



IBD2025



Identification of bacteria in the gut of black soldier fly larvae (*Hermetia illucens* L.) from breeding farms and its application.

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Overview

Introduction



Results & Discussion



Research Objectives



Conclusion & Recommendations



Methodology





source: <https://betterorigin.co.uk/2021/05/black-soldier-fly-guide/>

“Black soldier fly; BSF”

- BSF found in native of the **tropical** and **warm temperate zone**.
- BSF **is not** considered as **the vector** and **plant pests**.



Black soldier fly larvae; BSFL



source: <https://extension.entm.purdue.edu/publications/E-276/E-276.html>

“Saprotrophic insect”

- **BSFL consumed and decompose** various organic matter.



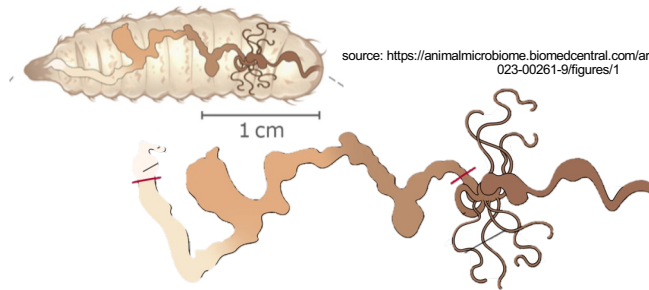
changed to high value biomass
rich in proteins and fats.



source: <https://checksammy.com/blog/5-benefits-of-organic-recycling-and-why-its-important/>



Black soldier fly larvae; BSFL



source: <https://animalmicrobiome.biomedcentral.com/articles/10.1186/s42523-023-00261-9/figures/1>

“Microbe-rich environments”

- As the BSFL is found in decaying environments, which contain various of microorganisms.



Beneficial



Pathogenic

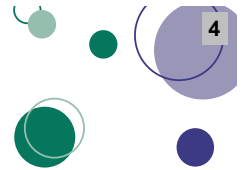


(Nayduch and Burrus 2017; Rotheray 2019)

source: <https://www.undp.org/india/stories/hail-black-soldier-fly-turning-waste-animal-feed>



Bacterial diversity and importance



• **Hydrolytic activities** | enzyme synthesis



source: <https://animalmicrobiome.biomedcentral.com/articles/10.1186/s42523-023-00261-9/figures/1>

• **BSFL digestion** | digestion, detoxification

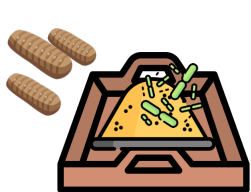
• **Promoted growth and development** | insect immunization supplement

Bacterial diversity and importance

Gorren et al. (2021)

- *Providencia* spp. = playing a role in protein &
- *Klebsiella* spp. = lipid conversion in gut.

- *Klebsilla* supplemented. (Li et al., 2023)



BSFL's weight





BSFL in Thailand

- Enhance the efficiency of **BSFL's utilization** for food & feed.



source: <https://www.dubideli.com/products/black-soldier-fly-larvae-large>

“ There has been **no study on the bacterial diversity** & **classification of bacterial communities** in the gut of BSFL in Thailand.”





Objectives



- ✓ Identify bacteria in the BSFL's gut.
- ✓ Evaluate their enzyme production for **selecting beneficial microbes** to supplement BSFL feed.



Methodology

1st Identify **bacteria diversity** in the BSFL's gut.

2nd The study of **bacterial enzyme production**.





Methodology 1st

1 BSFL sample collected

- The collection of 5th BSFL from farms in 6/8 provinces.

Nakhon Pathom; NPT



Ratchaburi; RBR



Roi Et.; RET



Surin; SRN



Maha Sarakham; MKM



Chiang Mai; CMI



Chonburi; CHB



Chumphon; CPN



culture



non-culture



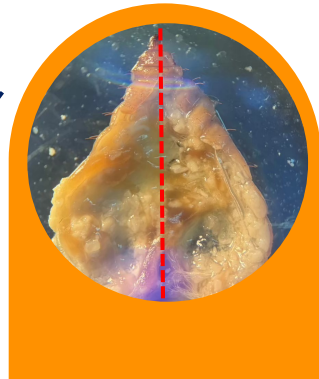
Methodology 1st

► BSFL's gut samples dissection.



- Select **BSFL** with **dark brown** color.

- Cut off the rear end and longitudinal incision along the body to expose the gut.



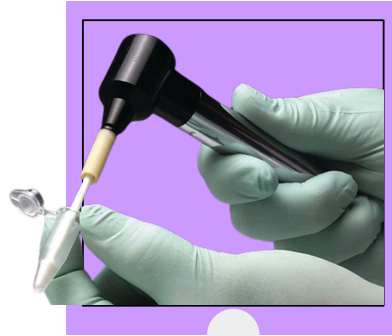


Methodology 1st

► BSFL's gut samples dissection.



- Extract the gut & rinse with ethyl alcohol 2–3 times.



- Grind the gut using a pellet pestles in solution.



Methodology 1st

2 Bacterial culture method

Spread the gut sample using the **spread-plate technique** on 3 types of medias:



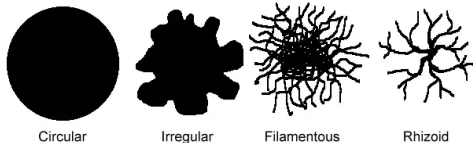
Incubate in 27°C, 1-2 days



Observe the colony growth on medias including:

- Size
- Shape
- Texture
- Margin
- Elevation
- Color

+Gram staining

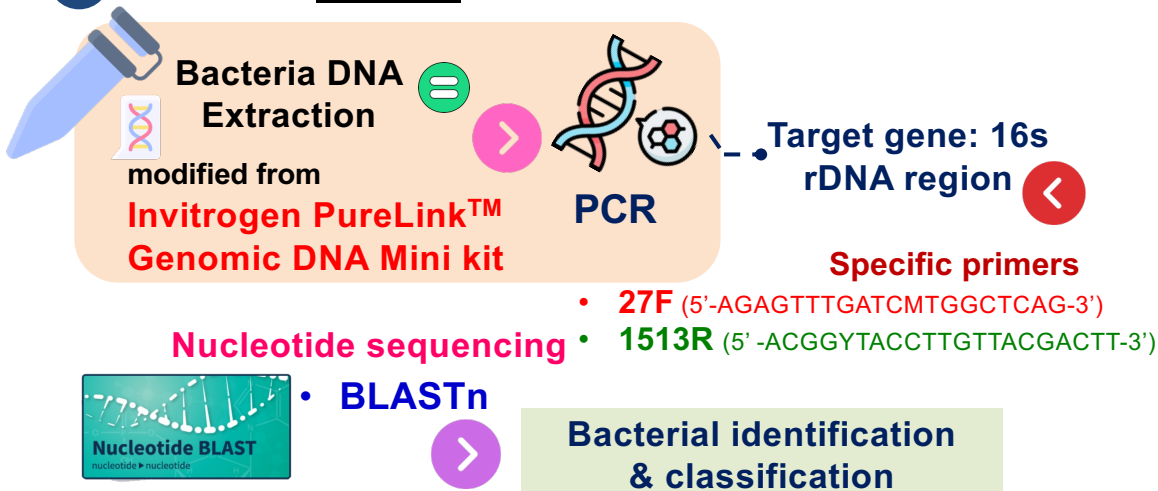


source: <https://microbeonline.com/colony-morphology-bacteria-describe-bacterial-colonies/>



Methodology 1st

2 Bacterial culture method





Methodology **1st**

3 Bacterial non-culture method



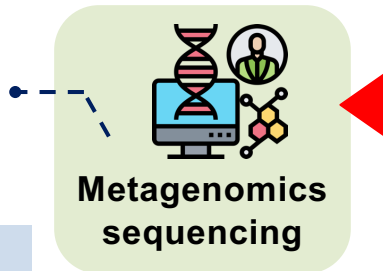
modified from
Invitrogen PureLink™ Genomic DNA Mini kit

Nucleotide sequencing analysis

**“16s rDNA gene”
(V3+V4 region)**



**Bacterial identification
& classification**

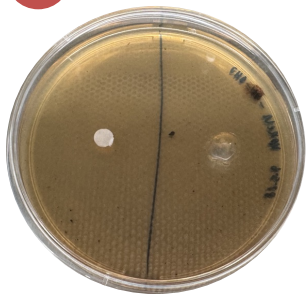




Methodology **2nd**

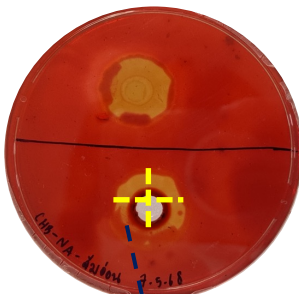
- The study of **bacterial enzyme production.**

1 Cellulase production test



CMC agar

Incubate in 37°C, 72 hr.



Stain with 1% Congo red solution for 30 minutes, rinse the surface with 1 M NaCl solution.

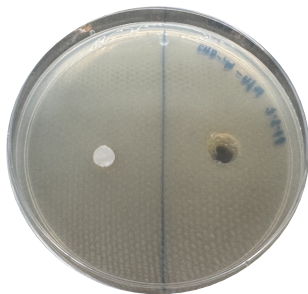
• **Measure the diameter of the clear zone.**



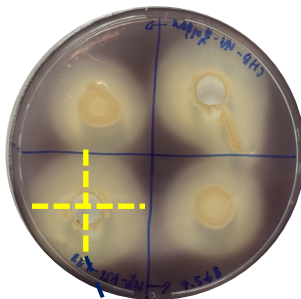


Methodology **2nd**

1 Amylase production test



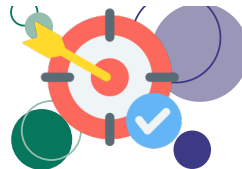
Starch agar
Incubate in 37°C, 48 hr.



**Stain with Iodine solution
for 1 minutes and rinse off.**

**Measure the diameter of the
clear zone.**





Results & Discussion



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



Bacterial culture method

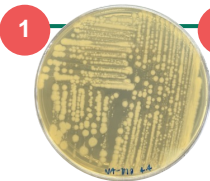


- > Total of 4,120 bacterial isolates.
- > Isolates were classified into 15 groups based on colony characteristics.

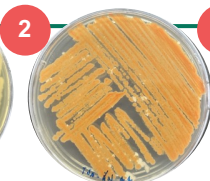


Bacterial culture method

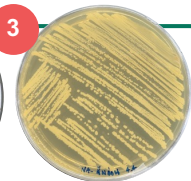
→ Bacterial colony



White

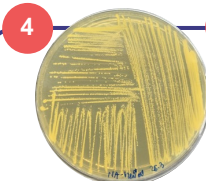


Orange

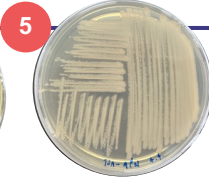


Light orange

Circular, Entire, Flat and Smooth

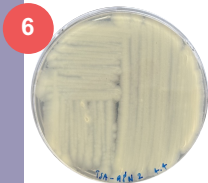


Yellow

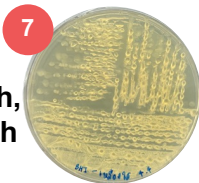


Cream

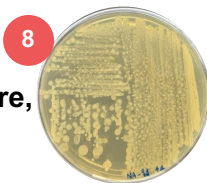
Circular, Entire, Raised and Smooth



Cream
**Circular, Notch,
Flat and Rough**



Clear yellow
**Circular, Entire,
Raised and
Mucoid**

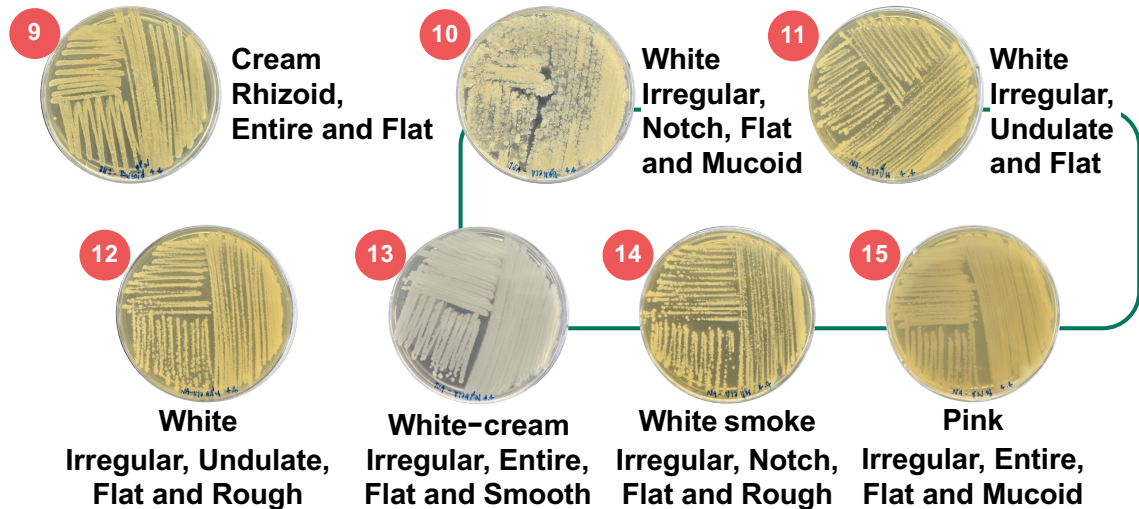


Clear
**Circular, Entire,
Umbonate and
Mucoid**



Bacterial culture method

Bacterial colony





Bacterial culture method

↳ Bacterial identification

58 isolates

NPT: 9 isolates

RBR: 7 isolates

RET: 11 isolates

SRN: 8 isolates

CHB: 10 isolates

CPN: 13 isolates



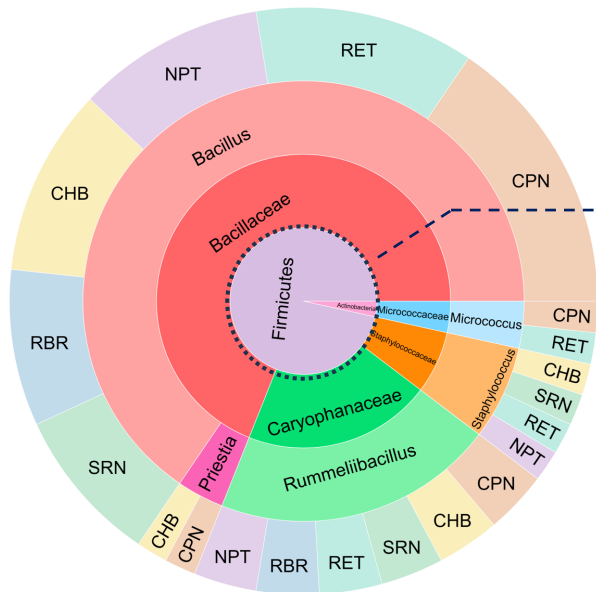
>98% similarity with the sequences in the database.

1,358 – 1,486
base pairs





Bacterial culture method :Bacterial community in Phylum



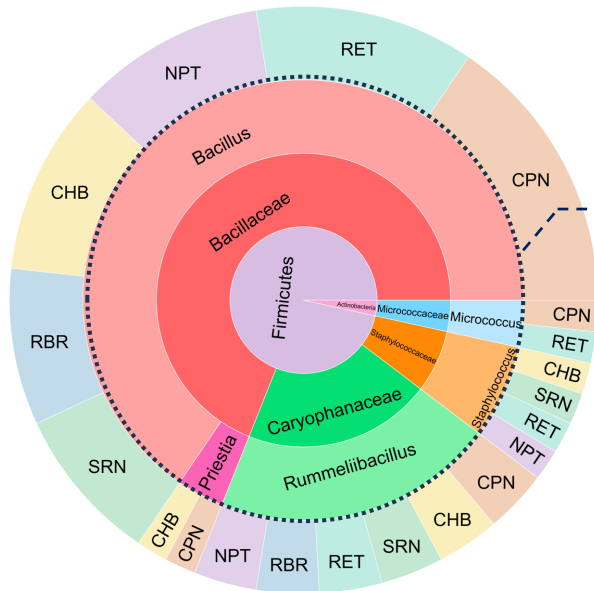
> 2 phyla

- Firmicutes
= 6 farms

- Actinobacteria
= 2 farms; RET&CPN



Bacterial culture method :Bacterial community in Genus



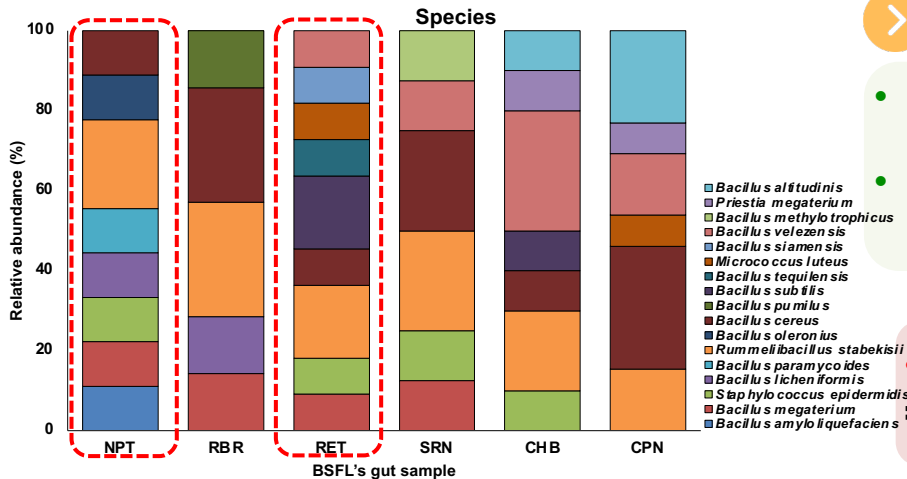
> 5 genera

- **RET, CHB & CPN**
= highest diversity

- ***Bacillus***
- ***Rummeliibacillus***
= had a higher %relative abundance



Bacterial culture method :Bacterial community in Species



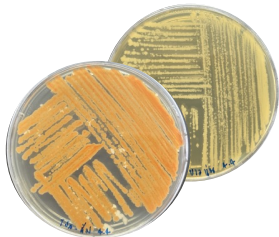
> 17 species

- *Bacillus cereus* (18.97%)
- *Rummeliibacillus stabekisii* (20.69%)

• **NPT & RET**
= highest diversity



Bacterial culture method



- **Phylum Firmicutes** is the most abundance.
- **Genus *Bacillus* & *Rummeliibacillus*** are more commonly found in each locations.

Gold et al. (2022); Tegtmeier et al. (2021)

✓ genus *Bacillus*, *Rummeliibacillus* and *Staphylococcus* from BSFL gut.

Pei et al. (2022)

✓ *Bacillus* spp. can produce enzyme for degrade organic substrates or BSFL's diets and stimulate nutrients absorption.



Bacterial culture method



Tegtmeier et al. (2021) ✓ genus *Bacillus* & *Rummeliibacillus* are classified as the **core microbiome**.”

***Staphylococcus* spp.** was found in low abundance.

- It is a common microorganism in the environment and animal gut.
- Reported to **compete with pathogens** and **help maintain microbial balance** in the digestive system.

(Gold et al., 2022)



Bacterial non-culture method: Bacterial diversity

Table 1 Diversity indices of bacterial community in BSFL's alimentary tract from six locations..

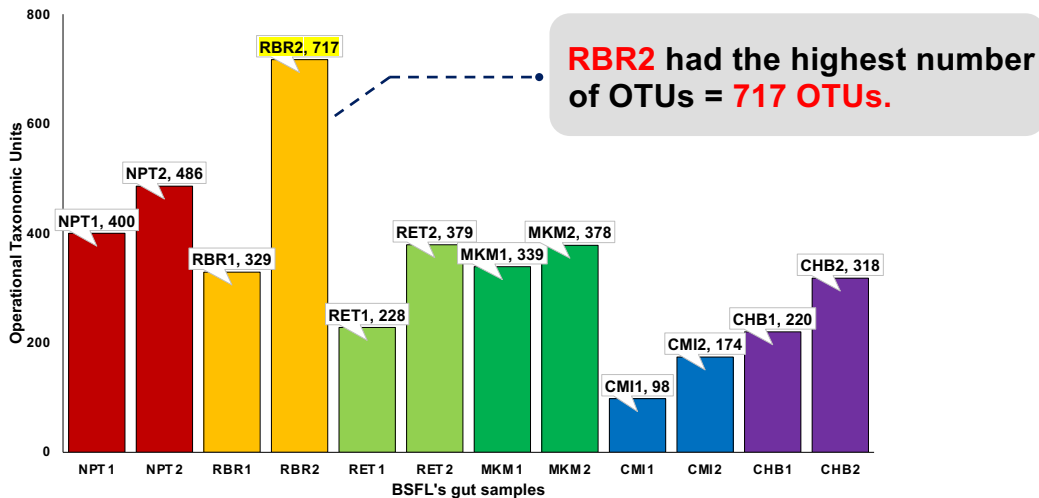
Sample Locations	Number of reads		Alpha diversity		
	Raw reads	Clean reads	Choa1	Shannon	Simpson
NPT1 ^{1/}	80,032	73,922	400.05	5.82	0.96
NPT2	79,939	73,929	486.15	4.55	0.85
RBR1	79,966	73,108	329.04	5.89	0.97
RBR2	79,789	74,603	717.00	6.07	0.97
RET1	79,821	73,600	228.00	4.94	0.94
RET2	Number of reads ranging from 73,108 to 75,871	75,613	379.00	5.42	0.95
MKM1		74,996	339.16	4.52	0.93
MKM2		74,987	378.00	4.64	0.93
CMI1		75,871	98.04	3.51	0.86
CMI2		75,329	174.05	3.55	0.86
CHB1	80,221	74,117	220.25	4.77	0.93
CHB2	79,917	75,100	318.00	3.93	0.82

RBR2 showed the highest number of choa1 index.

^{1/} NPT; Nakhon Pathom, RBR; Ratchaburi, RET; Roi Et., Maha Sarakham, CMI; Chiang mai and CHB; Chonburi.



Bacterial non-culture method :Bacterial diversity





Bacterial non-culture method: Bacterial diversity

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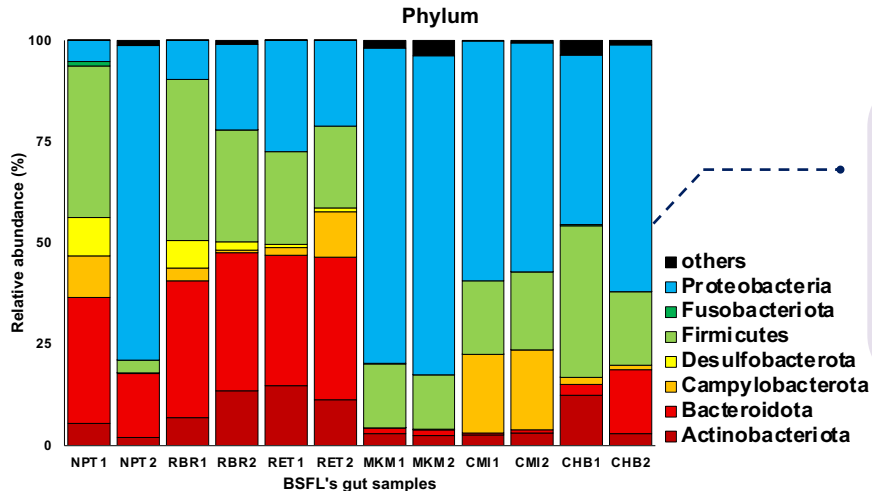
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**NPT1 & RBR1-2
showed the
highest number
of Shannon &
Simpson index.**

^{1/} NPT; Nakhon Pathom, RBR; Ratchaburi, RET; Roi Et., Maha Sarakham, CMI; Chiang mai and CHB; Chonburi.



Bacterial non-culture method: Bacterial community in Phylum

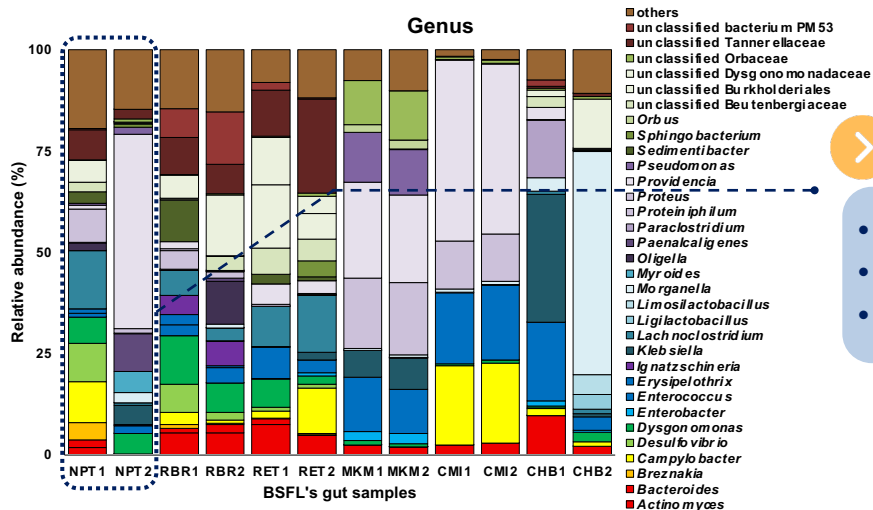


relative abundance $\geq 10\%$

- Proteobacteria
 - Firmicutes
 - Bacteroidota
- = had a higher relative abundance.



Bacterial non-culture method: Bacterial community in Genus



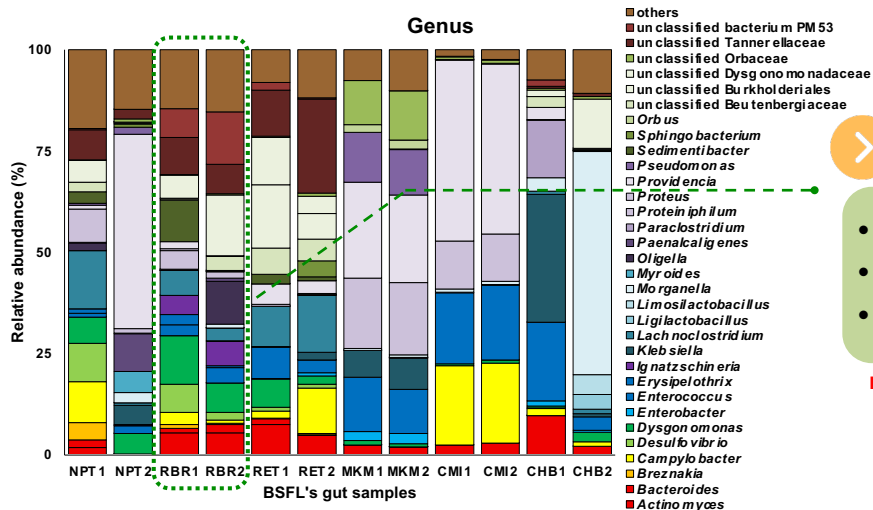
Nakhon Pathom; NPT
Rep.1-2

- *Lachnoclostridium*
- *Providencia*
- *Campylobacter*

relative abundance $\geq 10\%$



Bacterial non-culture method: Bacterial community in Genus



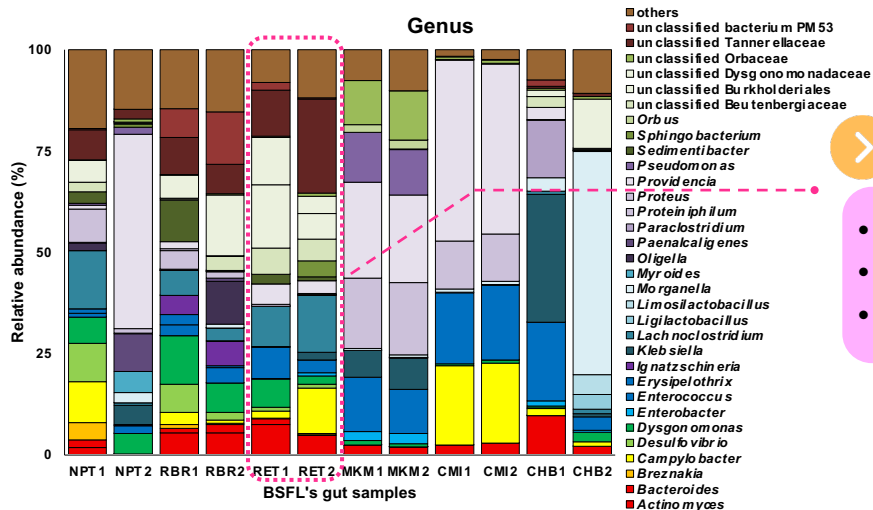
Ratchaburi; RBR
Rep.1-2

- *Dysgonomonas*
- *Sedimentibacter*
- *Oligella*

relative abundance $\geq 10\%$



Bacterial non-culture method: Bacterial community in Genus

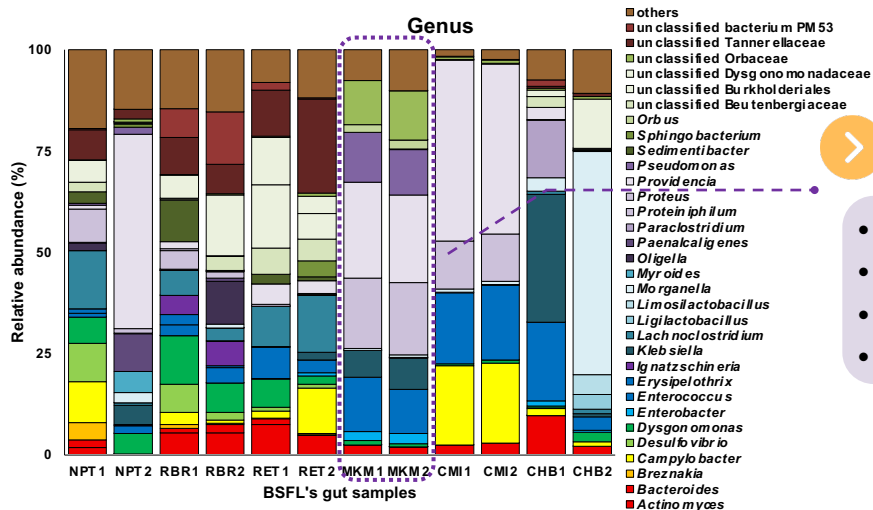


Roi Et.; RET
Rep.1-2

- **Campylobacter**
- **Lachnoclostridium**
- **Actinomyces**
relative abundance $\leq 10\%$



Bacterial non-culture method: Bacterial community in Genus



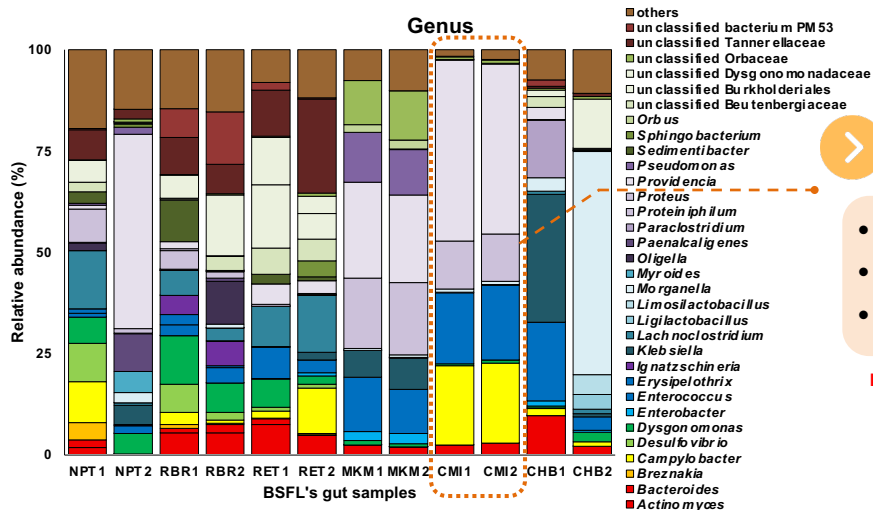
Maha Sarakham; MKM Rep.1-2

- Proteus***
- Providencia***
- Enterococcus***
- Pseudomonas***

relative abundance $\geq 10\%$



Bacterial non-culture method: Bacterial community in Genus



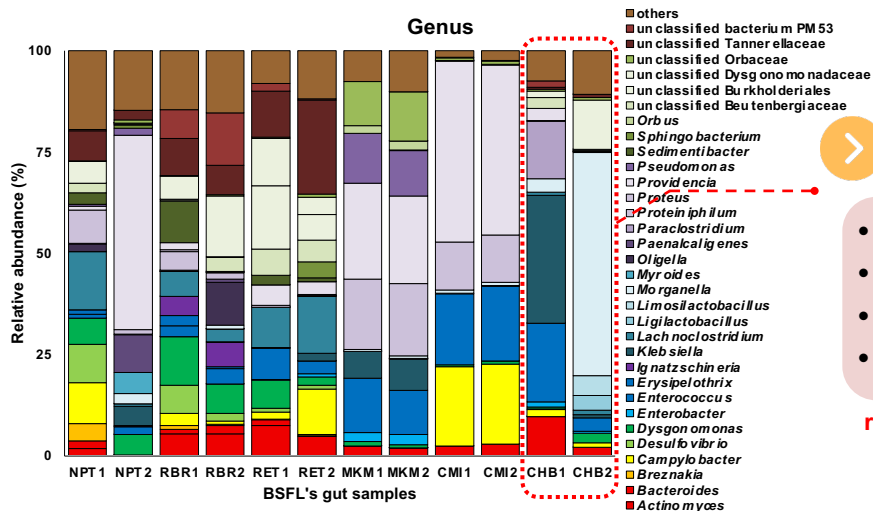
Chiang Mai; CMI
Rep.1-2

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

relative abundance $\geq 10\%$



Bacterial non-culture method: Bacterial community in Genus



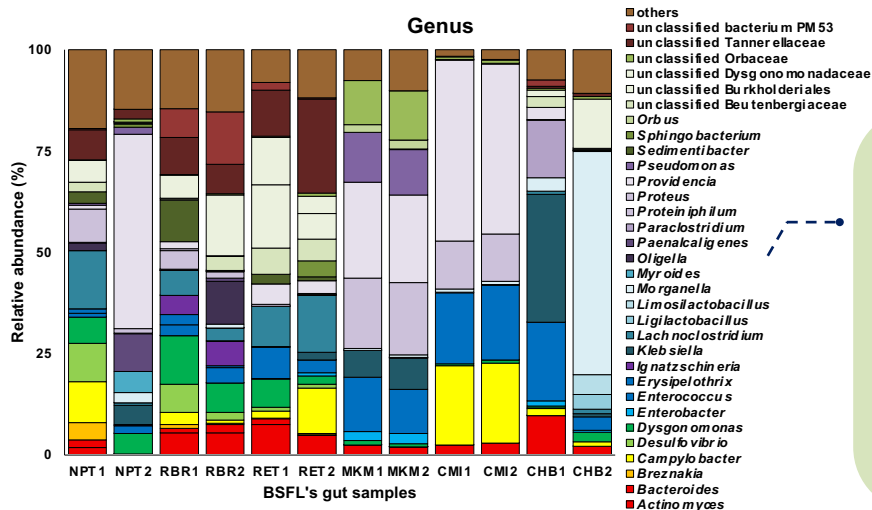
Chonburi; CHB
Rep.1-2

- *Klebsiella*
- *Enterococcus*
- *Paraclostridium*
- *Morganella*

relative abundance $\geq 10\%$



Bacterial non-culture method: Bacterial community in Genus



- *Providencia*
- *Enterococcus*
- *Campylobacter*
- *Morganella*
- *Proteus*
- *Klebsiella*

= had a higher relative abundance.



Bacterial community by non-culture method



Callegari et al. (2020)



phylum **Proteobacteria**, **Firmicutes**,
Actinobacteria & **Bacteroidota**.

found in all farms, but
low relative abundance

(Schreven et al., 2021)

Each phylum was found in different
amounts depending on the farm locations.



“The **phyla** of bacteria found are classified
as the core microbiome.” (Klammsteiner et al., 2021)



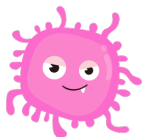


Bacterial community by non-culture method



- genus *Providencia*, *Enterococcus*, *Klebsiella*, *Morganella*, *Proteus*, *Pseudomonas*, *Actinomyces*, *Dysgonomonas*, *Lachnoclostridium*, *Campylobacter*

Tanga et al. (2021); Li et al. (2023); Klammsteiner et al. (2020)

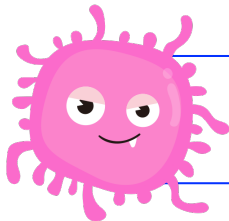


“The **genera** of bacteria found are classified as the core microbiome.” (Silvaraju et al., 2024)





Bacterial community by non-culture method



“The **phyla & genera** of bacteria found are classified as the **core microbiome**.”

It was consistently detected under various conditions for larval rearing.

- **Environmental**
- **Feeding types**
- **Locations**





Bacterial community

(Tanga et al., 2021)

Campylobacter spp.

Providencia spp.

Morganella spp.

(Klammsteiner et al., 2020)

Klebsiella spp.

Enterococcus spp.

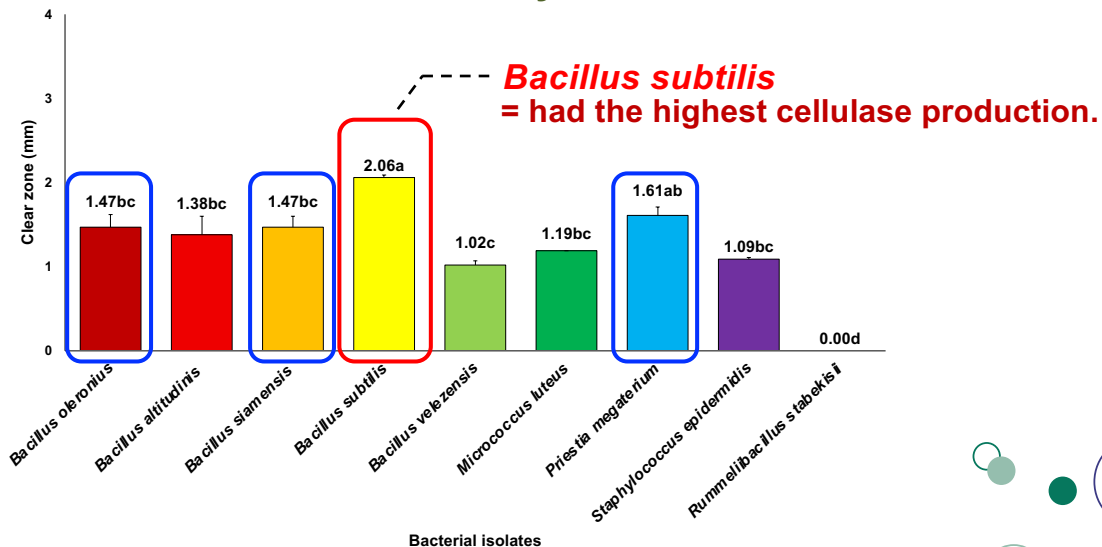
are considered opportunistic bacteria that can **cause disease in humans and animals.**





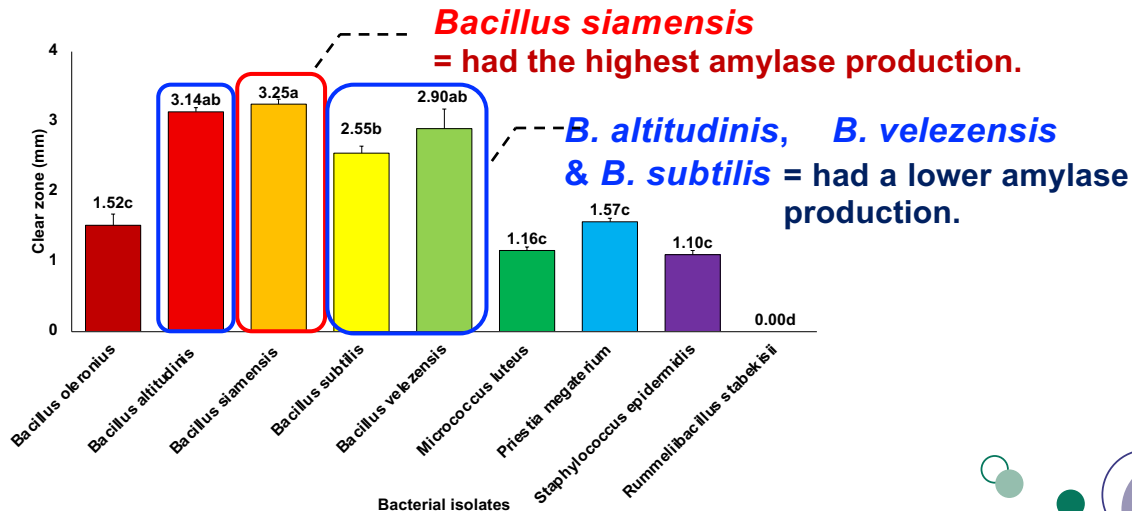
The study of bacterial enzyme production.

Bacterial Cellulase enzyme test

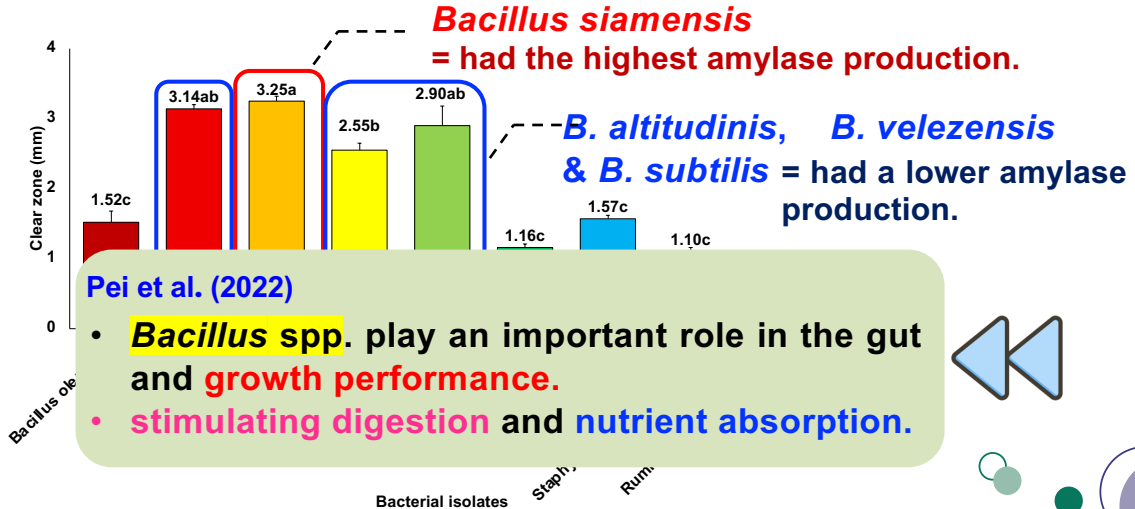




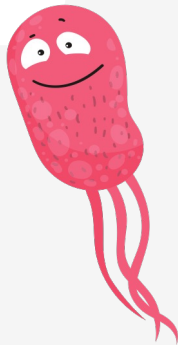
Bacterial Amylase enzyme test



Bacterial Amylase enzyme test



● Conclusion & Recommendation





Conclusion.....

Bacteria diversity in BSFL's gut



- **Culture method**

RET had the **highest** bacterial diversity.

--- phylum **Firmicutes**
genus ***Bacillus*** & ***Rumeliibacillus*** } in all farm sites.

- **Non-culture method**

RBR2 had the **highest** bacterial diversity.

--- phylum **Proteobacteria, Firmicutes** & **Bacteroidota** } in all farm sites.
genus ***Providencia, Enterococcus, Campylobacter, Dysgonomonas, Morganella, Proteus & Klebsilla***

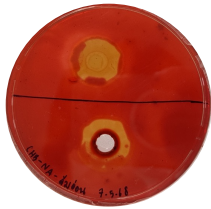




Conclusion.....

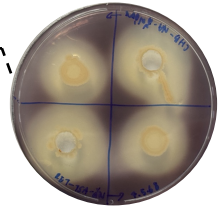
38

Bacterial enzyme production



- ***Bacillus subtilis***
= had the **highest cellulase** production.

- ***Bacillus siamensis***
= had the **highest amylase** production.





Recommendation...

As information for precautions regarding **beneficia & pathogenic** during the processes of breeding, harvesting, and utilization of BSFL.



Further works...



Effect of bacteria supplementation on BSFL's growth.

Thank You For your attention

 **Pongpan Phuengmun**

Faculty of Science and Technology,
Thammasat University