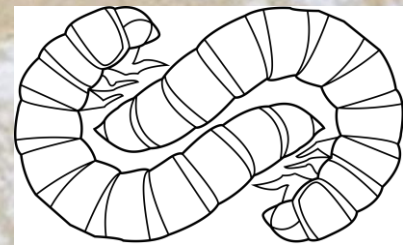


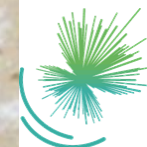
Improving mealworm health through nutrition

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UNIVERSITY OF COPENHAGEN



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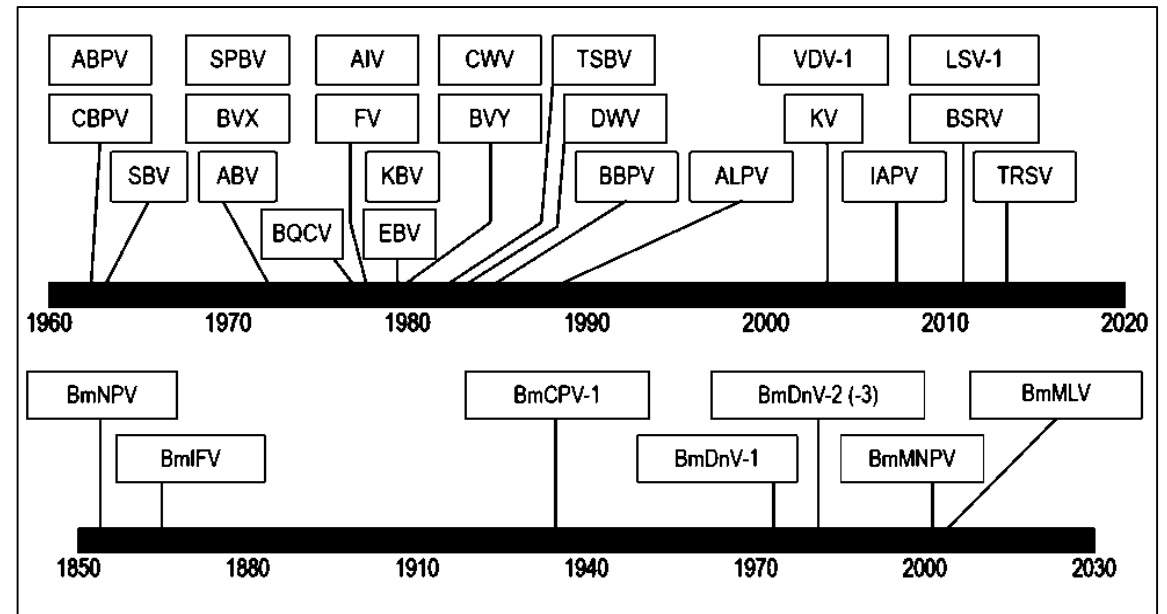
Why is this relevant? Why should we try to improve mealworm health?

No real dangers yet

Why is this relevant?

No real dangers yet **BUT:**

- **Learning** from past domestication attempts / Failures / Successes (?)

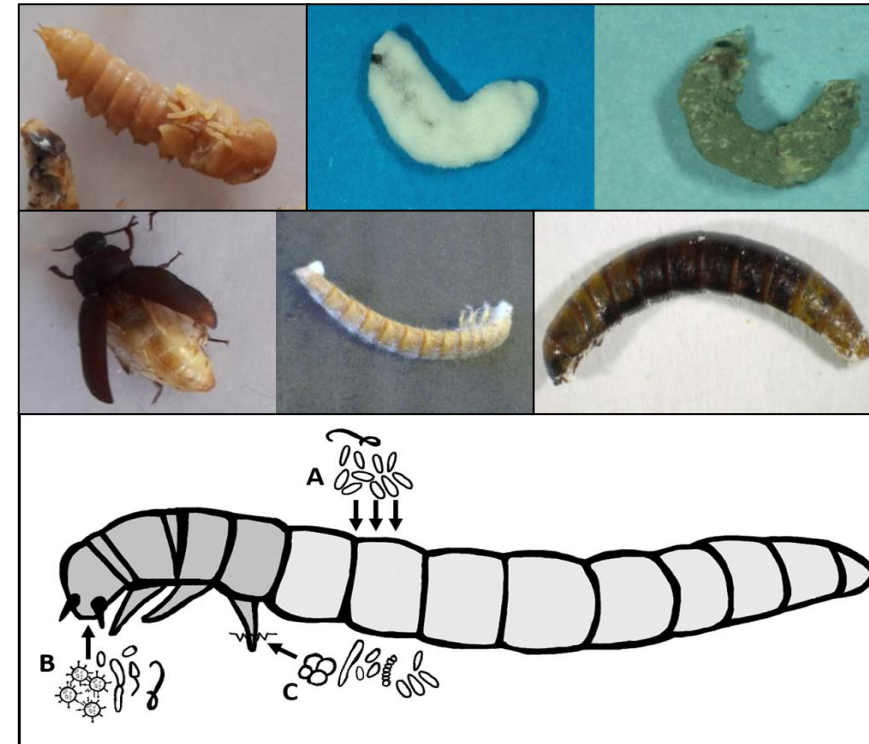


Timeline of discovery of new virus diseases in honeybees (*Apis mellifera*) and silkworm (*Bombyx mori*) Source: Eilenberg et al. 2015

Why is this relevant?

No real dangers yet **BUT:**

- **Learning** from past domestication attempts / Failures / Successes (?)
- **Future-proofing** the industry



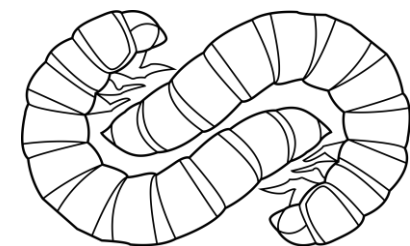
inVALUABLE: The focal areas and work packages

- **Production** (work packages 1-3)

Focus on optimization of the production of mealworms (WP1); improving the understanding of mealworm health and nutrition (WP2); and development of innovative technologies for implementing cost-effective production systems through automation and monitoring of mealworm health (WP3).

- **Processing** (work packages 4-5)

- **Product Application** (work packages 6-8)



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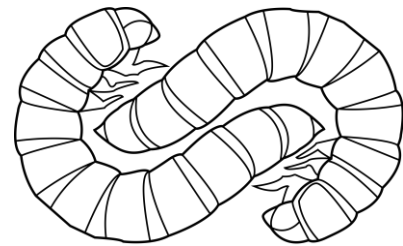
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inVALUABLE: Health and Nutrition

The aim is to better our understanding of the nutritional needs of mealworms and assess the feed potential of a range of selected by-products.

Strong focus on:

- **Preventing mealworm diseases**
- **Improving overall fitness**



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How?

Investigating the Effect of Different Acid Bacteria on the Fate of *Listeria Staphylococcus aureus* Infection in G

Beneficial Protective Role of Endogenous Lactic Acid Bacteria Against Mycotic Contamination of Honeybee Beebread

Irakli Janashia¹ · Yvan Choiset² · Damian Jozefiak³ · Franck Déniel⁴ · Emmanuel Coton⁴ · Chanishvili¹ · Thomas Haertlé^{2,3,5} 

Isolation and Partial Characterization of Lactic Acid Bacteria from the Gut Microbiota of Marine Fishes for Potential Application as Probiotics in Aquaculture

Sergio Alonso¹ · M. Carmen Castro¹ · Margarita Berdasco¹ · Inés García de la Banda² · Xabier Moreno-Ventas³ · Alma Hernández de Rojas¹

Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*)

Exploitation of the Medfly Gut Microbiota for the Enhancement of Sterile Insect Technique: Use of *Enterobacter* sp. in Larval Diet-Based Probiotic Applications



Antonios A. Augustinos^{1,2} , Georgios A. Kyritsis^{1,3} , Nikos T. Papadopoulos³, Adly M. M. Abd-Alla¹, Carlos Cáceres¹, Kostas Bourtzis¹ *

Probiotic *Enterococcus mundtii* Plate Protects the Model Insect *Diabroloium castaneum* against *Spizillus thuringiensis*

Grau¹, Andreas Vilcinskas^{1,2} and Gerrit Joop¹ *

Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae*

Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish

José L. Balcázar^{a, b}  , Daniel Vendrell^a, Ignacio de Blas^a, Imanol Ruiz-Zarzuola^a, José L. Muzquiz^a, Olivia Girones^a

Gut lactic acid bacteria as additives for mass rearing of *Tenebrio molitor*

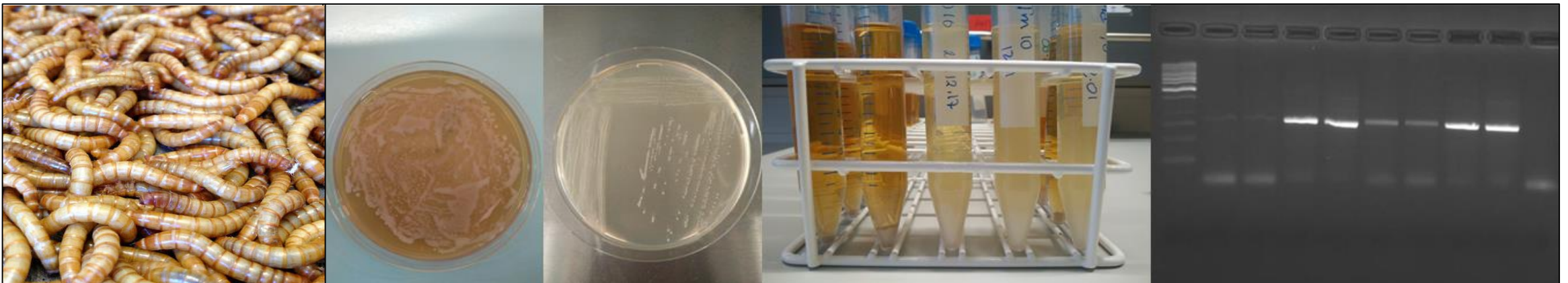
Expected benefits:

- **Improved survival**
- **Enhanced growth**
- **Nutritional benefits (to final consumer)**



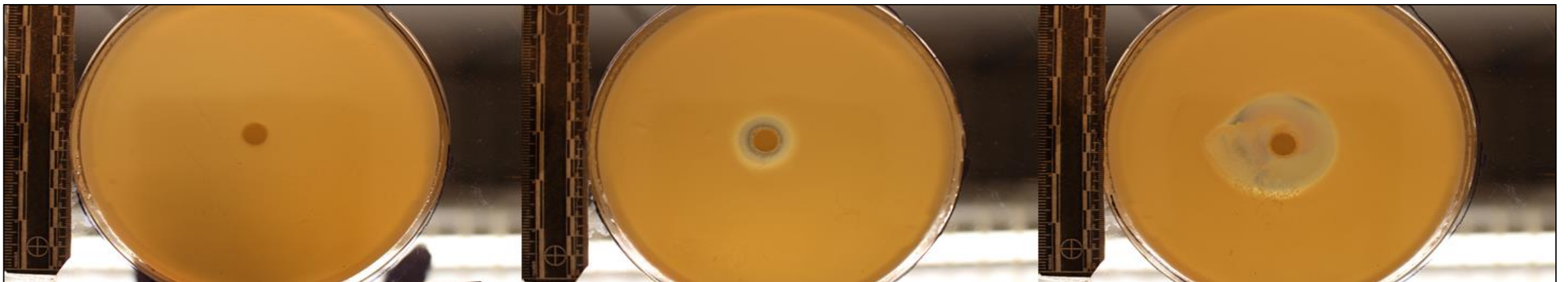
Screening, Isolation and Identification

- **Dissection** of mealworm guts (focus on gut flora)
- **Culture** on selective media for Lactic Acid Bacteria (MRS)
- **Identification** using MALDI biotyper and Sanger Sequencing



In vitro antimicrobial activity

- Focus on **inhibition potential**
- Selected **pathogenic bacteria**: *Bacillus thuringiensis* X3, *Serratia marcescens*, *S. plymuthica*, *Pseudomonas aeruginosa*



In vitro antimicrobial activity

- **Candidate selection** partly based on **growth requirements**
- ***Pediococcus pentosaceus*** strain selected for *in vivo* experiments

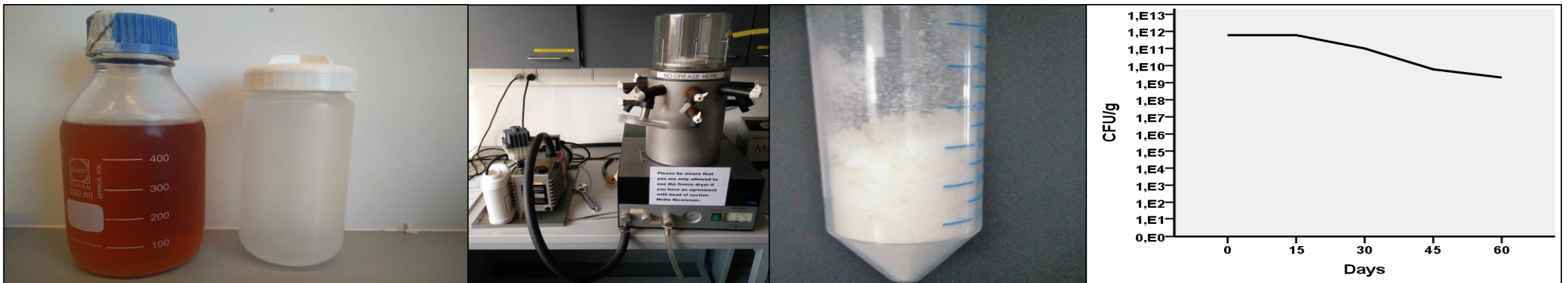
Strain	<i>Bt12</i>	<i>BtUK</i>	<i>Bt34</i>	<i>S. marcescens</i>	<i>S. plymuthica</i>	<i>P. aeruginosa</i>
Mean inhibition zone (mm)	11,3	11,7	11,5	7,8	12	8,5



The image shows six petri dishes arranged horizontally, each containing a bacterial culture with a central well. The wells show varying sizes of clear inhibition zones. Below each dish is a red label with the strain name: Bt 12, Bt UK, Bt 34, S. marcescens, S. plymuthica, and P. aeruginosa.

Formulation of Selected LAB Strain, *P. pentosaceus*

- **Freeze drying** enables **easy delivery** of bacteria in **existing diet**
- Improves **storage stability** and **cell viability** at rearing temperatures

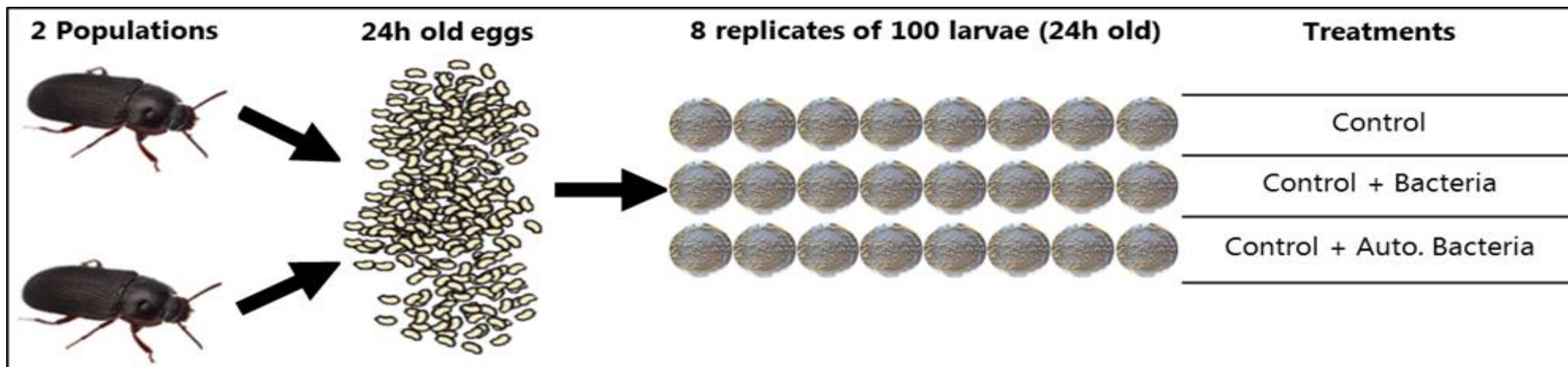


In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Aim:

To test the effect of *P. pentosaceus* as a supplement on the fitness and development of *T. molitor*

Treatments:



In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Aim:

To test the effect of *P. pentosaceus* as a supplement on the fitness and development of *T. molitor*

Treatments:

C	Control = ad libitum dry food (50g) and brewers' grain (variable)
CB	Control + Bacteria ($1 \cdot 10^9$ C.F.U./g)
CB ⁻	Control + Bacteria Autoclaved



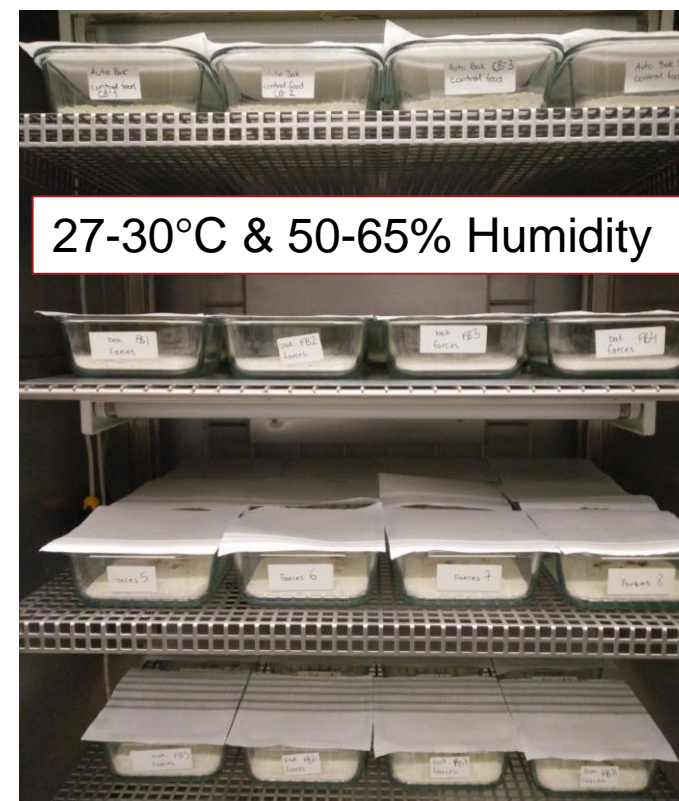
In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Aim:

To test the effect of *P. pentosaceus* as a supplement on the fitness and development of *T. molitor*

Variables:

- Larval survival and weight
- Number of pupae and emerged intact adults
- Number of eggs per adult female (over 7 days)
- Adult weight
- Gut microbiome analysis (at larval harvest weight)



In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Results:

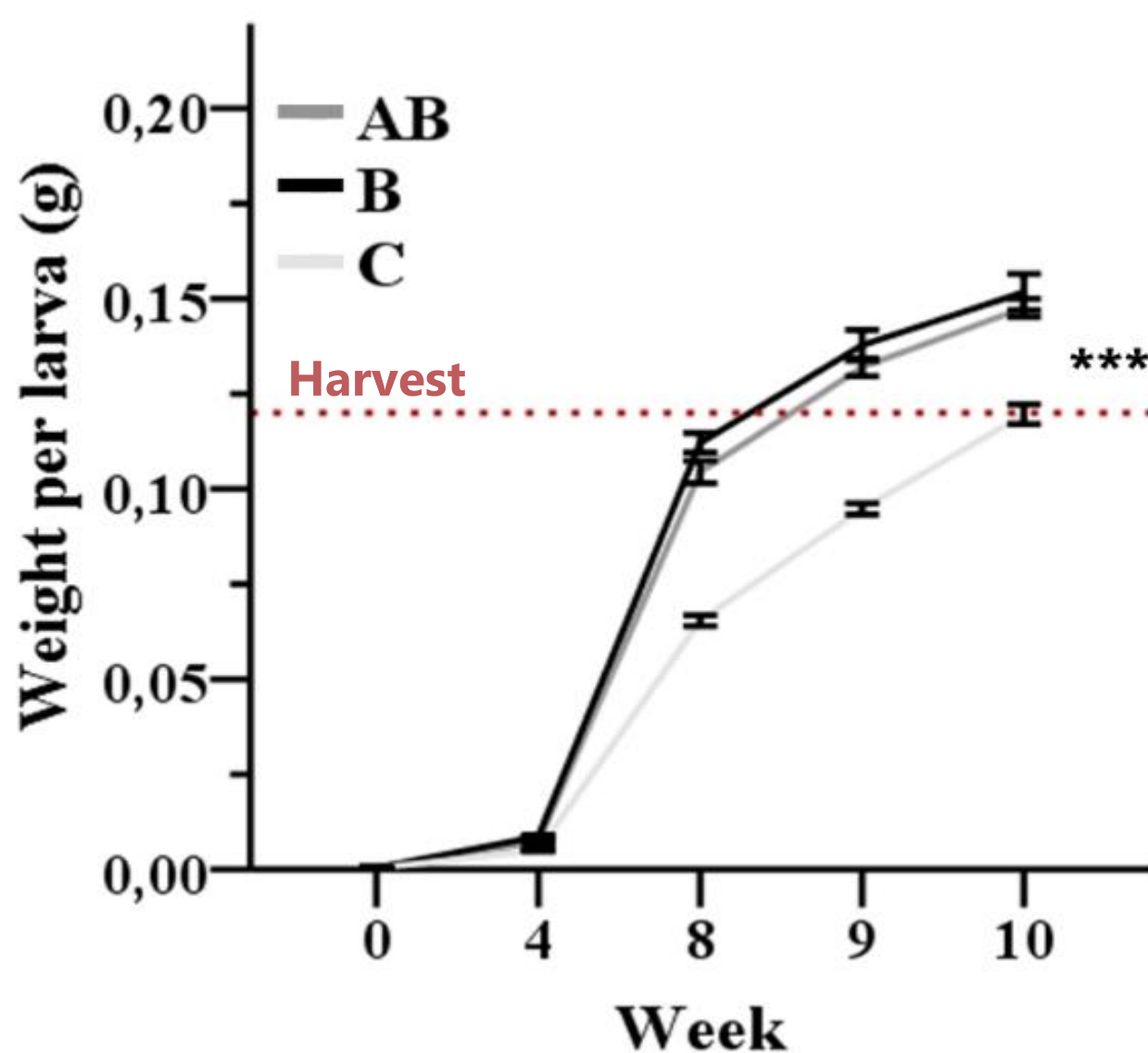
Larval weight



Bacteria

Autoclaved Bacteria

Control



In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Results:

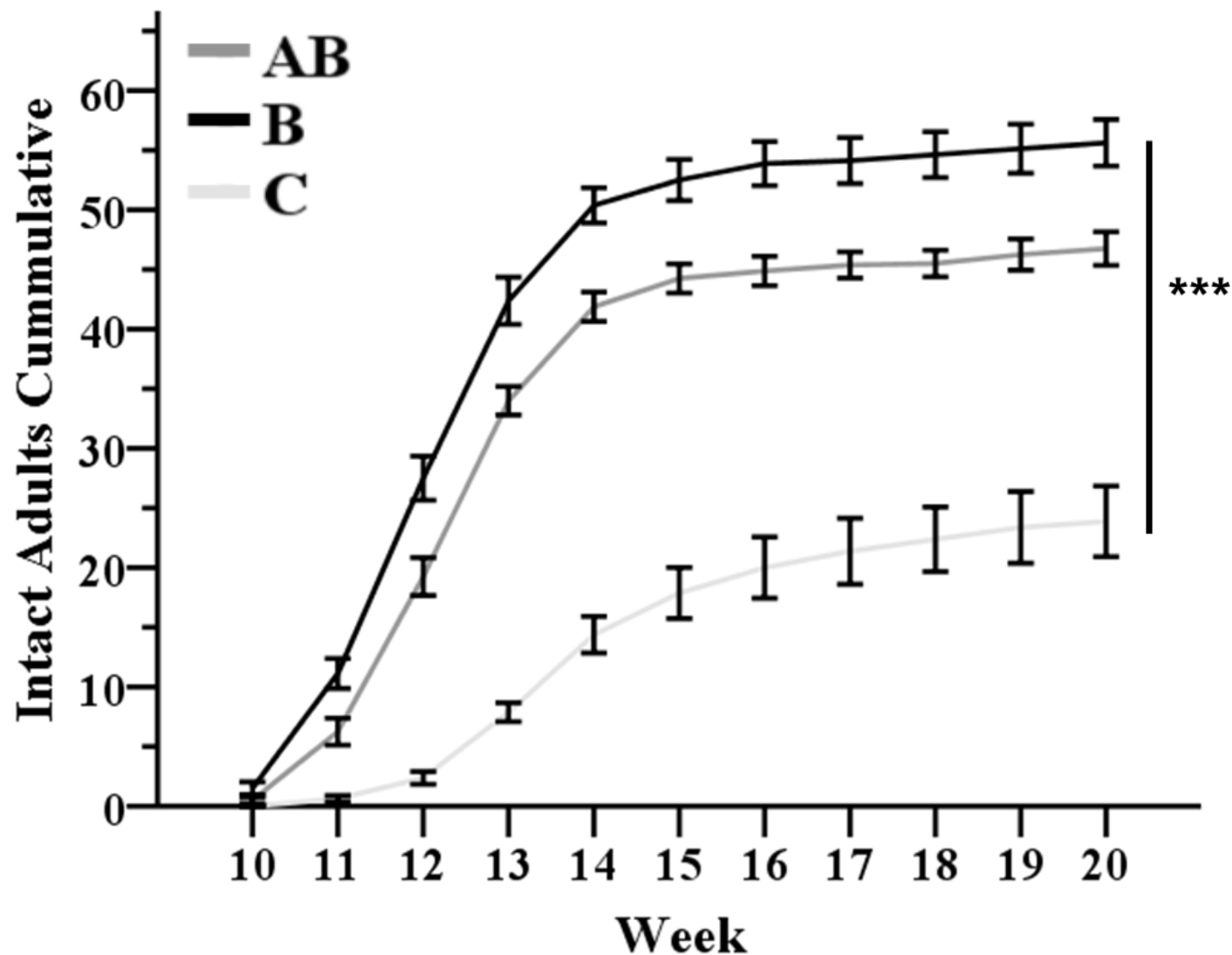
Number of intact emerged adults



Bacteria

Autoclaved Bacteria

Control

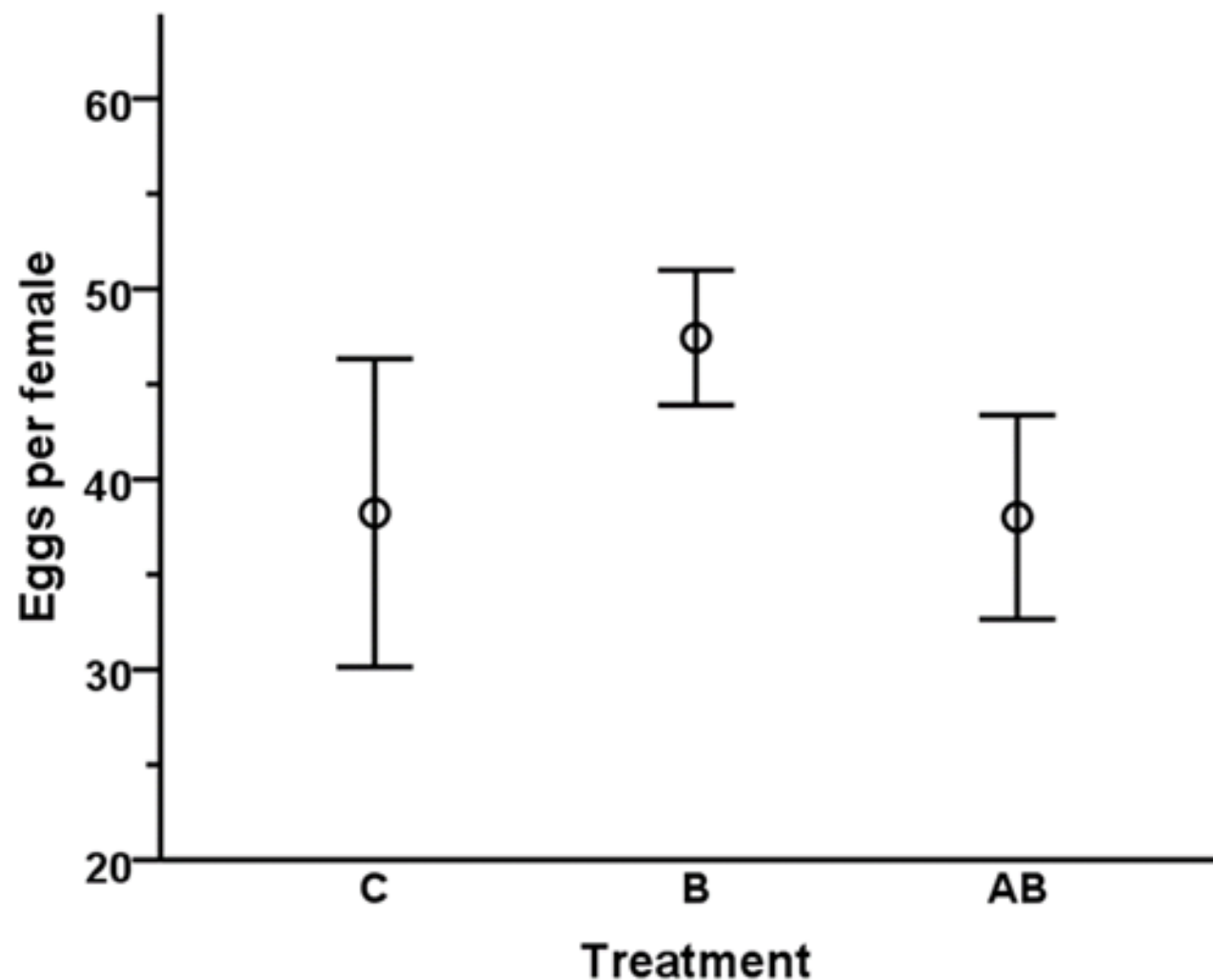


In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Results:

Number of eggs
per female over 7 days

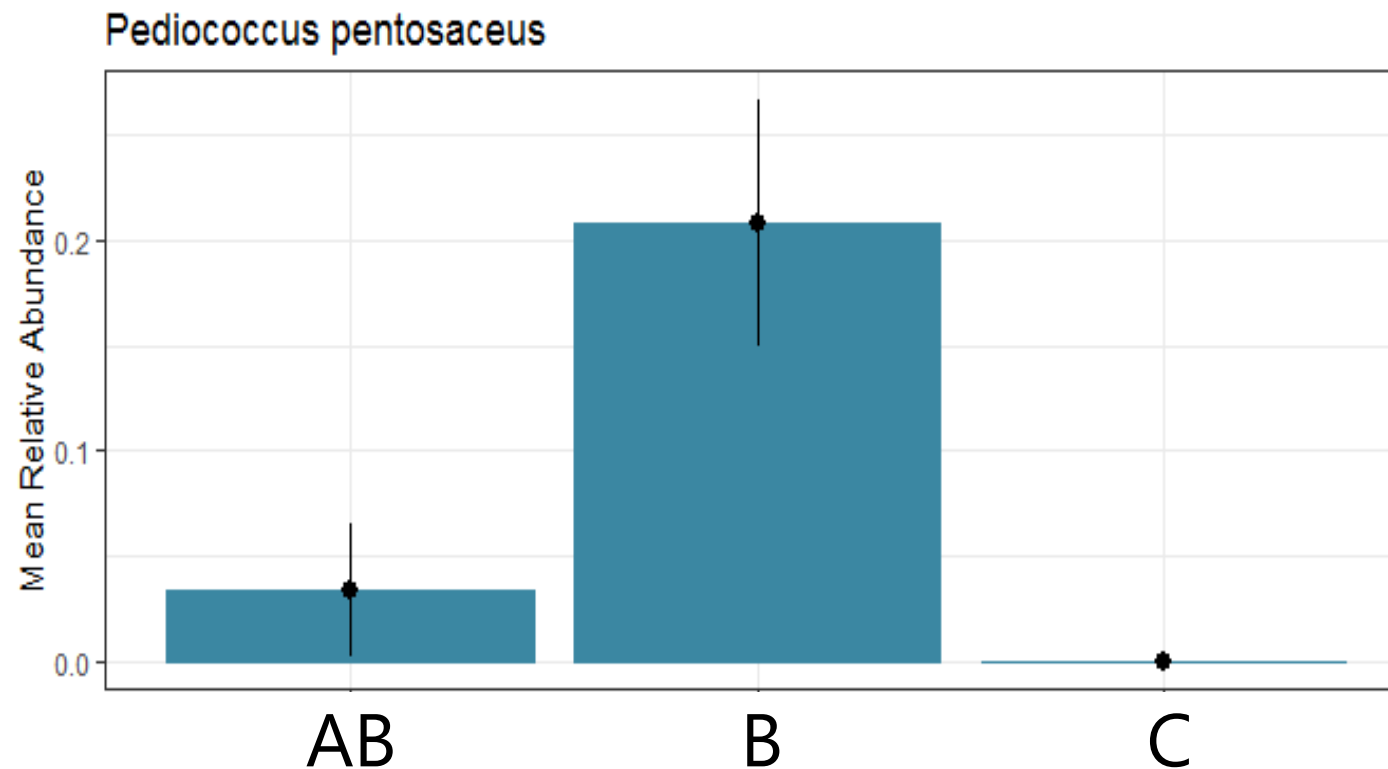
X No significant
differences:
- More replicates?



In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Results:

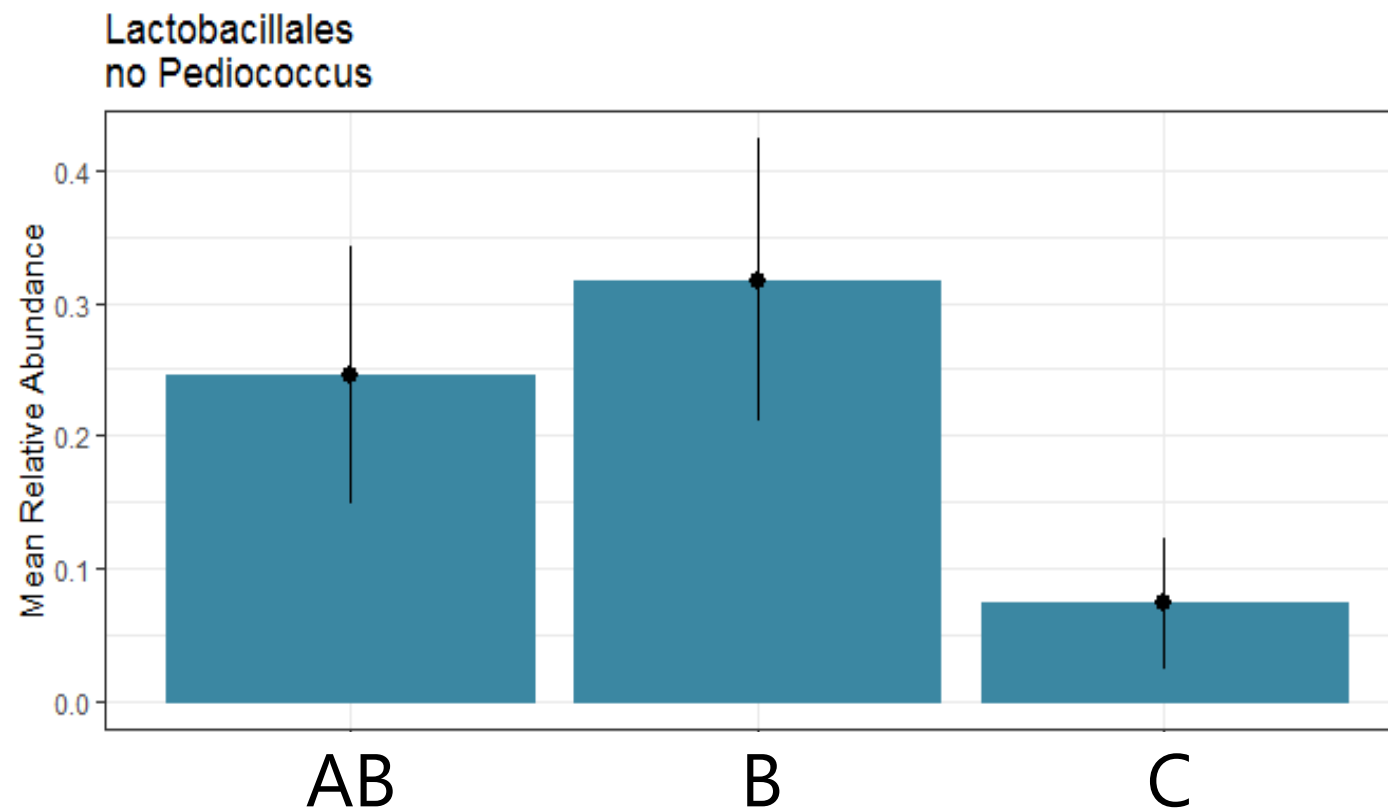
Microbiome sequencing, Relative abundance



In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Results:

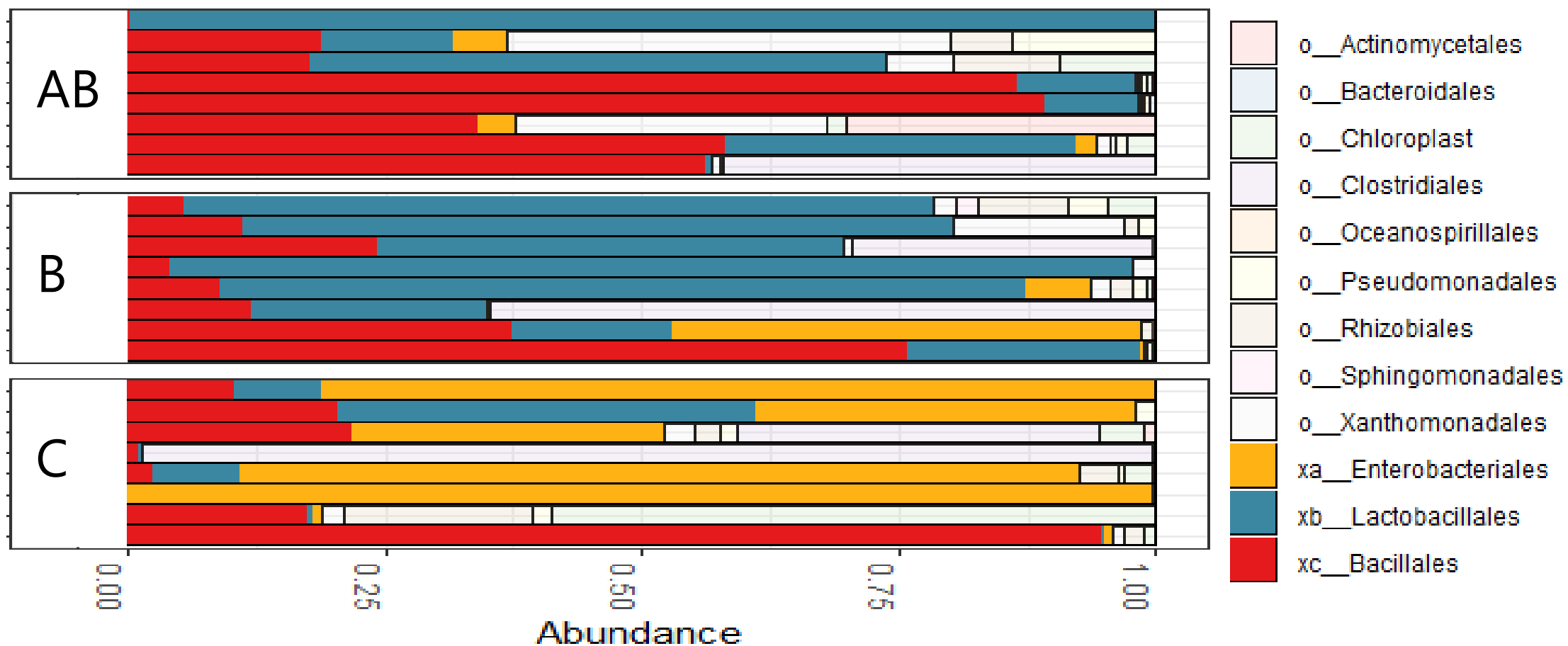
Microbiome sequencing, Relative abundance



In vivo effects of *P. pentosaceus* on *T. molitor* fitness

Results:

Microbiome sequencing, Relative abundance (Order level)



Conclusions and future perspectives

- Identification of pathogens and threats ongoing
- Lactic acid bacteria showing potential as probiotic additives but:
- Further *in vivo* experiments needed to determine

Pathogen protection

Dosage

Inter-generational effects

Effects at production scale



THANK YOU

Everyone from inVALUABLE

Jørgen Eilenberg

Annette Bruun Jensen

Myrsini Eirini Natsopoulou



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