae – rinsing II

Sampling:	**Total aerobic count** (II) Log CFU/g No rinse	Rinse
Start	7,86±0,11	7,83±0,06
Starvation 24 h	8,13±0,29	7,81±0,27
Starvation 48 h	8,10±0,35	7,74±0,18

~ Opposite tendency in experiment II compared with I

i.e. no effect starvation &
– some effect rinsing!? (not significant)

Results:
Starvation of larvae – rinsing

	Enterobacteriaceae Log CFU/g			
	I		II	
Sampling:	No rinse	Rinse	No rinse	Rinse
Start	7,06 ±0,06	6,65 ±0,61	6,71 ±0,32	6,80 ±0,21
Starvation 24 h	5,73 ±0,40	6,09 ±0,17	7,18 ±0,33	7,07 ±0,19
Starvation 48 h	6,73 ±0,31	7,22 ±0,22	7,13 ±0,41	6,70 ±0,30

Generally a high level of bacteria from the *Enterobacteriaceae* familiy

- an apparent decrease at 24 h (exp. I) not supported by exp. II

Results:
Sterile feeding of larvae (II)

	Total aerobic count Log CFU/g		Enterobacteriaceae Log CFU/g	
Sampling:	No rinse	Rinse	No rinse	Rinse
Start	7,86 ±0,11	7,83 ±0,06	6,71 ±0,32	6,80±0,21
Sterile feed 24 h	8,05 ±0,24	7,83 ±0,12	6,58 ±0,15	7,12±0,41
Sterile feed 48 h	8,22 ±0,39	8,08 ±0,31	6,64 ±0,16	6,62±0,60

- For total aerobic count / *Enterobacteriaceae*
  - no clear effect of
    - starvation 24 - 48 h
    - rinsing with tap water
    - feeding with sterile substrate

Results:
Other bacterial groups in larvae

- Level of other bacterial groups in larvae

  Again no clear effect of 'treatment' i.e. starvation/rinsing/sterile feeding

- Psychrotrophs i.e. growing at 6.5°C
  - 3.5-5.5 log CFU/g
- Lactic acid bacteria
  - 5-7 CFU/g
- Bacterial endospores
  - close to or below detection limit <2 log CFU/g

Level of bacterial groups in substrate

(log CFU/g¤)

Bacterial group:	Substrate 0 h	Substrate 48 h	'Sterile' substrate 48 h
Total aerobic count	**4,19**±0,17	**7,28±0,37**	**8,35±0,58**
Psychrotrophs'	**4,39**±0,04	4,50±0,45	2,93±0,41
Enterobacteriaceae	**3,84**±0,54	**7,02±0,57**	**7,61±0,15**
Lactic acid bacteria	**2,35**±0,08	**5,90±0,19**	**5,86±0,09**
Bacterial endospores	**0,67**±1,15	0,67±1,15	1,65±1,43

¤Mean 3 replicates ±SD

Start at 0 h: Bacterial levels **<4.5** Log CFU/g

After 48 h larval feeding: Bacterial levels increases in both normal / sterile substrate - **depending on bacterial group!**
~ reflects larval content…

Summary:
Mealworm post-harvest practices

Neither, rinsing with tap water nor feeding with sterile substrate seemed to reduce bacterial load in mealworm larvae markedly

→ starvation to remove substrate from gut may still be desirable!!?

Generally high bacterial numbers in larvae

→ application of heat treatment necessary

Bacterial numbers in substrate apparently 'reflects' the level in the larvae (~balance)

Thanks to:

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Technical University of Denmark
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InnovationsFonden
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