IBD2025

มหาวิทยาลัยธรรมศาสตร์
THAMMASAT UNIVERSITY

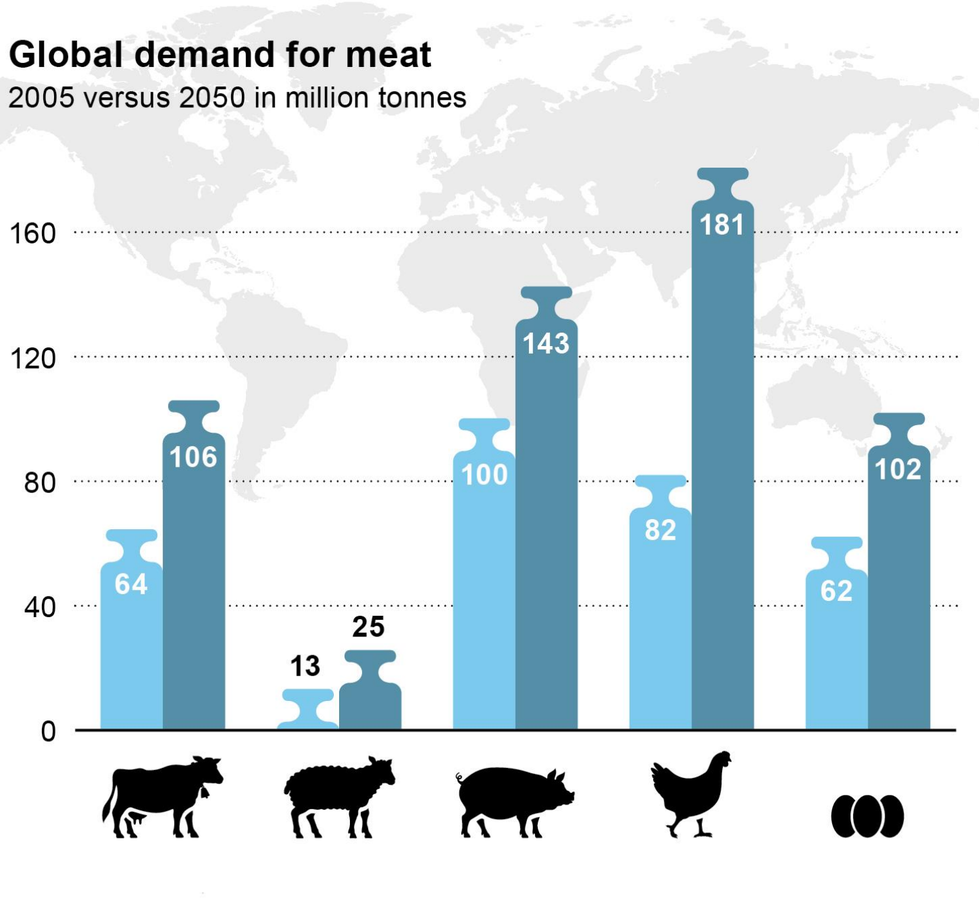
International Conference on Biodiversity 2025
Session 1: Biodiversity & Food Security

Composition and diversity of bacterial communities in the gut of black soldier fly larvae (*Hermetia illucens* L.) reared on organic wastes

Presented By **Sutinee Plodprong**

Faculty of Science and Technology,
Thammasat University Rangsit Campus

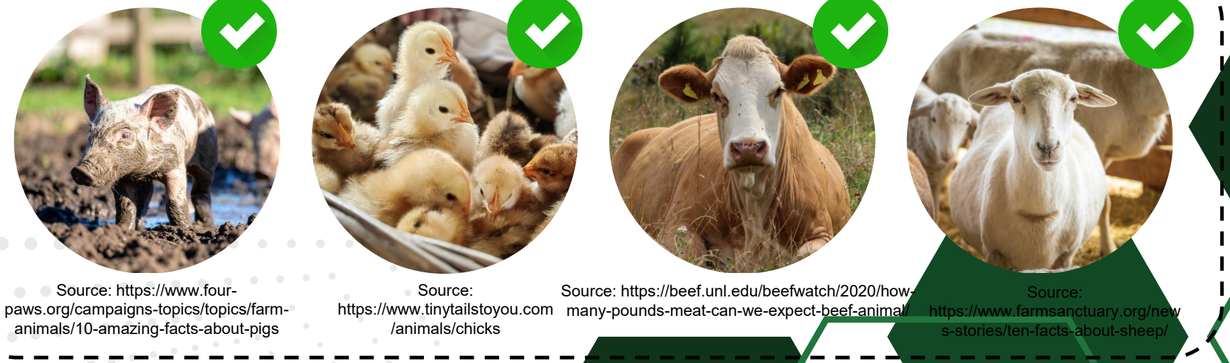
INTRODUCTION



Source: Food and Agriculture Organization of the United Nations, ESA Working paper No. 12-03, p. 131

Source: https://bluehorizon.com/insight/high-increase-in-demand-per-meat-category-by-2050-show-chicken-with-the-largest-increase-and-total-volume/

- **Rising global population drives higher consumption of animal-based protein.** (United Nations, 2019)
- **Increased livestock & feed demand emphasize the need for sustainable feed sources.** (Lu et al., 2022)



Source: https://www.four-paws.org/campaigns-topics/topics/farm-animals/10-amazing-facts-about-pigs

Source: https://www.tinytailstoyou.com/animals/chicks

Source: https://beef.unl.edu/beefwatch/2020/how-many-pounds-meat-can-we-expect-beef-animal

Source: https://www.farmsanctuary.org/news-stories/ten-facts-about-sheep/

Protein-rich edible insects: a sustainable protein alternative.

(Nowak et al., 2016)

BSFL; Black soldier fly larvae

Saprophagous insect:

It converts low-value organic waste.

Source: https://www.livinginsider.com/profile/65246/member_info.html

Source: https://www.sanook.com/women/229989/

Source: https://www.scghome.com/living-ideas/articles.

Source: https://growlandscapes.com.au/product/chook-manure/

Nutrient-rich “insect biomass”

BSFL; Black soldier fly larvae

Source: https://www.freepik.com/premium-photo/close-up-black-soldier-fly-bsf-larvae-maggot-hermetia-illucens_133636932.htm

- **Protein** 35-49%
- **Fat** 29-35%
- **Energy** 2,900 Kcal/Kg
- **Amino acid profile** = fish meal

“Dietary supplement” for livestock animals.

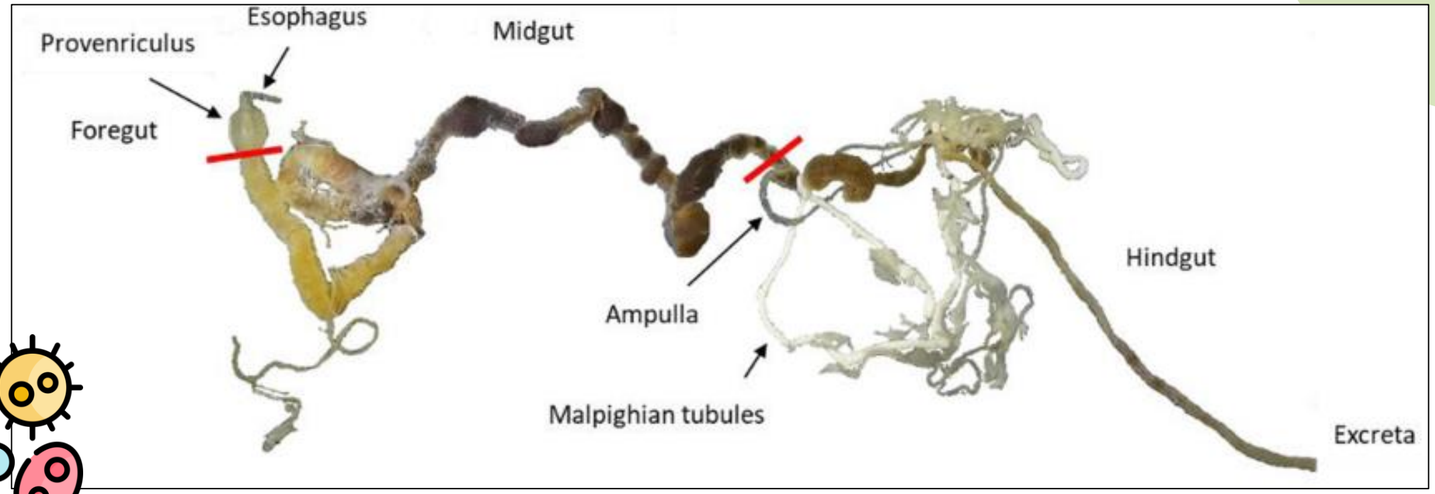
(Abd El-Hack et al., 2020; Pornsuwan and Hanboonsong, 2023)

Effective growth of BSFL as a protein source depends on “substrates”.

(Gligorescu et al., 2018)

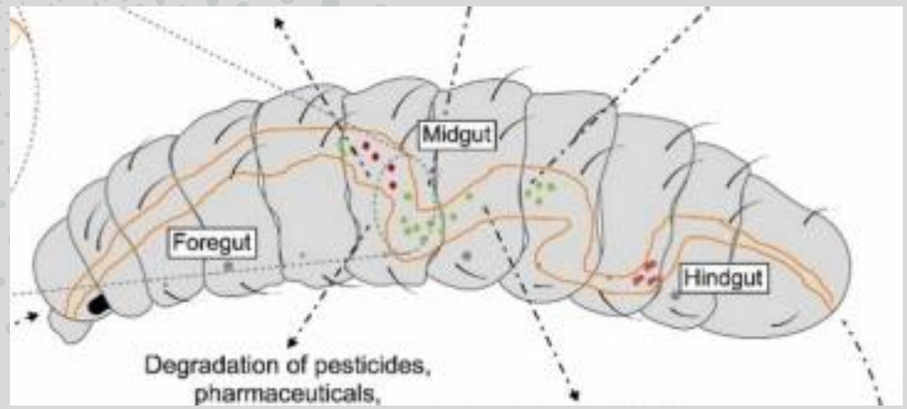
Source: https://flukerfarms.com/reptile-u/blog/black-soldier-fly-larvae-soldierworm-grubs-/

Gut microbiota (Chen et al., 2023)



- Digestion
- Antimicrobial peptide (AMPs) synthesis
- Overall insect health (Silvaraju et al., 2024)

Source: https://www.mdpi.com/1422-0067/21/14/4955



Other factors

affect changes in gut microbiota.

Biotic factors

- Developmental stage
- Genetic background
- Gut regions
- Initial substrate microbes

Abiotic factors

- Geographic locations
- Temperature
- Substrates pH
- Density

(Bruno et al., 2019;Wynants et al., 2019; Auger et al., 2023)

AMPs properties of BSFL & its gut microbiota: lead to alternative valorization of the BSF system.

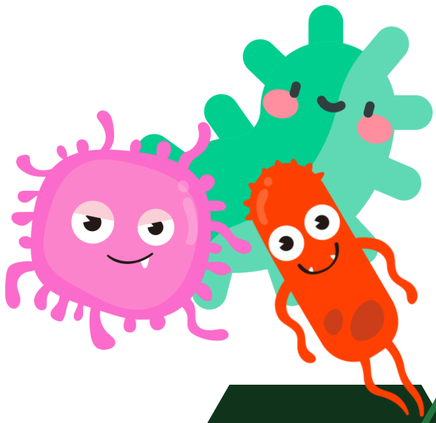
Bruno et al. (2019) & Khamis et al. (2020)

- **BSFL** can serve as reservoirs for various significant **foodborne pathogens.**

Biosafety assessments should be conducted.

- **Substrates = "Microbial contamination"** resulted in a significant shift in the **BSFL gut microbiota.** (Chen et al., 2023)

Bacterial communities in the gut of BSFL reared on organic wastes that locally available in **Thailand remains unexplored.**



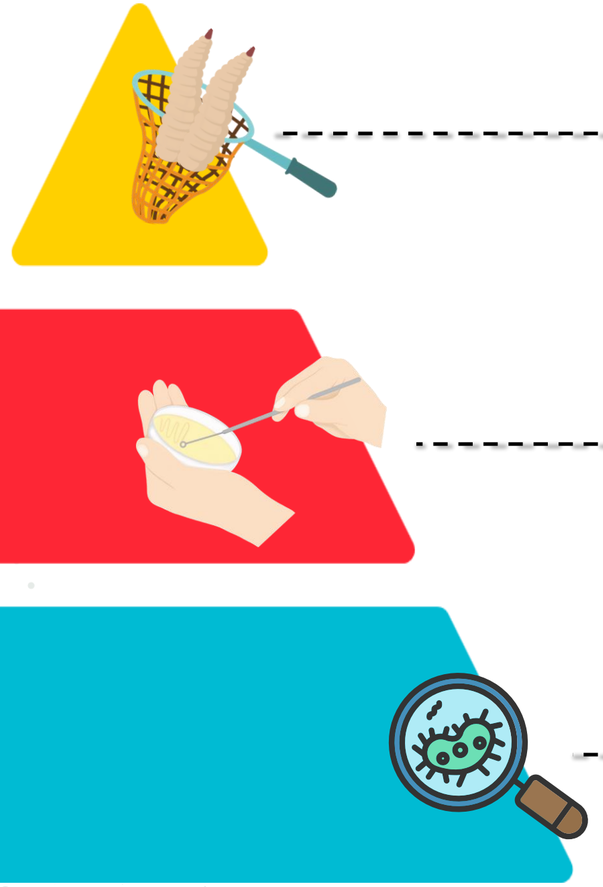
OBJECTIVES

To investigated effect of substrates on the gut bacterial communities of BSFL.

Source: https://extension.entm.purdue.edu/publications/E-276/E-276.html

Source: https://www.undp.org/india/stories

METHODOLOGY



1. Collecting BSFL samples
2. Bacterial culture method
3. Bacterial non-culture method

1 Collecting BSFL samples

1

FOW; Food waste

2

FSM; Food waste 1: Supermarket waste 1

3

FRW; Fruit waste

4

FSB; Fruit waste 1: Soybean residue 1

5

CHM; Chicken manure

6

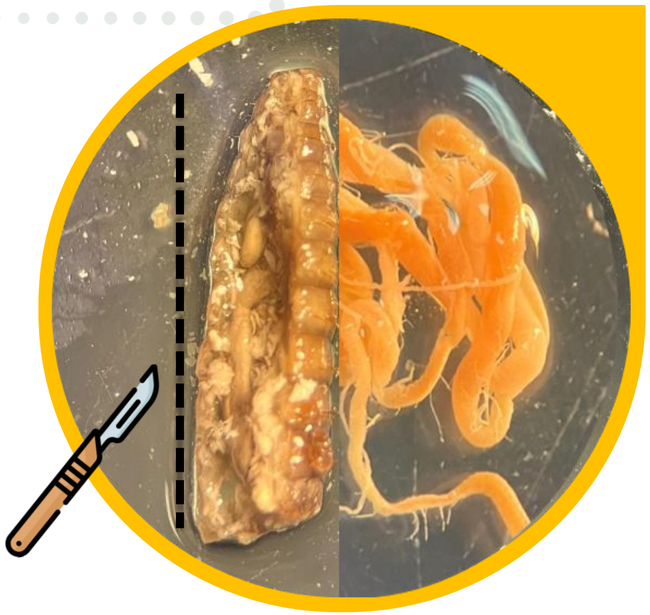
STD; Control diets (chicken feed 1: soybean residue 1)

1 Collecting BSFL samples

- Larvae 13-17 days old.
- Dark brown color.

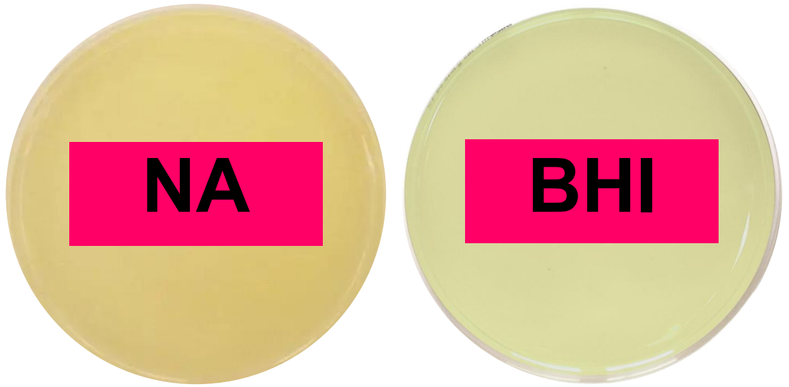
2 Bacterial culture method

2.1 Gut sample preparation



Dissection of BSFL gut
& grinding with solution.

2.2 Bacteria cultivation



- 30 °C
- 24–48 h

“Observation” of colony morphology

- Form
- Margin
- Elevation
- Color
- Gram staining

2 Bacterial culture method

2.3 Bacteria DNA Extraction

Bacteria DNA Extraction

- Invitrogen PureLink™ Genomic DNA Mini Kit

PCR

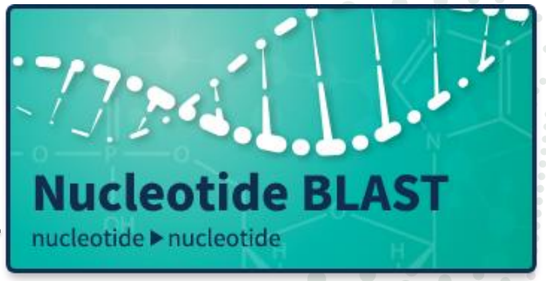
Target gene: 16s rRNA region

Specific primers:

- **27F: 5-AGAGTTTGATCMTGGCTAG-3**
- **1513R: 5-ACGGYTACCTTGTTACGACTT-3**

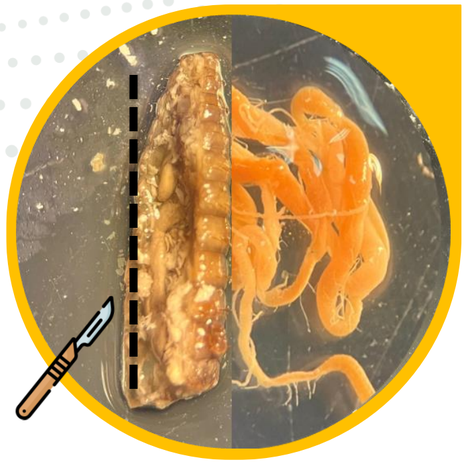
Bacterial identification

Nucleotide sequence analysis



Source: https://blast.ncbi.nlm.nih.gov/Blast.cgi

3 Bacterial non-culture method



3.1 Dissection of BSFL gut

3.2 Gut **DNA Extraction**

- Invitrogen PureLink™ Genomic DNA Mini Kit

Metagenomics technique

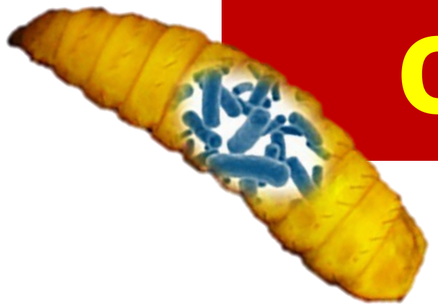
3.3

- **Bacterial diversity analysis**

BMKGENE

RESULTS & DISCUSSION

“Bacterial communities: culture method”



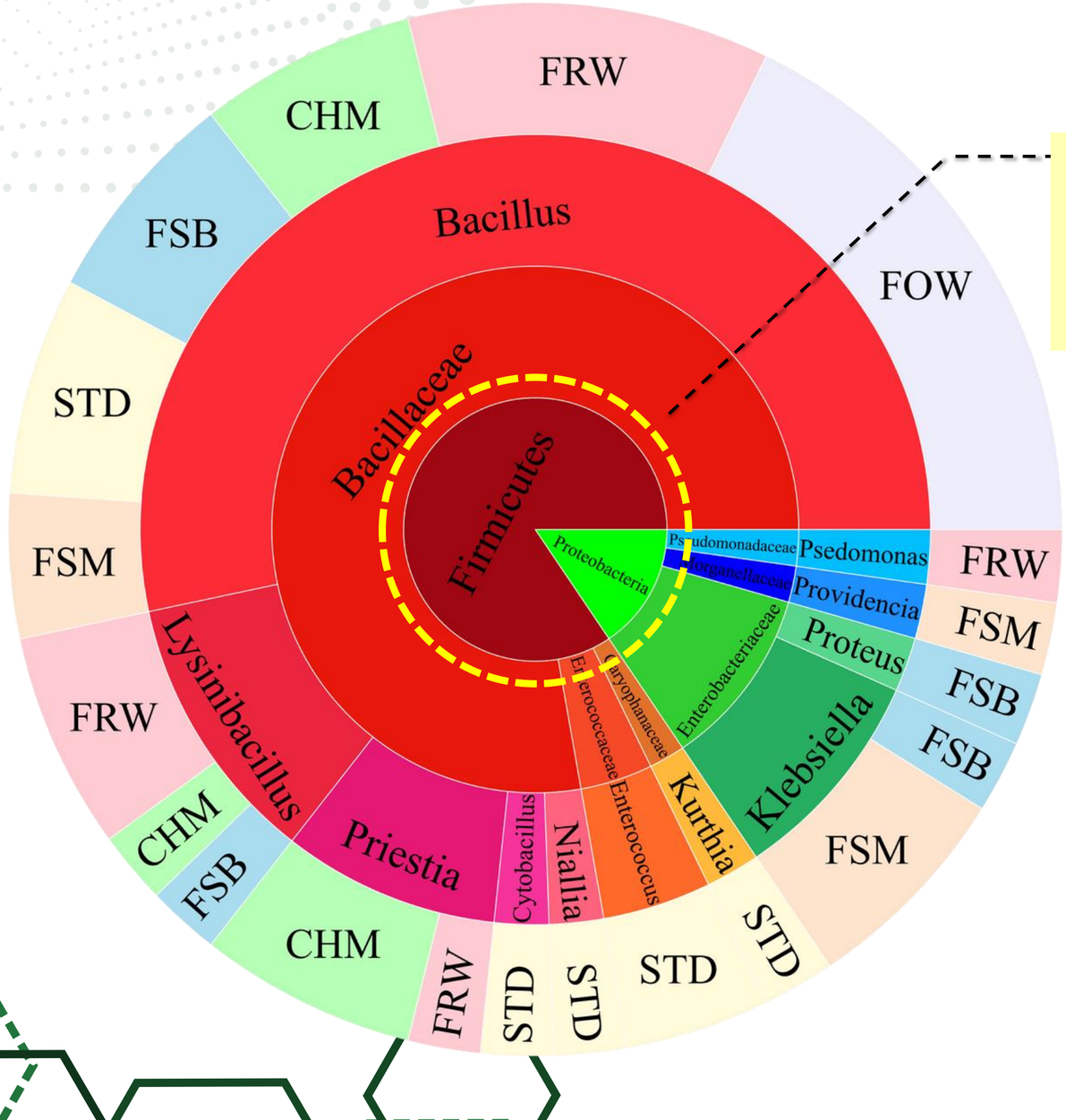
Source: https://www.globalseafood.org/advocate/improving-the-lipid-profile-of-black-soldier-fly-larvae/

“Bacterial culture method”

A total of **45 isolates** were obtained from different substrates, selected based on distinct colony morphologies.

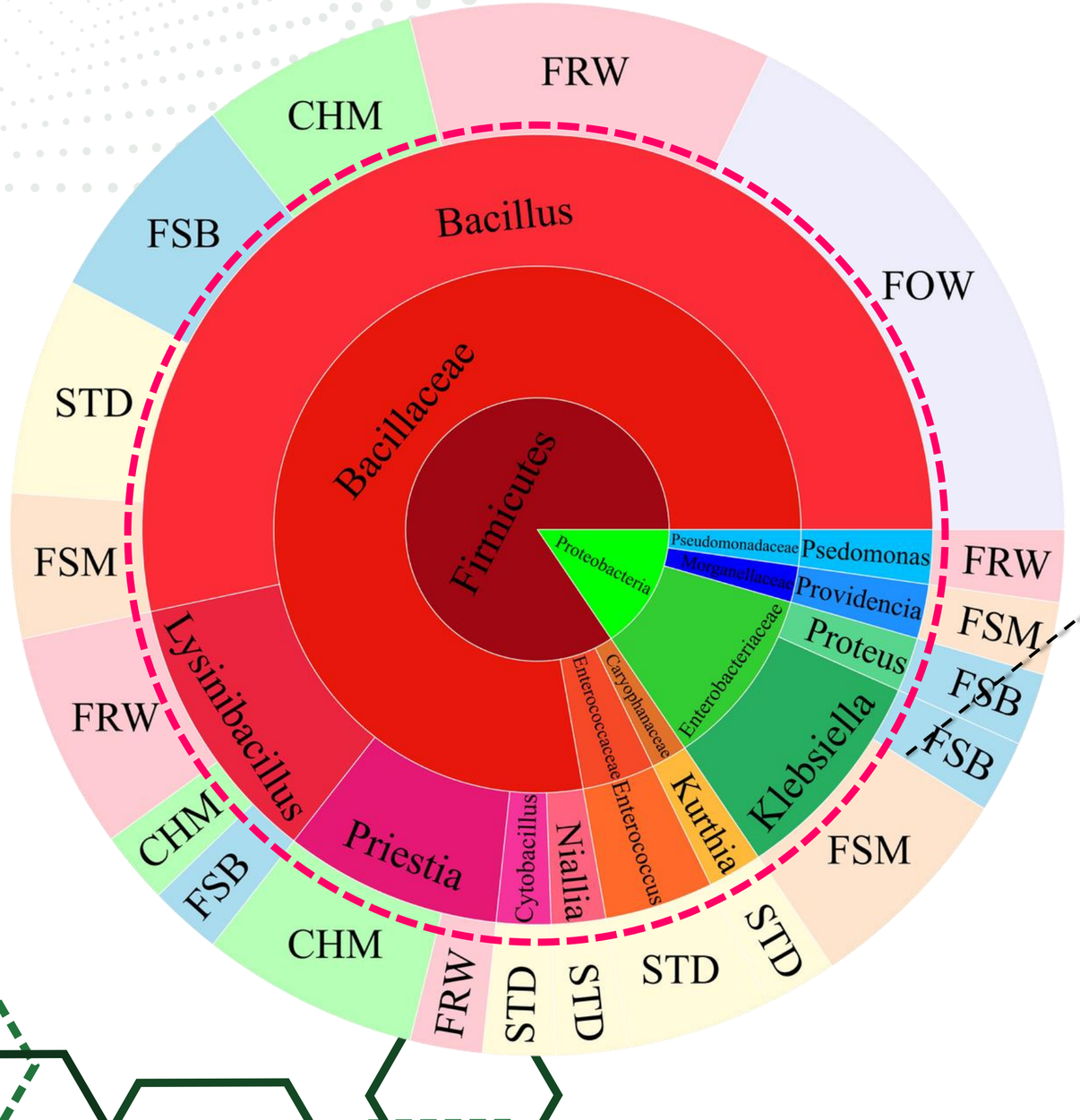
- **STD & FOW: 8 isolates**
- **FSM & FSB: 6 isolates**
- **FRW: 10 isolates**
- **CHM: 7 isolates**

“Bacterial culture method” - Phylum level



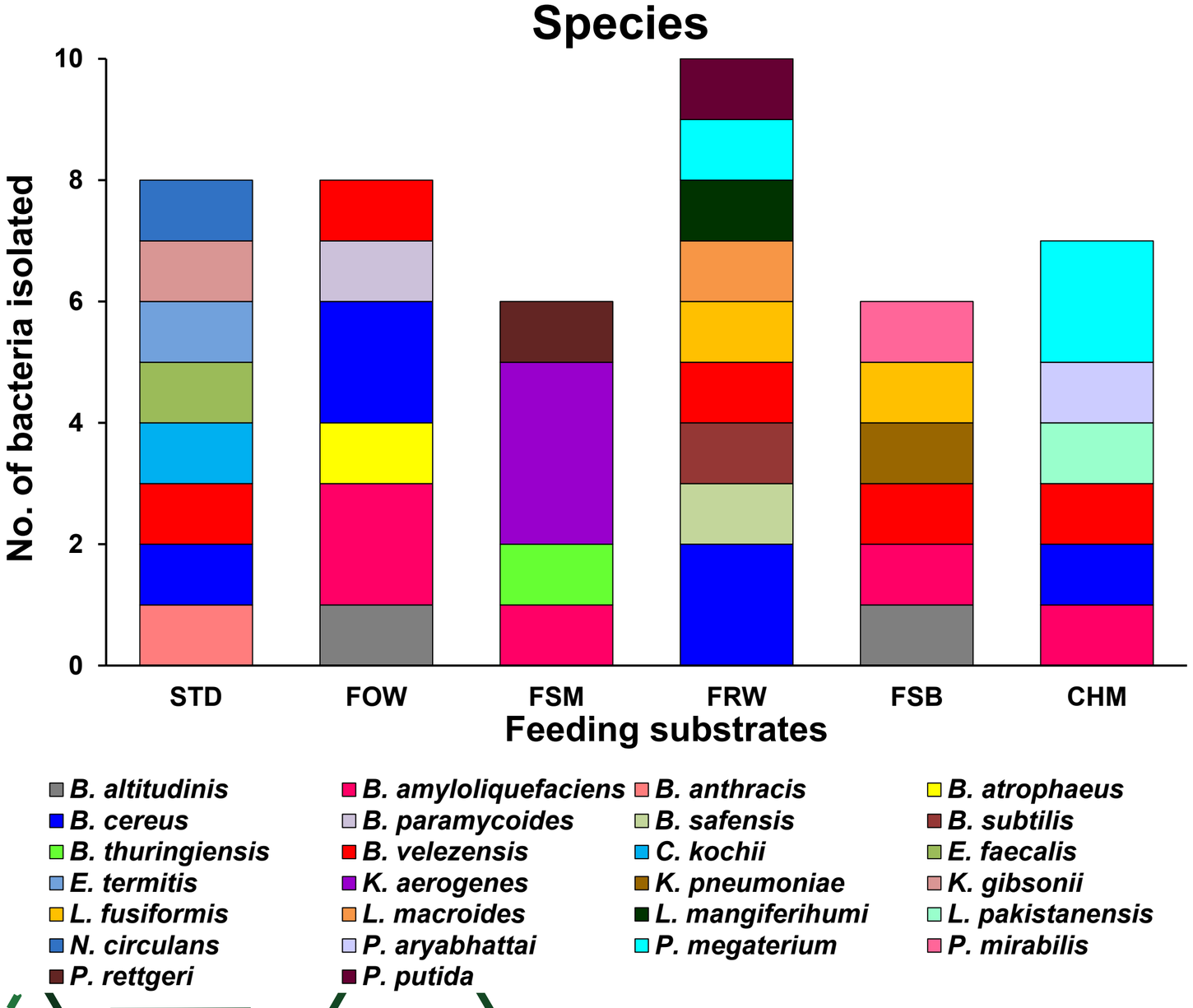
- **2 Phyla**
  - **Firmicutes:** 6 substrates
  - **Proteobacteria:** FSM, FRW & FSB

“Bacterial culture method” - Genus level



- **11 Genera**
  - ***Bacillus*: 6 substrates**
  - ***Lysinibacillus*: FRW, FSB & CHM**
  - ***Klebsiella*: FSM & FSB**
  - ***Enterococcus*: STD**
  - ***Providencia*: FSM**
  - ***Pseudomonas*: FRW**

“Bacterial culture method” - Species level



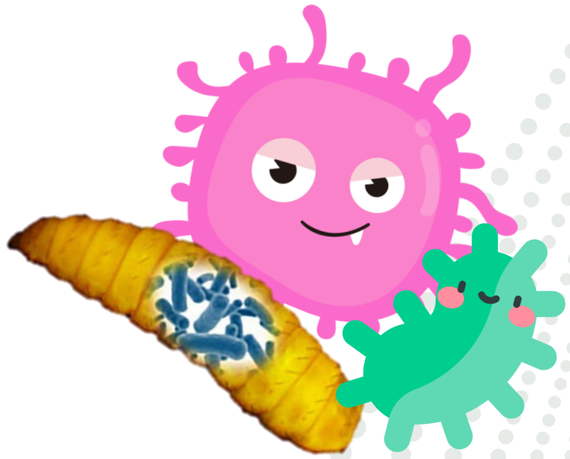
- 28 Species
  - *B. cereus*
  - *B. amyloliquefaciens*
  - *B. velezensis*

FRW: the highest diversity. (9 species)

Dominate bacteria….

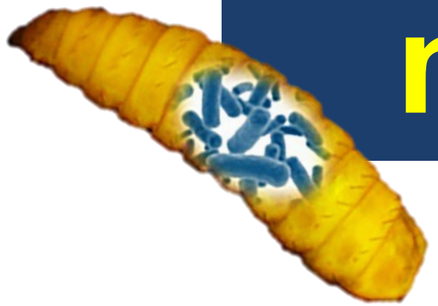
genus *Bacillus* (Shumo et al., 2021; Pei et al., 2024; Van Kessel et al., 2024)

- **Enhances production of digestive enzymes. (protease, amylase, cellulase & lipase)**
- **Antibiotic synthesis**



RESULTS & DISCUSSION

“Bacterial communities: non-culture method”



Source: https://www.globalseafood.org/advocate/improving-the-lipid-profile-of-black-soldier-fly-larvae/

“Bacterial non-culture method”

Table 1 Summary on raw data processing and alpha diversity of the gut bacterial communities of BSFL fed on different substrates.

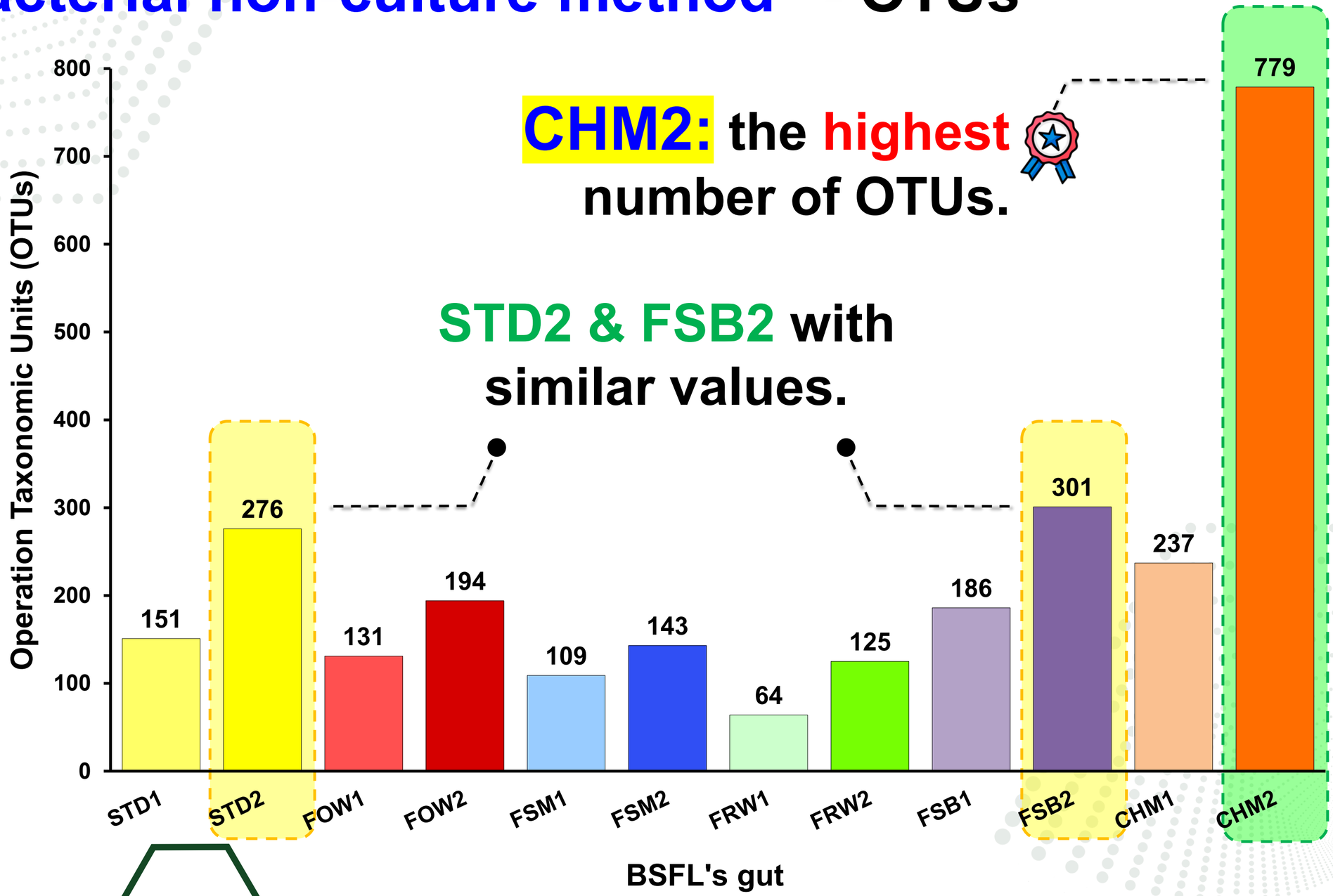
Feeding substrates	Raw reads	Clean reads	Shannon	Simpson	Choa1
STD1	79,876	74,408	4.70	0.94	151.27
STD2	80,363	75,172	**5.12**	**0.95**	276.02
FOW1	80,019	73,436	2.12	0.53	131.18
FOW2	79,837	74,809	2.79	0.76	194.00
FSM1	80,406	74,215	2.13	0.55	109.03
FSM2	79,960	75,158	3.19	0.83	143.03
FRW1	80,186	74,136	2.09	0.55	64.00
FRW2	79,898	75,265	3.35	0.75	125.04
FSB1	79,893	73,494	4.88	0.92	186.03
FSB2	80,234	75,521	3.70	0.74	**301.01**
CHM1	79,886	73,818	3.69	0.79	237.00
CHM2	**80,141**	**76,095**	**6.20**	**0.97**	**779.00**

CHM2: the highest gut bacterial diversity.

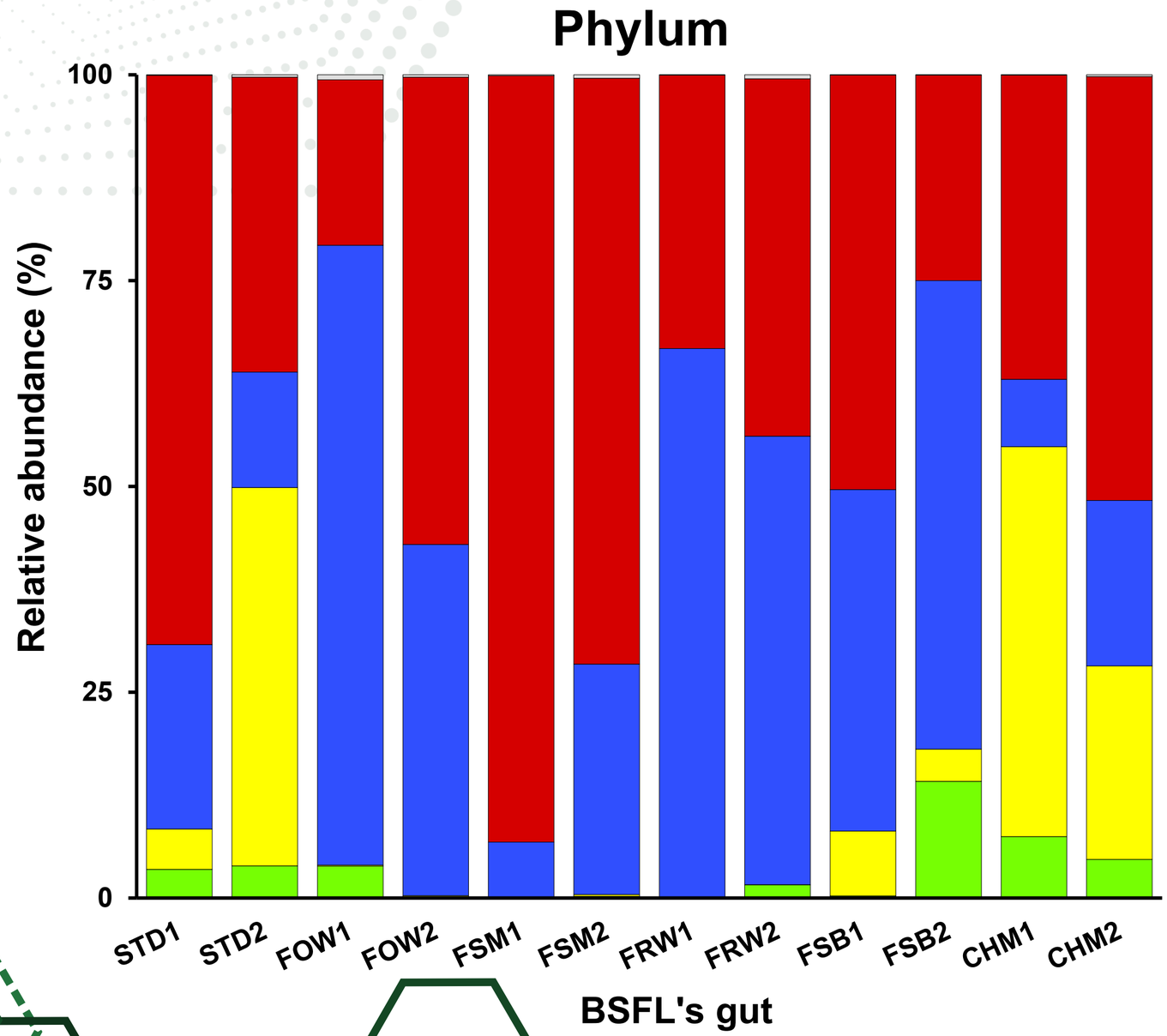
“Bacterial non-culture method” - OTUs

CHM2: the highest number of OTUs.

STD2 & FSB2 with similar values.



“Bacterial non-culture method” - Phylum level



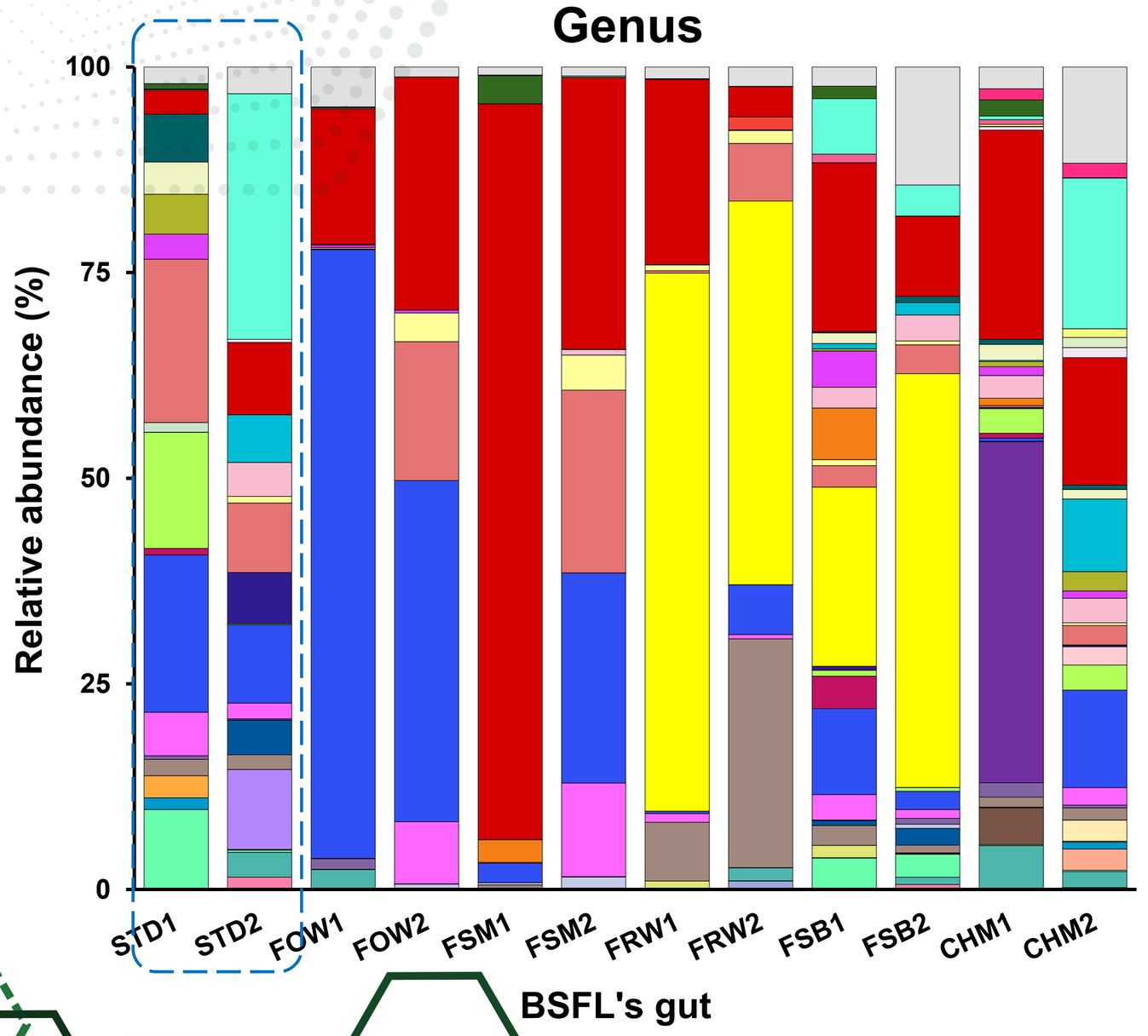
All substrates

- **Proteobacteria**
- **Firmicutes**

Some rearing substrates

- **Bacteroidota**
- **Actinobacteriota**

“Bacterial non-culture method” - Genus level



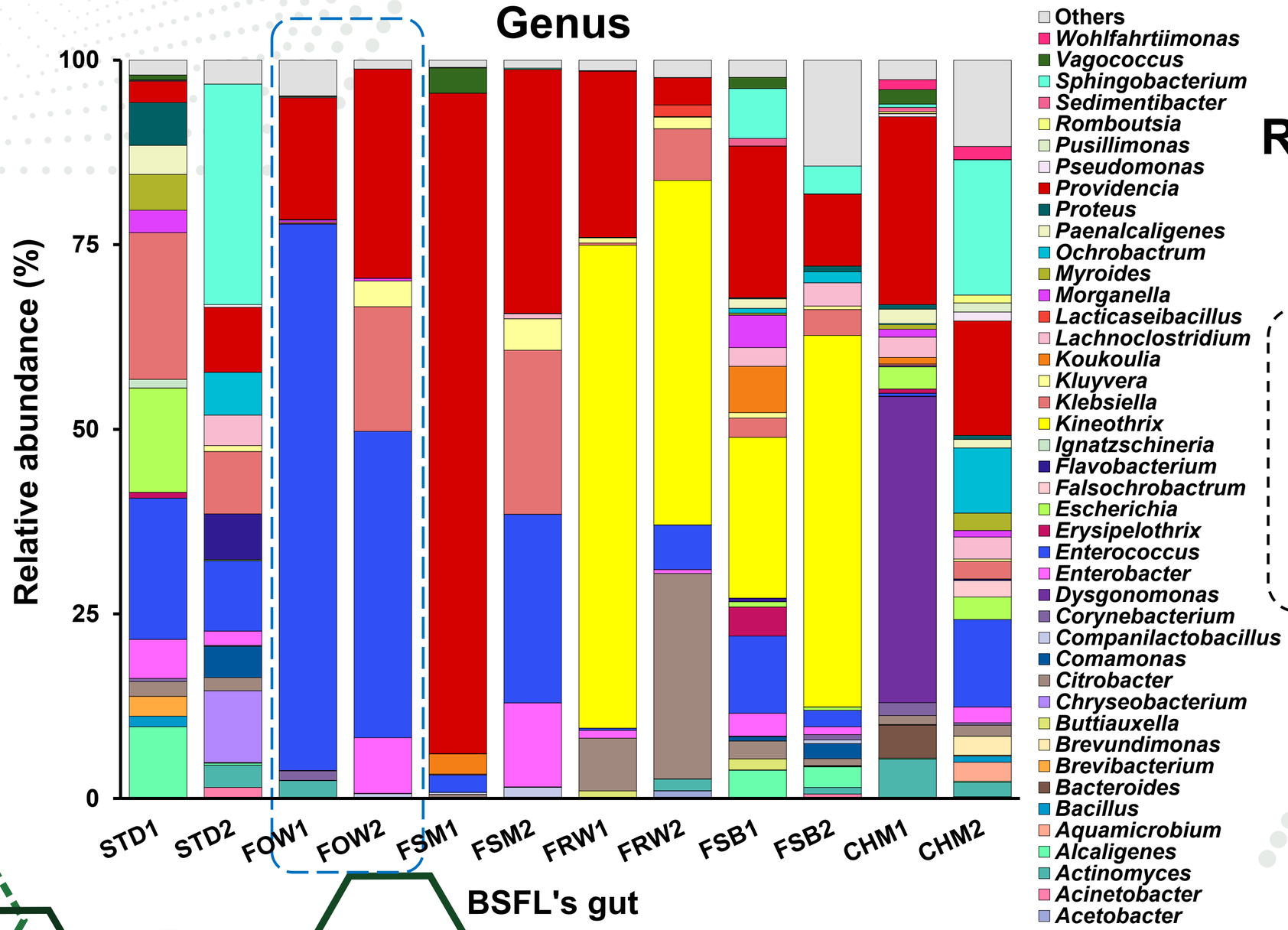
Relative abundance ≥ 1%

STD

rep. 1&2

- *Klebsiella*
- *Enterococcus*
- *Sphingobacterium*

“Bacterial non-culture method” - Genus level



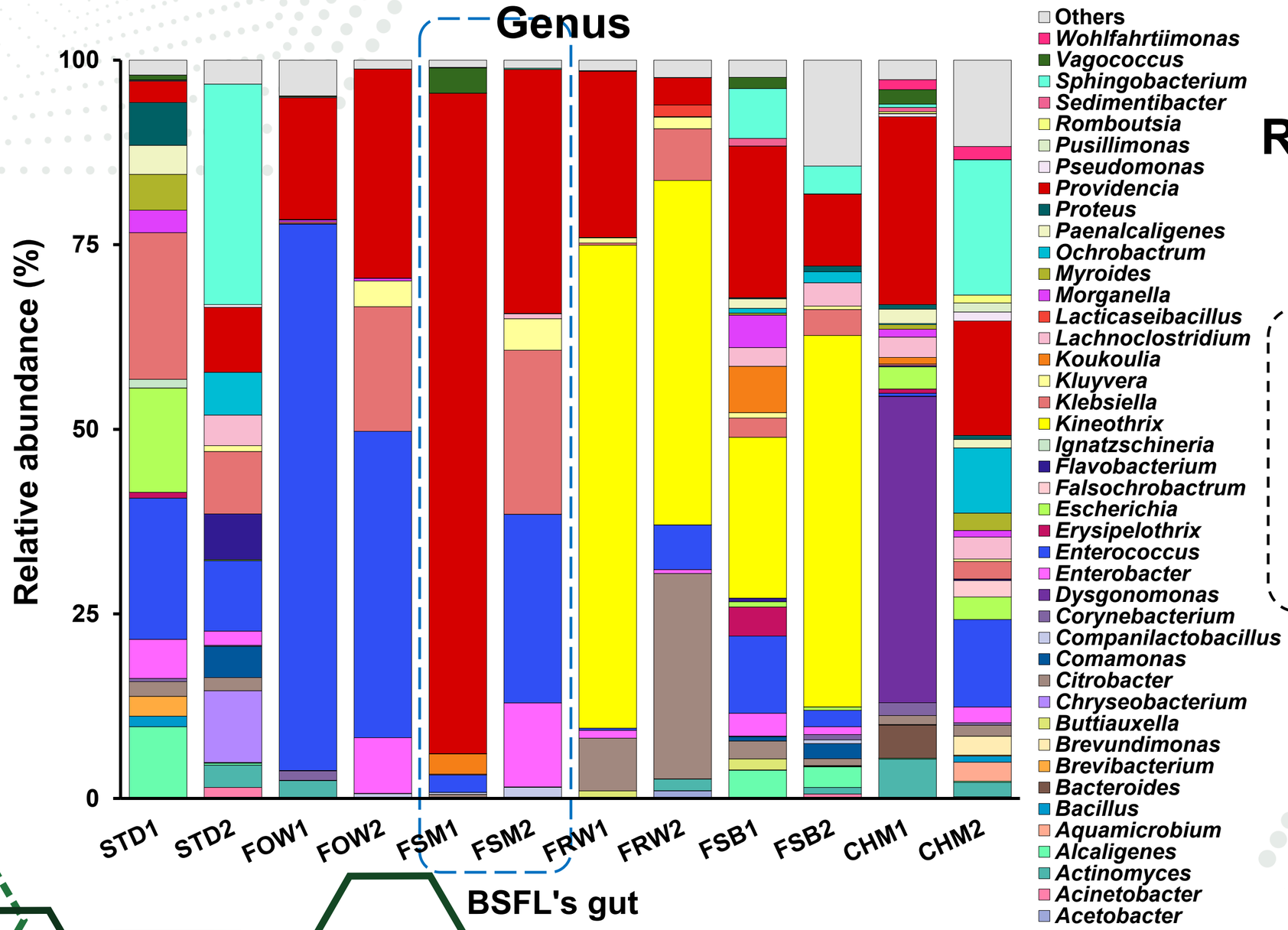
Relative abundance ≥ 1%

FOW

rep. 1&2

- ***Enterococcus***
- ***Providencia***

“Bacterial non-culture method” - Genus level



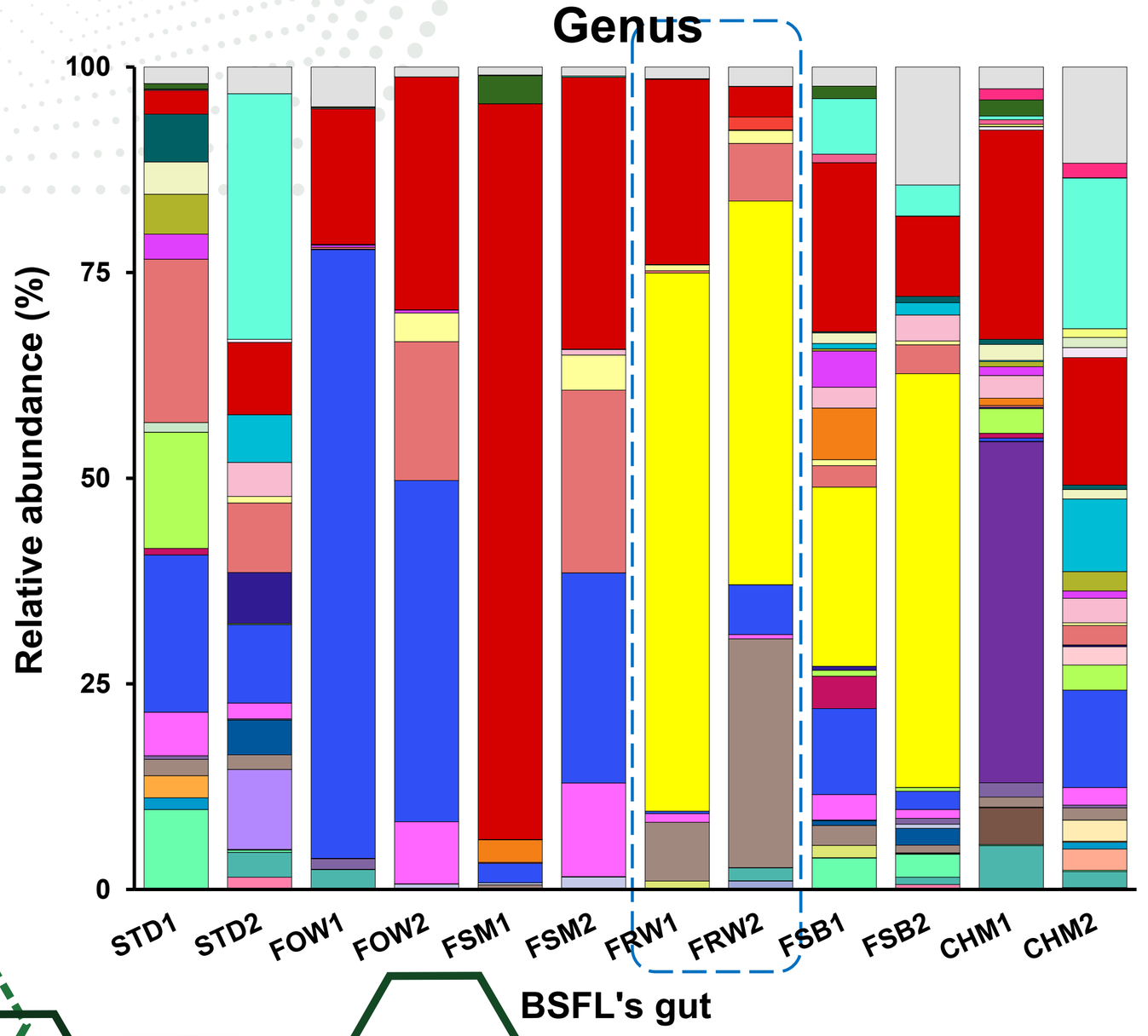
Relative abundance ≥ 1%

FSM

rep. 1&2

- ***Providencia***
- ***Enterococcus***

“Bacterial non-culture method” - Genus level



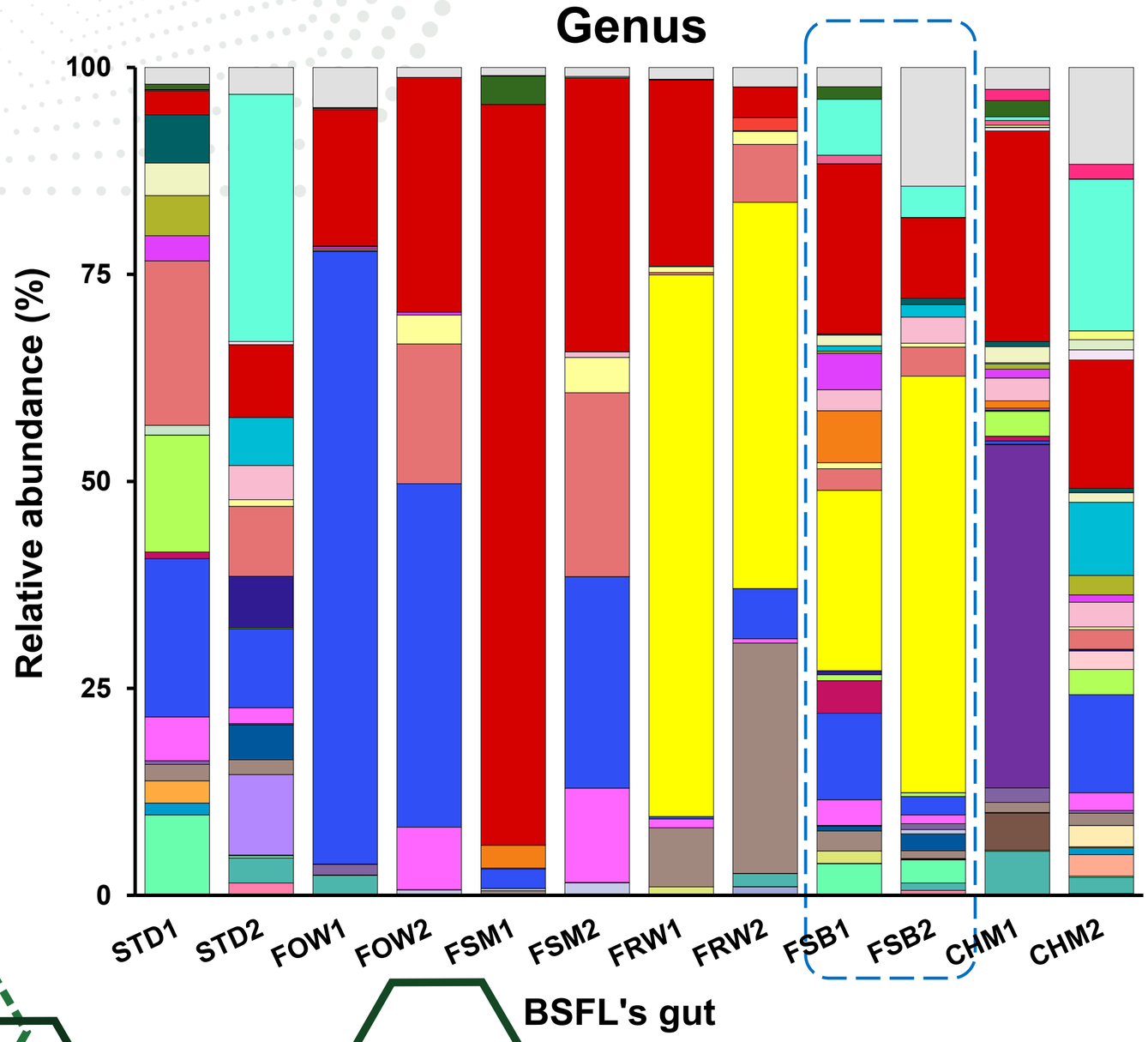
Relative abundance ≥ 1%

FRW

rep. 1&2

- *Kineothrix*
- *Providencia*
- *Citrobacter*

“Bacterial non-culture method” - Genus level



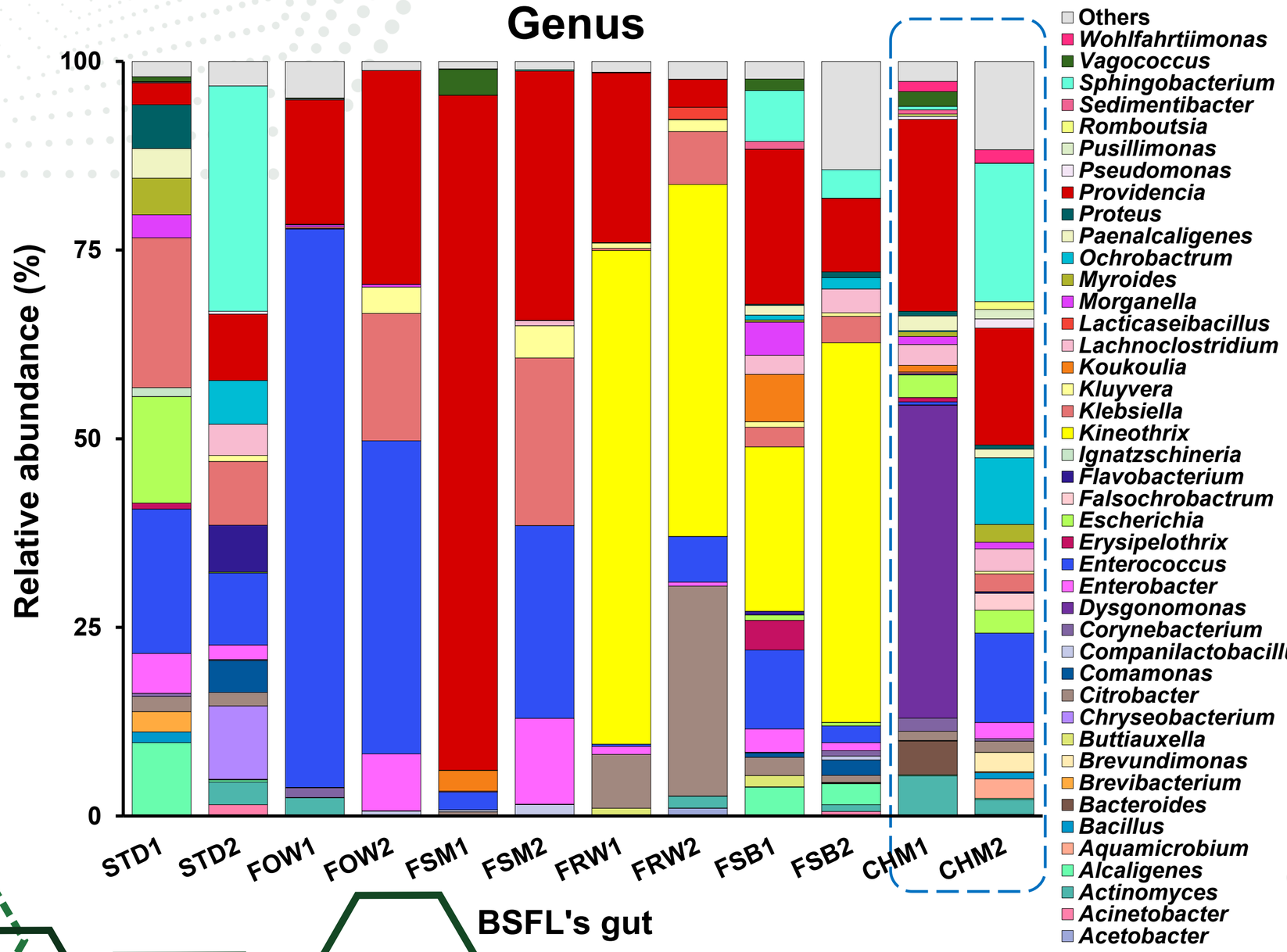
Relative abundance ≥ 1%

FSB

rep. 1&2

- *Kineothrix*
- *Providencia*

“Bacterial non-culture method” - Genus level



Relative abundance ≥ 1%

CHM

rep. 1&2

- *Dysgonomonas*
- *Providencia*
- *Sphingobacterium*

- **4 main phyla: Actinobacteriota, Bacteroidota, Firmicutes & Proteobacteria were found in this study.**

= Commonly found as dominant bacteria in the larval gut. (Bruno et al., 2019; Tanga et al., 2021; Schreven et al., 2022)

The abundance of each bacterial phyla differ across studies.

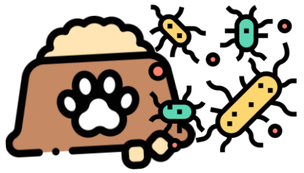
Klammsteiner et al. (2020)

- **phylum Actinobacteriota & Bacteroidota**

Gorrens et al. (2021)

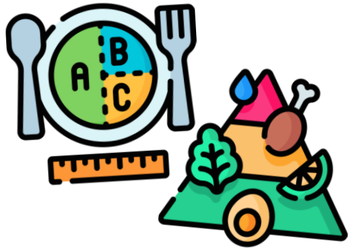
- **phylum Proteobacteria & Firmicutes**

Gut bacteria varies depending on the "substrates."



Substrates microbes

- It can enter the gut & become part of the larval gut microbiota. (Tanga et al., 2021)



Nutrient composition

- Protein, Fat & Carbohydrate.

- It influences bacterial diversity & enzyme production for digestion. (Chen et al., 2023; Bonelli et al., 2020)

Bacteria that degrade protein- & fat-rich substrates

- ***Providencia***
- ***Enterococcus***
- ***Morganella***

Bacteria that degrade carbohydrate- & fiber-rich substrates

- ***Providencia*** (Hemicellulose)
- ***Klebsiella*** (Cellulose, Hemicellulose & Pectin)
- ***Enterococcus***
- ***Dysgonomonas*** (Cellulose& Hemicellulose)

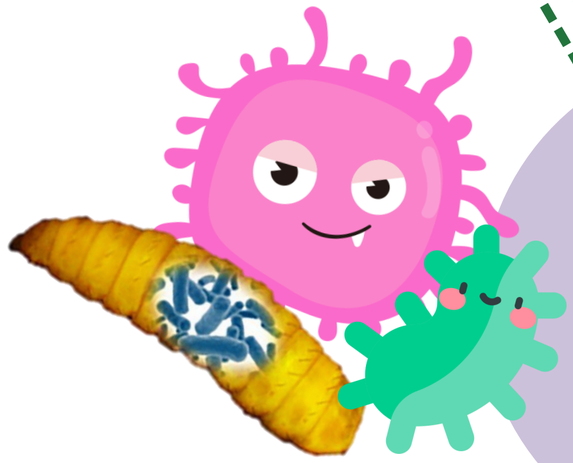
= “Core microbiota”

(Tanga et al., 2021; Tegtmeier et al., 2021)

(Ao et al., 2020; Sontowski et al., 2020; Jiang et al., 2019; Gorren et al., 2021; Bruno et al., 2019; Vandeweyer et al., 2023)

“Biosafety perspective”

Bacteria ⊕ **Positive association with BSFL.**



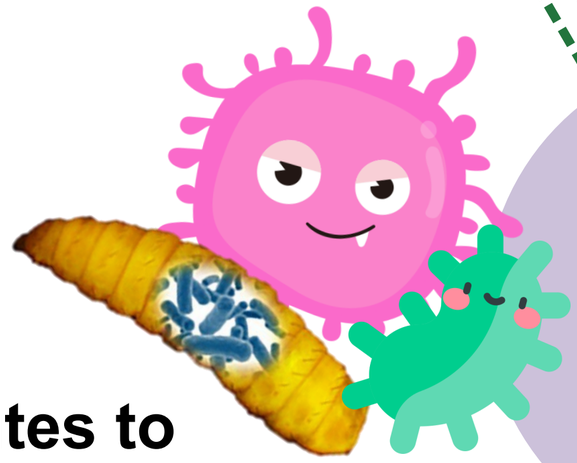
genus *Providencia & Morganella*

⊕ **It can stimulate oviposition & be vertically transmitted in BSF.** (Shumo et al., 2019)

genus *Enterococcus*

⊕ • **Increasing the BSF egg-hatching rate & larval growth rate.**
(Shumo et al., 2019)

“Biosafety perspective”



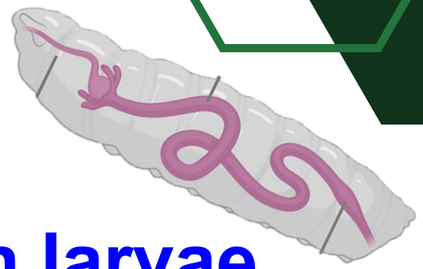
genus *Klebsiella*

- It supports early larval growth & contributes to pre-pupal weight gain. (Li et al., 2022b)

genus *Dysgonomonas*

- It promotes faster larval growth & enhances pupation efficiency. (Li et al., 2022b)

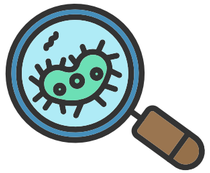
“CONCLUSION”



All substrates induce shifts gut bacteria in larvae.

- **The culture method**
  - genus *Bacillus* in all BSFL guts.
  - **FRW** had the highest diversity.

- **The non-culture method**
  - genus *Enterococcus, Klebsiella & Providencia* in all BSFL guts.
  - **CHM** had the highest diversity.



Some species may be “pathogenic to humans.”

“RECOMMENDATION”

“Ensuring Safety in BSFL Utilization”

Implement proper feed management.

- Hygienic feed storage
- Immediate use after preparation

Postharvest treatments

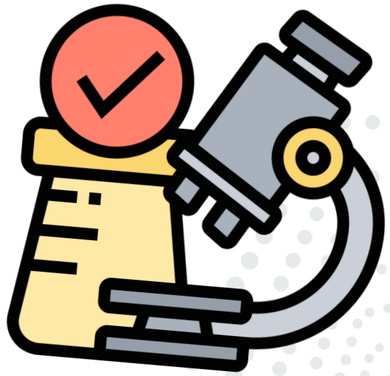
- Heating
- Drying
- Blanching
- Defatting
- Storage in hermetic bag

Reduce microbial contaminants.

“RECOMMENDATION”

“Research further”

- **Detection of the main foodborne pathogen strains in BSFL’ s gut and substrates.**
  - ***Campylobacter jejuni***
  - ***Clostridium botulinum***
  - ***Escherichia coli***
  - ***Listeria monocytogenes***
  - ***Salmonella spp.***
  - ***Staphylococcus aureus***



THANK YOU