

KU LEUVEN



MELiSSA Conference 2020

Microbial analysis of the MELiSSA waste degradation compartment 1 (C1) and Isolation and Identification of C1 dominant bacteria

Tinh Van Nguyen

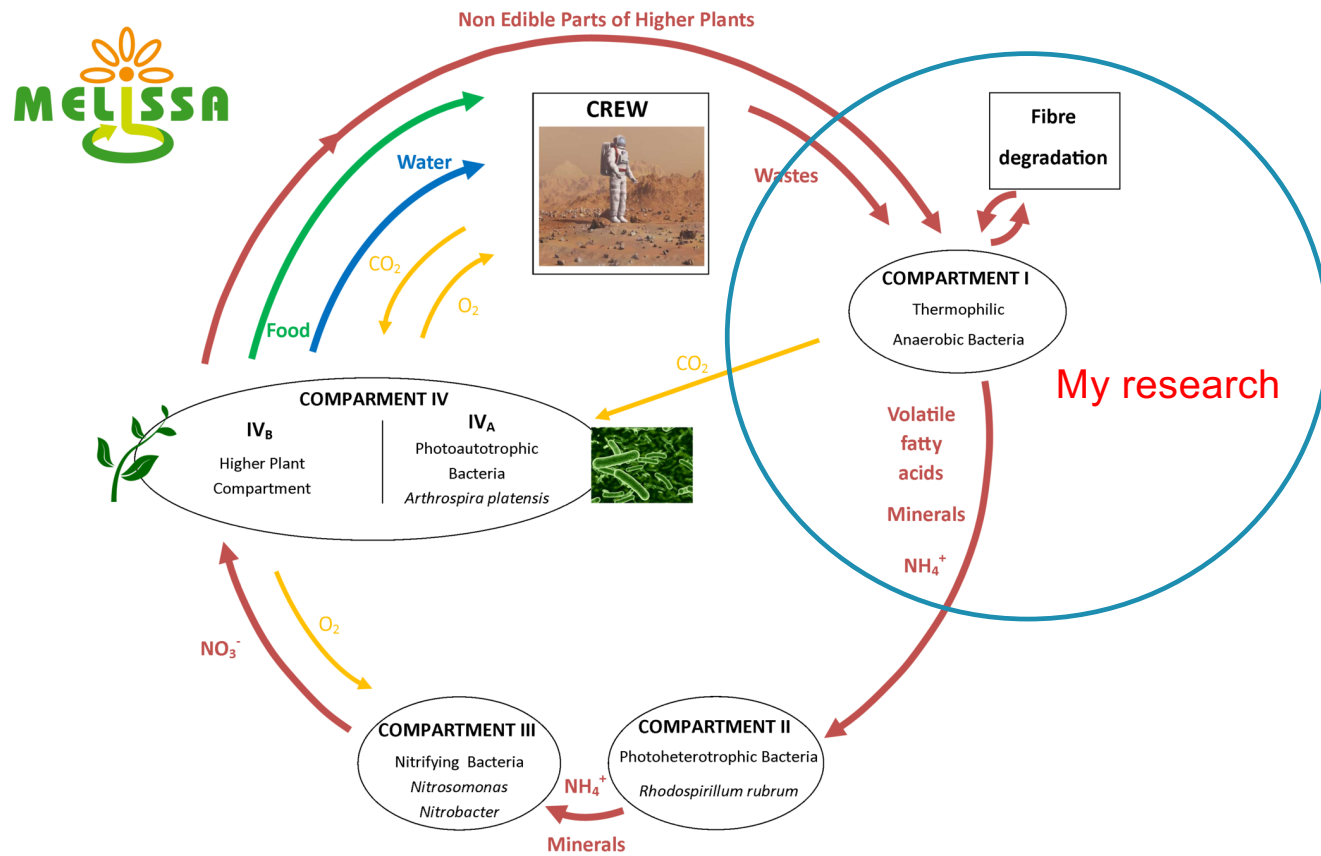
Supervisor: Prof. Dirk Springael

Co-supervisors: Prof. Karoline Faust, Prof. Kristel Bernaerts (KU Leuven)

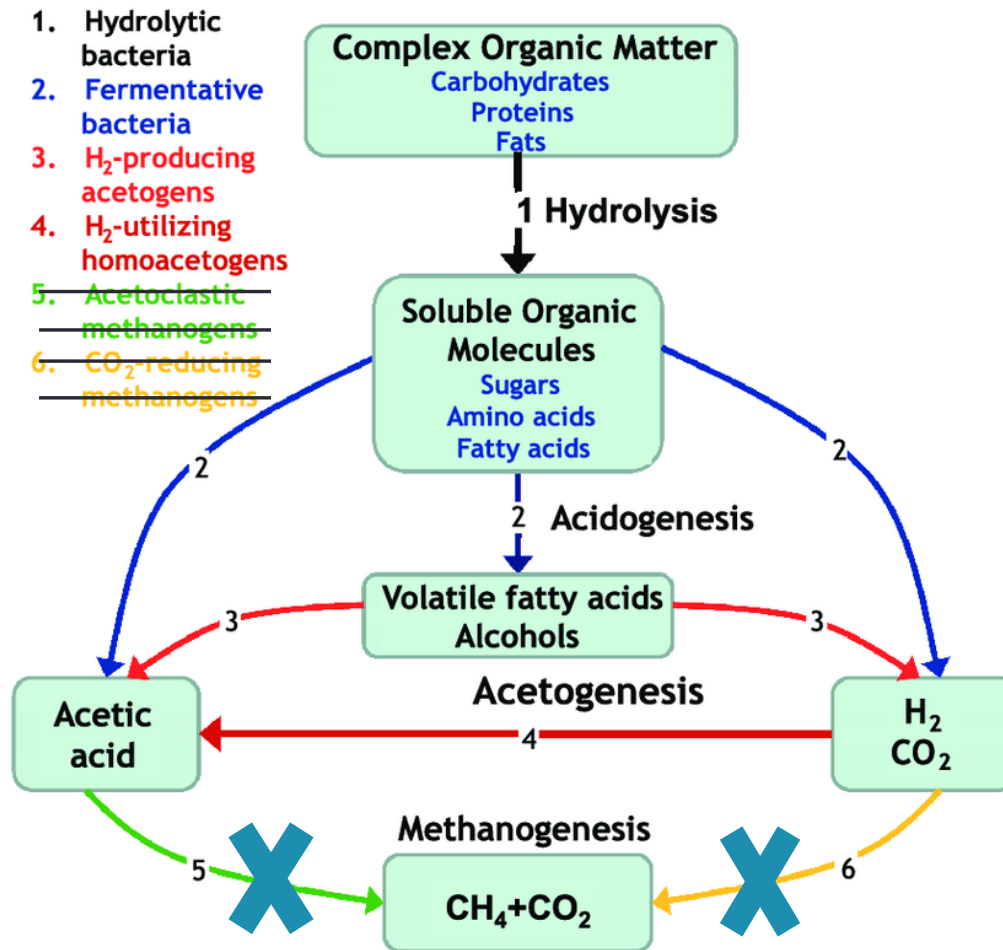
Prof. Claude-Gilles Dussap, Dr. Laurent Poughon (UCA)

Ghent, November 4th , 2020

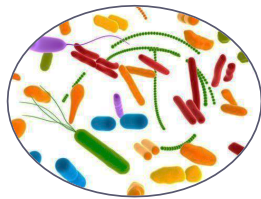
Micro Ecological Life Support Alternative - MELiSSA



MELiSSA Compartment 1

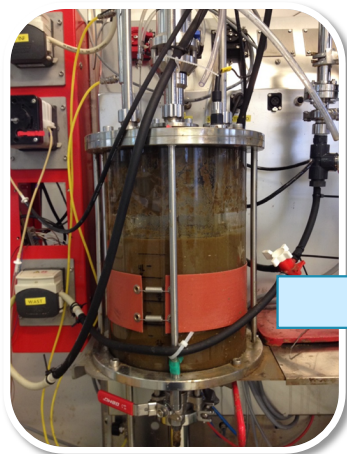


Two thermophilic - acidogenic AMBR



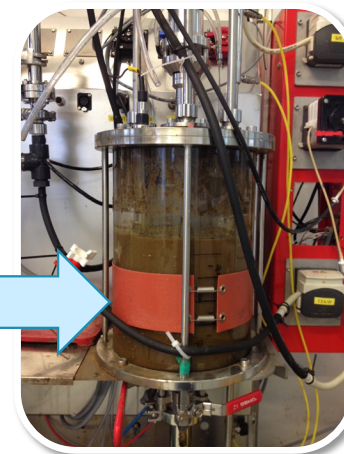
- MELiSSA Pilot Plant (Barcelona) (Stored)
- BELISSIMA (VITO) (Stored)
- KUL C1 (Fresh)
- DRANCO (Fresh)
- Thermophillic AD (Fresh)
- Lignocellulosic AD (Fresh)

Ghent-1



Running time: >1700 days

Ghent-2



Running time: >500 days

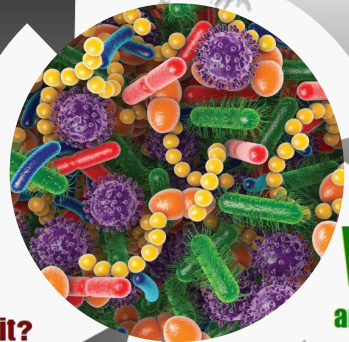
~460 days

Fresh + stored

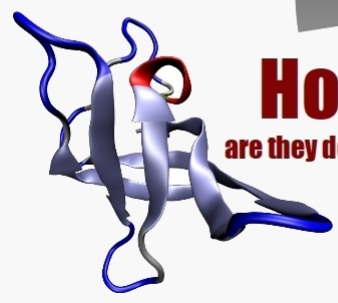
- 5L
- 55°C
- **pH 5.5**
- *HRT: 10 days*
- *SRT: ~80 days*
- Feed: MELiSSA mixture



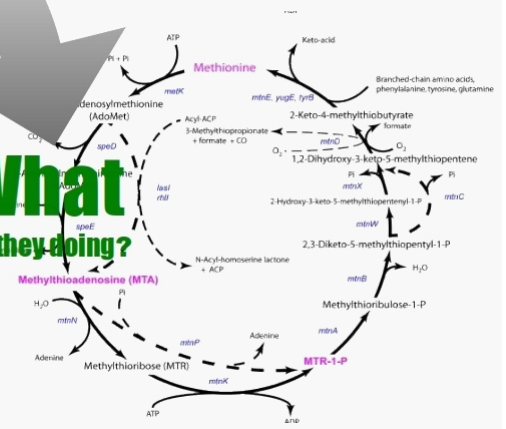
Who
is in there?



How
are they doing it?



What
are they doing?

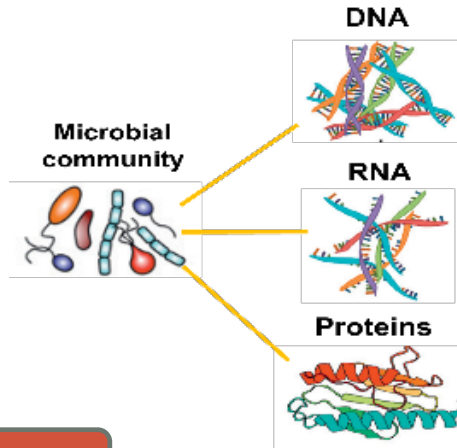


Microbial community analysis: META-OMICS & OMICS

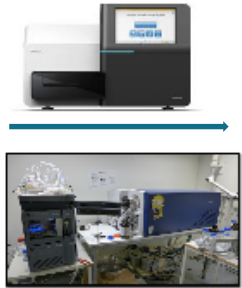
C1



Strain isolation and omics

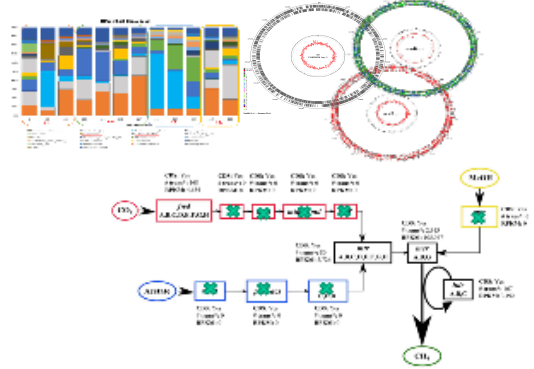


DNA/RNA Sequencing

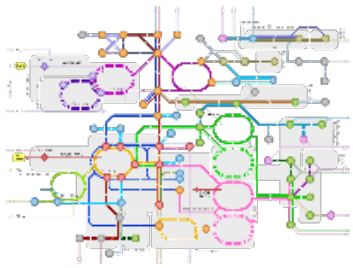


ESI-LC-MS/MS

Microbial community profiling



Gene / protein expression



Metabolic network

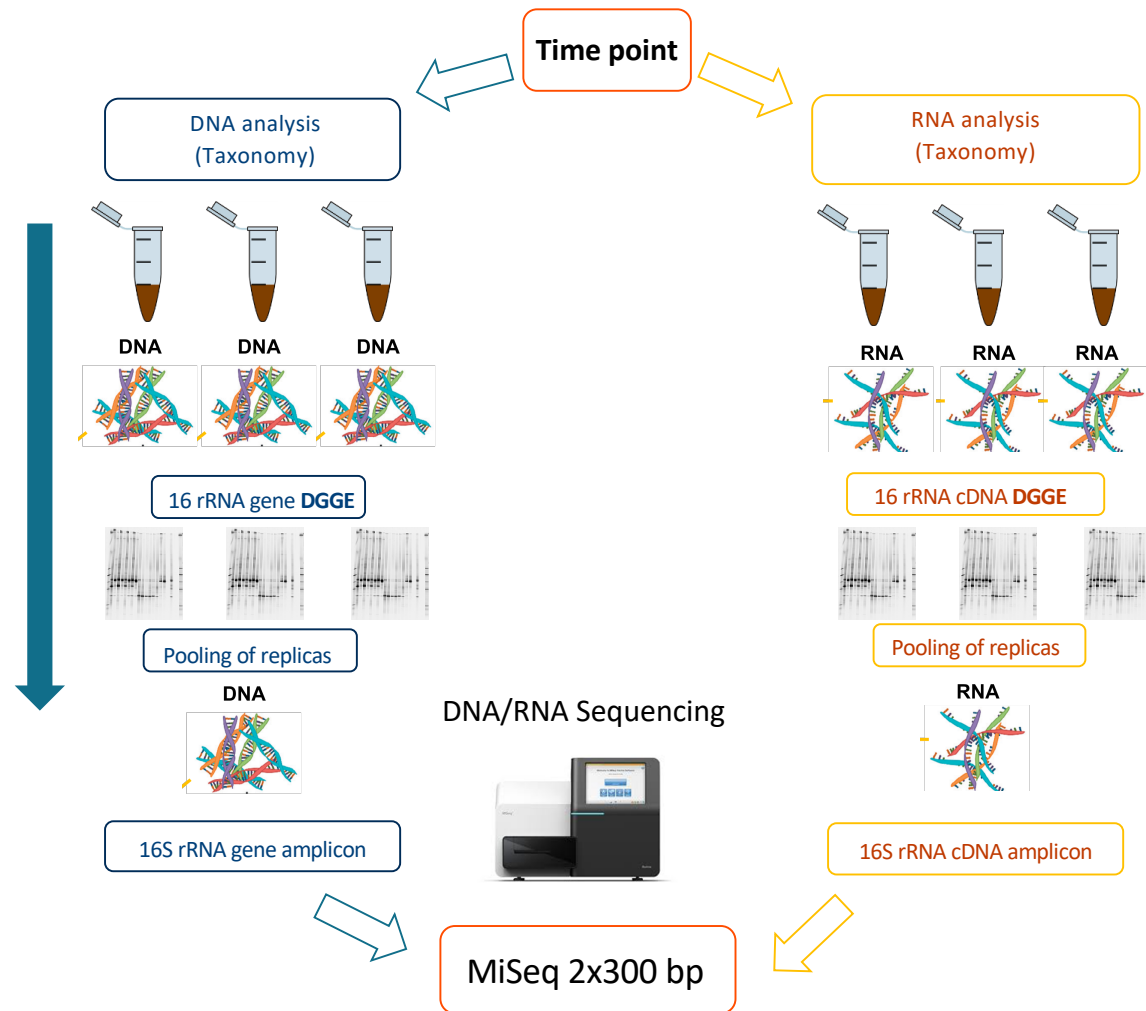
Meta-omics

Analysis of C1 microbial community

Core microbiome?

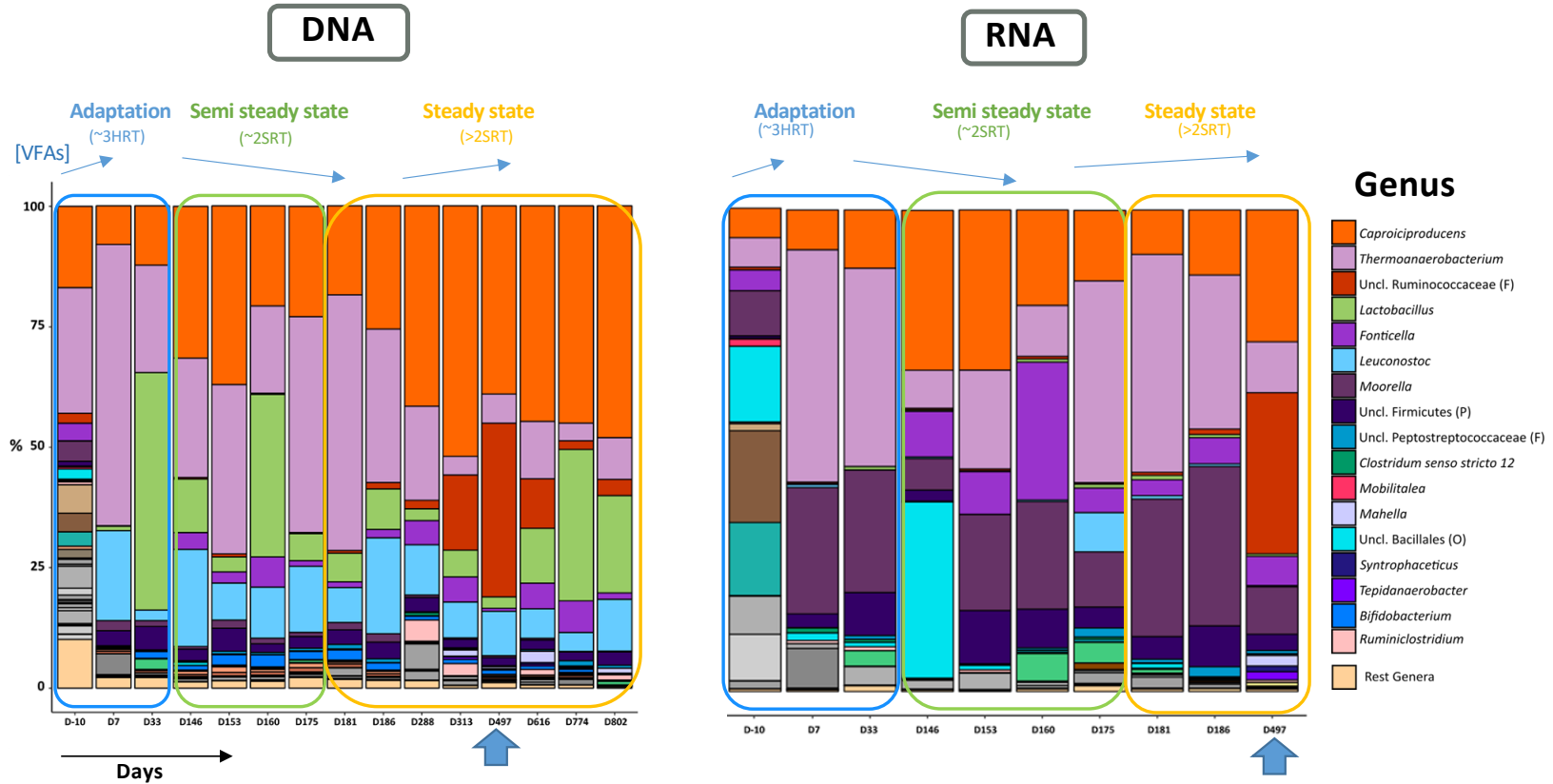


How stable?



- 16S DNA and cDNA
- Universal primers (V4)
- 100,000 reads / sample
- Mothur / QIIME analysis
- Silva v128 16S gene reference database

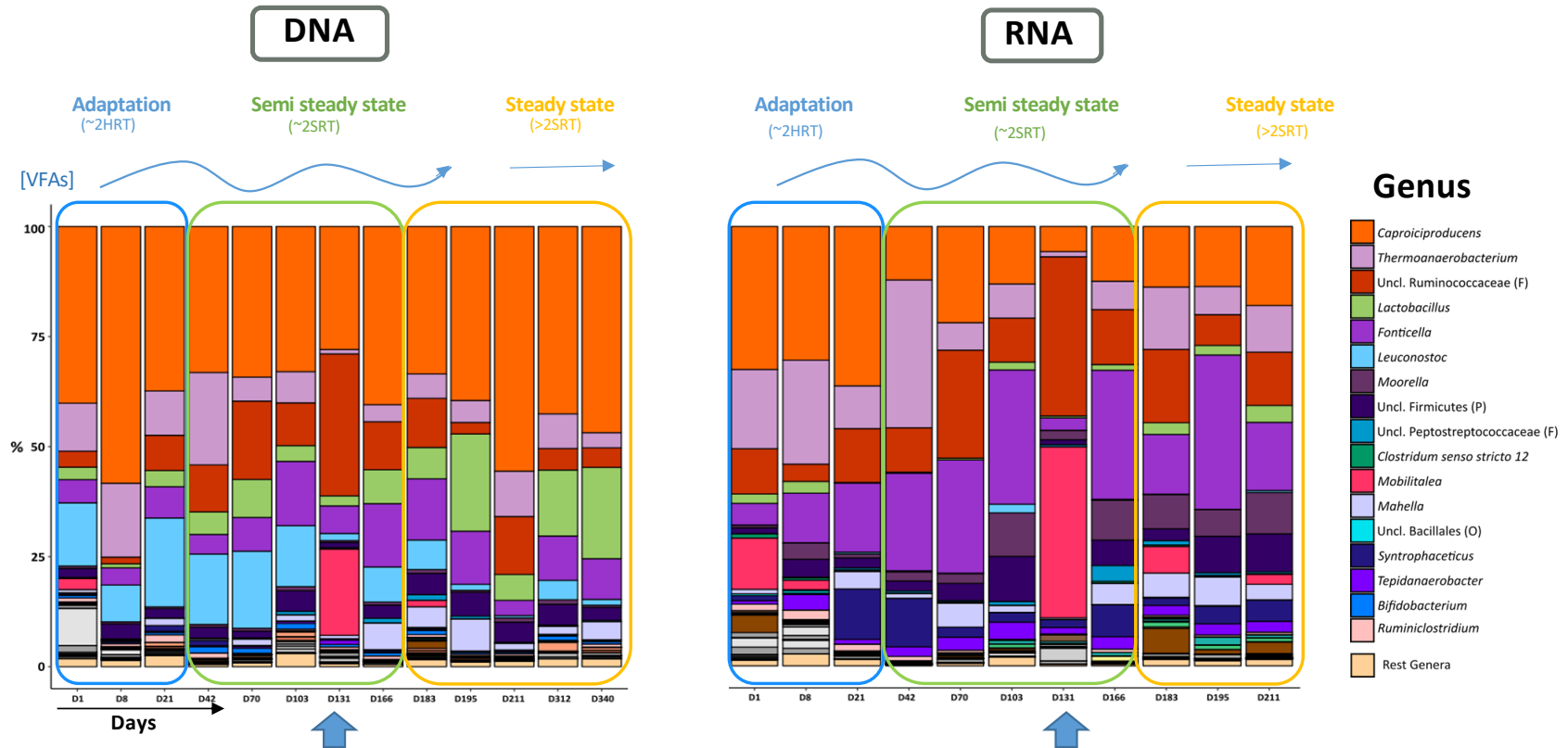
Ghent-1 – Microbial evolution



- ☐ Dominated by
- *Caproiciproducens*
 - *Thermoanaerobacterium*
 - *Mooreella / Lactobacillus*

Differences between DNA / RNA

Ghent-2 – Microbial evolution



□ Dominated by

- *Caproiciproducens*
- *Thermoanaerobacterium*
- Ruminococcaceae (F)
- *Fonticella*

Differences between DNA / RNA

Presence vs "activity"

- DNA = "presence"
- RNA = "active" (proxy)

"Present" LOW "activity"
 "Active" LOW "Presence"

RNA/DNA ratio: 0 1 >1

	D-10	D7	D33	D146	D153	D160	D175	D181	D186	D497
Caproiciproducenens	0,4	1,0	1,0	1,1	0,9	1,0	0,7	0,5	0,5	0,7
Thermoanaerobacterium	0,3	0,8	1,8	0,3	0,6	0,6	0,9	0,9	1,0	1,6
Uncl. Ruminococcaceae (F)	0,5	1,1	1,0	1,0	0,8	1,2	1,1	1,0	0,9	0,9
Lactobacillus	1,0	0,7	0,0	0,1	0,3	0,0	0,3	0,3	0,2	0,4
Fonticella	1,2	1,0	1,0	2,4	3,0	4,1	2,8	2,0	2,4	4,4
Leuconostoc	0,9	0,1	0,3	0,1	0,1	0,1	0,6	0,2	0,1	0,1
Moorella	2,0	8,8	11,9	4,9	7,8	10,8	6,6	11,9	12,2	7,6
Uncl. Firmicutes (P)	0,7	0,9	1,7	1,0	2,1	3,2	1,6	1,4	2,1	1,7
Uncl. Peptostreptococcaceae (F)	1,0	1,0	1,5	0,9	0,8	1,0	1,9	0,9	1,7	1,5
Clostridium sensu stricto 12	1,0	1,7	1,4	1,0	1,0	1,1	1,5	1,1	1,1	1,2
Mobilitalea	1,8	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
Mahella	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	2,3
Uncl. Bacillales (O)	5,4	2,4	1,8	22,7	1,7	1,4	1,3	1,8	1,2	1,0
Syntrophaceticus	0,8	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,9

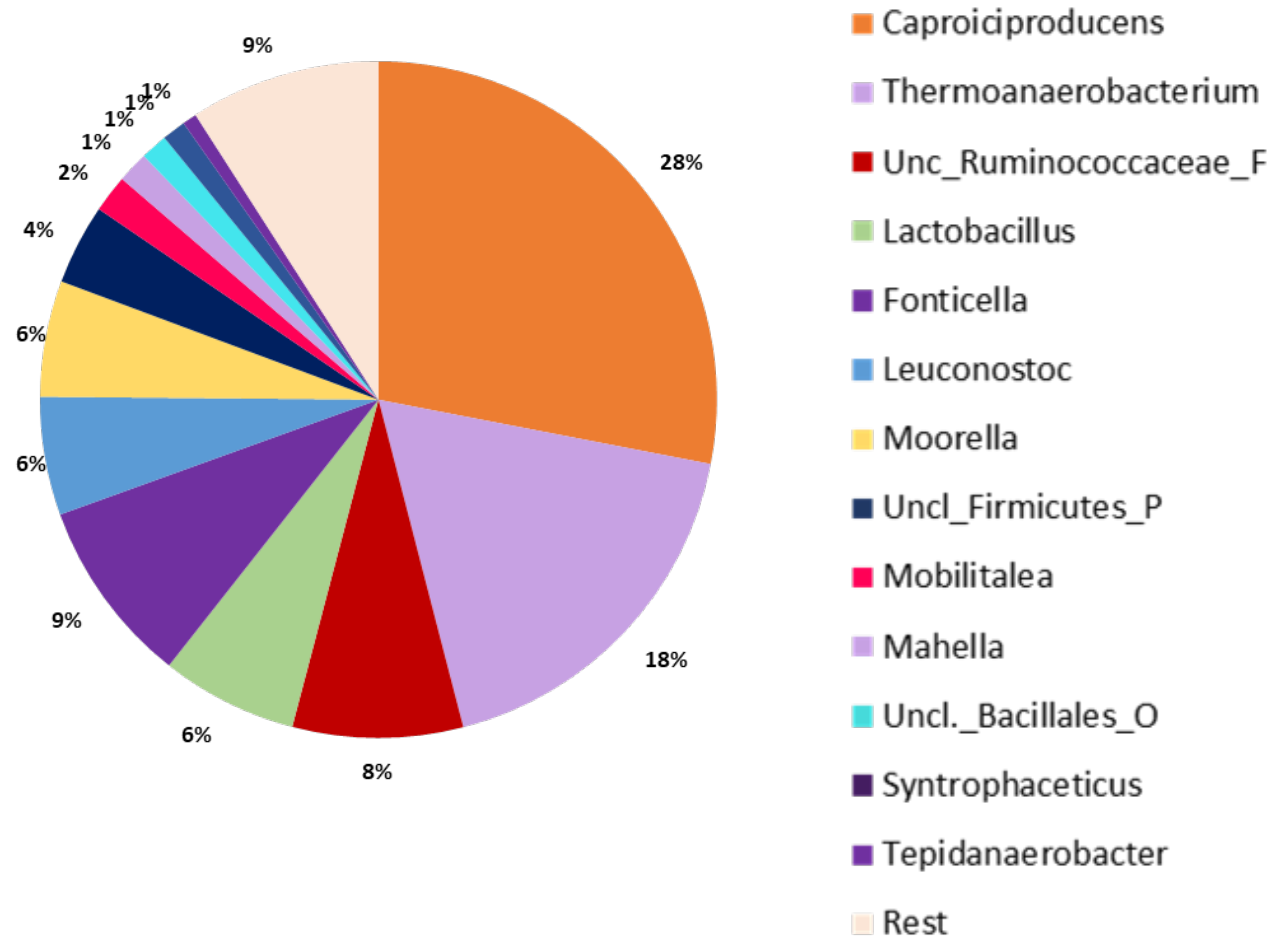
Ghent-1

Ghent-2

	D1	D8	D21	D42	D70	D103	D131	D166	D183	D195	D211
Caproiciproducenens	0,8	0,5	1,0	0,4	0,7	0,4	0,2	0,3	0,4	0,4	0,3
Thermoanaerobacterium	1,6	1,4	1,0	1,6	1,1	1,1	1,1	1,5	2,3	1,2	1,0
Uncl. Ruminococcaceae (F)	2,4	2,0	1,5	1,0	1,4	1,0	1,1	1,1	1,5	2,2	0,9
Lactobacillus	0,8	1,9	0,2	0,2	0,1	0,6	0,4	0,3	0,5	0,1	0,7
Fonticella	0,9	2,5	2,1	4,3	3,1	2,0	0,5	2,0	1,0	2,8	3,7
Leuconostoc	0,1	0,1	0,1	0,1	0,1	0,2	0,4	0,1	0,1	0,4	1,0
Moorella	1,0	3,2	1,4	1,9	2,0	5,7	2,2	6,1	4,7	4,9	5,3
Uncl. Firmicutes (P)	0,8	1,1	1,0	0,9	1,9	2,0	1,0	1,7	0,6	1,5	1,7
Uncl. Peptostreptococcaceae (F)	0,9	1,1	1,3	1,3	1,1	1,0	1,2	2,3	0,9	1,0	1,1
Clostridium sensu stricto 12	1,7	1,4	1,3	1,1	1,3	1,0	1,0	1,4	1,3	1,2	1,2
Mobilitalea	3,6	2,7	1,1	1,0	1,0	1,0	1,9	1,1	2,9	1,2	3,3
Mahella	1,2	1,2	1,9	1,5	2,6	1,1	0,8	0,8	1,1	0,9	1,8
Uncl. Bacillales (O)	1,3	1,4	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,2	1,1
Syntrophaceticus	1,6	1,3	5,5	5,5	2,6	2,8	2,1	5,8	2,0	4,5	4,4

C1 Core microbiome

Ghent-1 DNA/RNA & Ghent-2 DNA/RNA

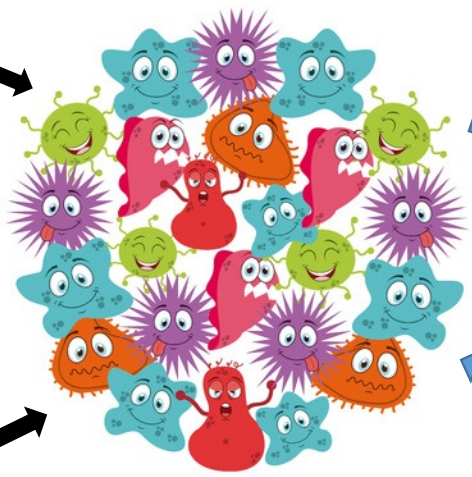


Validating a 16S rRNA gene based DGGE tool

Temperature

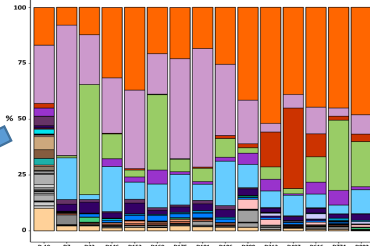
Substrate

pH



Who are they ?

2 months, expensive!!!



16S amplicon sequencing



(DGGE)
Denaturing Gradient Gel Electrophoresis

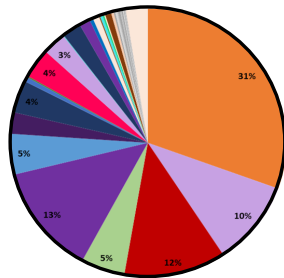
2 days, cheap!!!



Validating a **16S rRNA gene based DGGE tool** for monitoring bacterial community composition & screening isolates

Validating a 16S rRNA gene based DGGE tool

Objective: Identification of DNA bands of C1 dominant species on DGGE



- *Caproiciproducens*
- *Thermoanaerobacterium*
- *Fam. Ruminococaceae*
- *Lactobacillus*
- *Fonticella*
- *Leuconostoc*
- *Moorella*

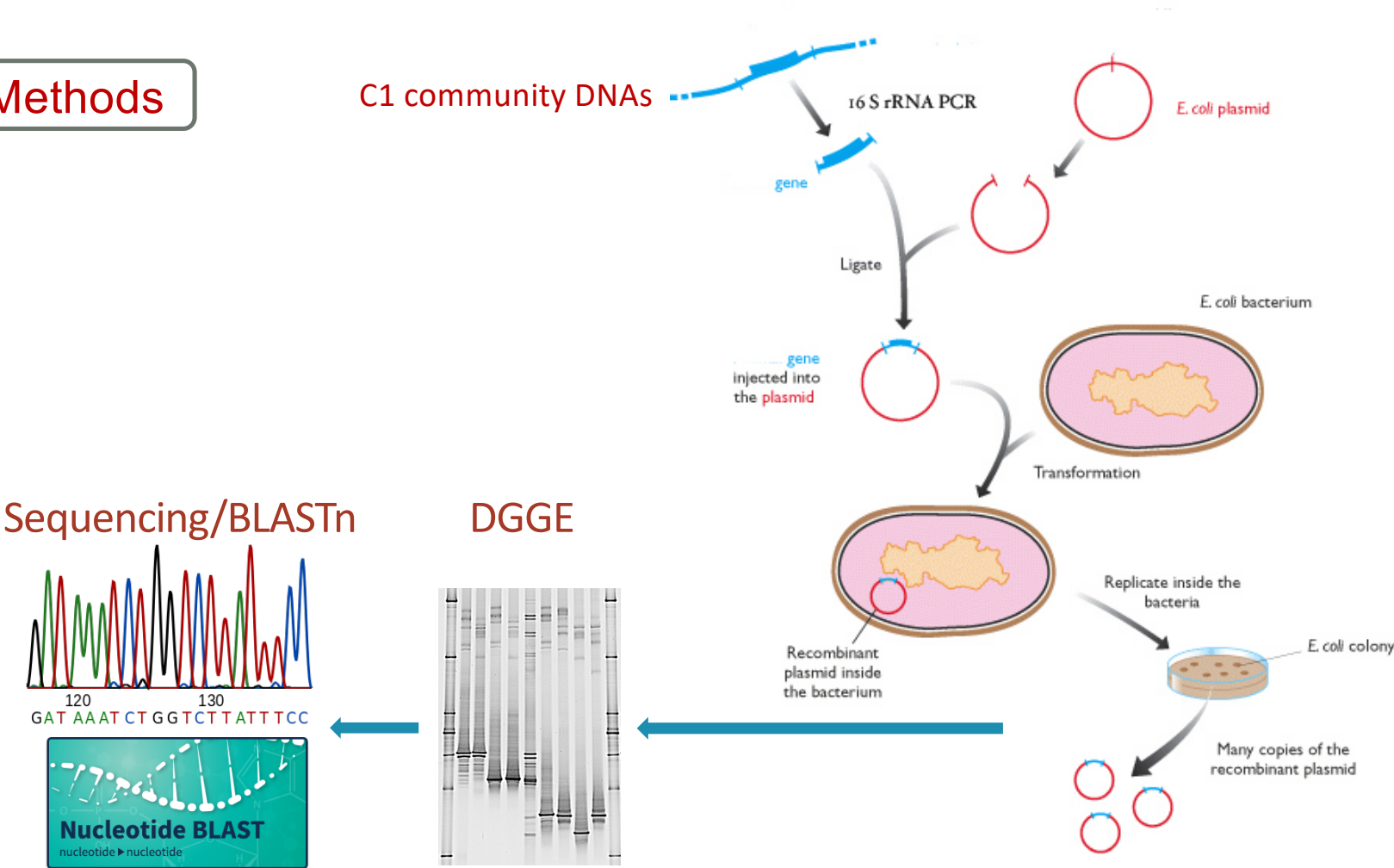


who is who?

- 16S rRNA amplicon sequencing

Validating a 16S rRNA gene based DGGE tool

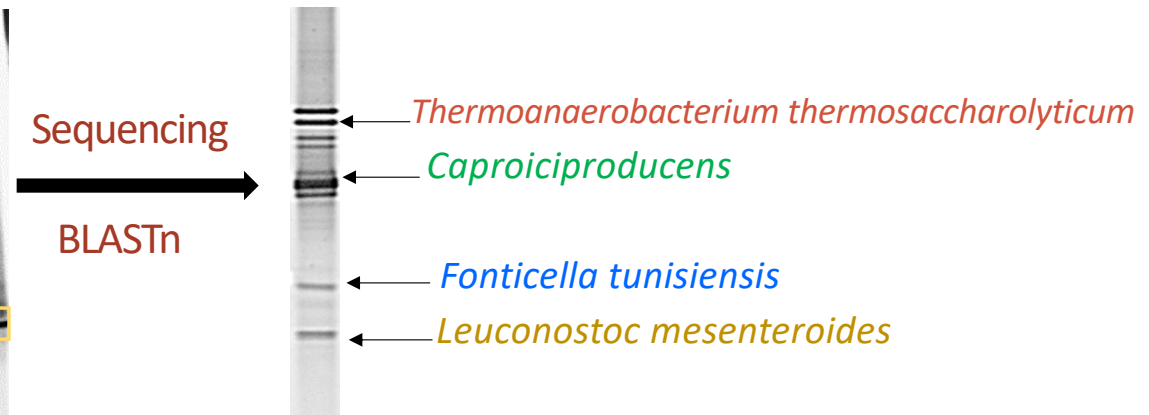
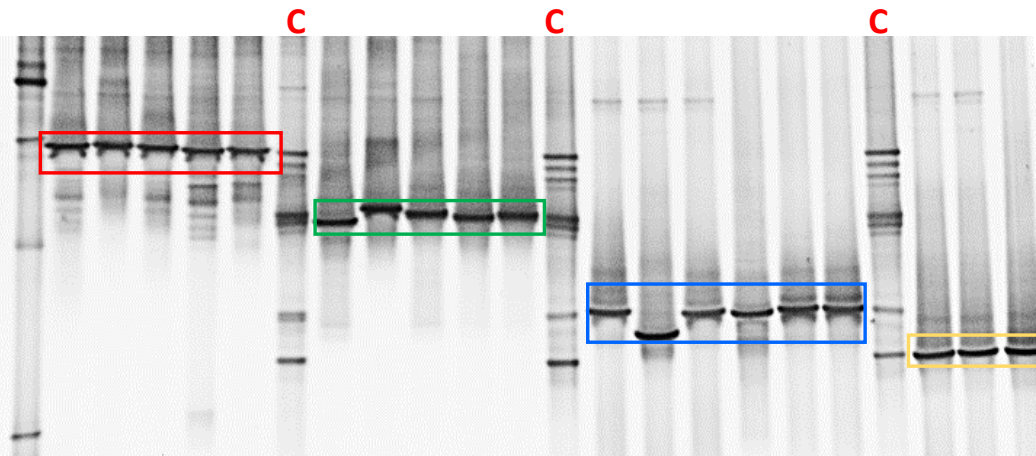
Methods



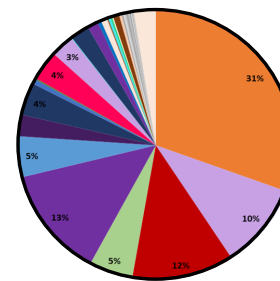
Validating a 16S rRNA gene based DGGE tool

Results

- 19 positive colonies analyzed by DGGE

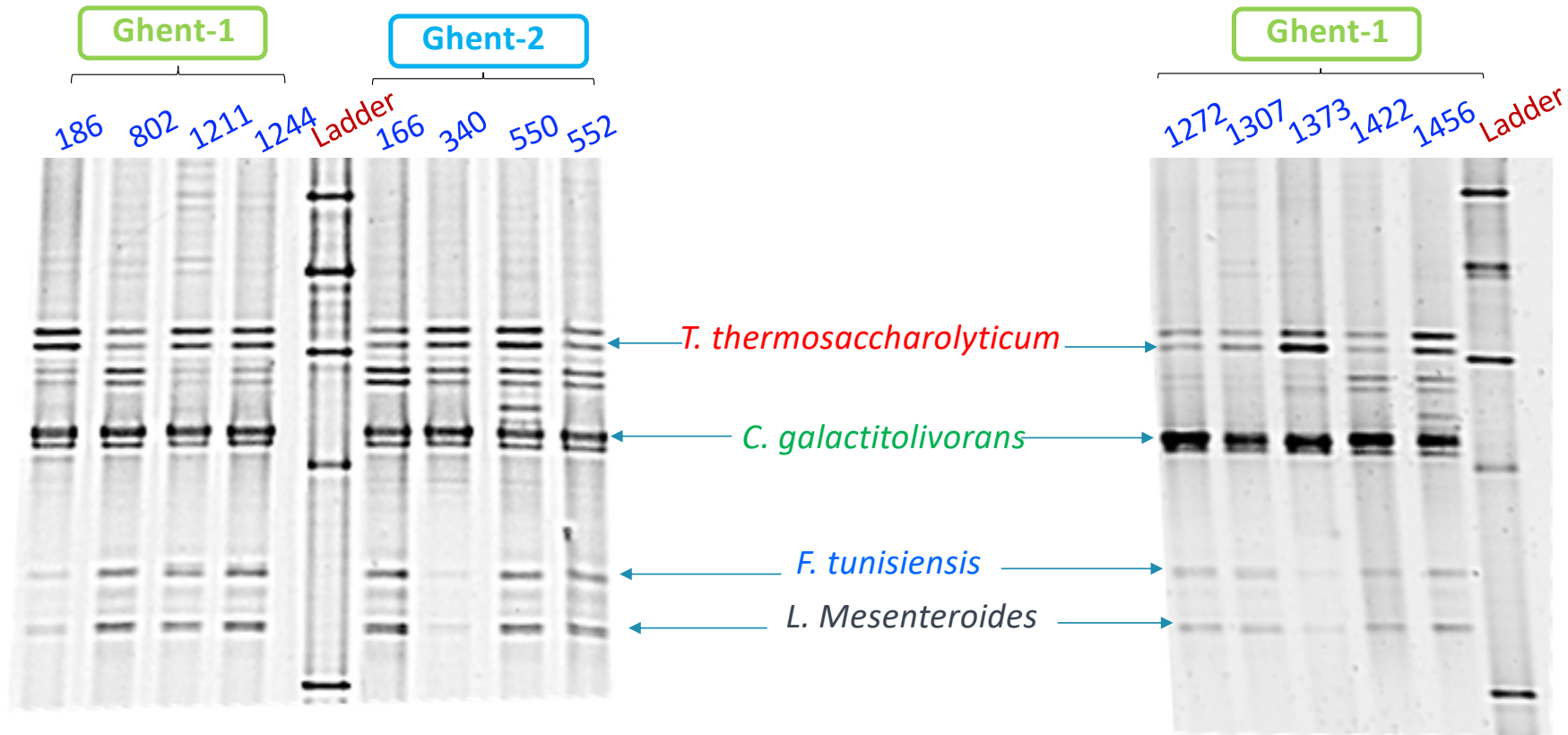


- The same dominant/potentially keystone species identified on by 16S rRNA gene amplicon sequencing
- 16S rRNA gene based DGGE translates 16S rRNA gene amplicon sequencing data
- Some dominant species were identified at species level

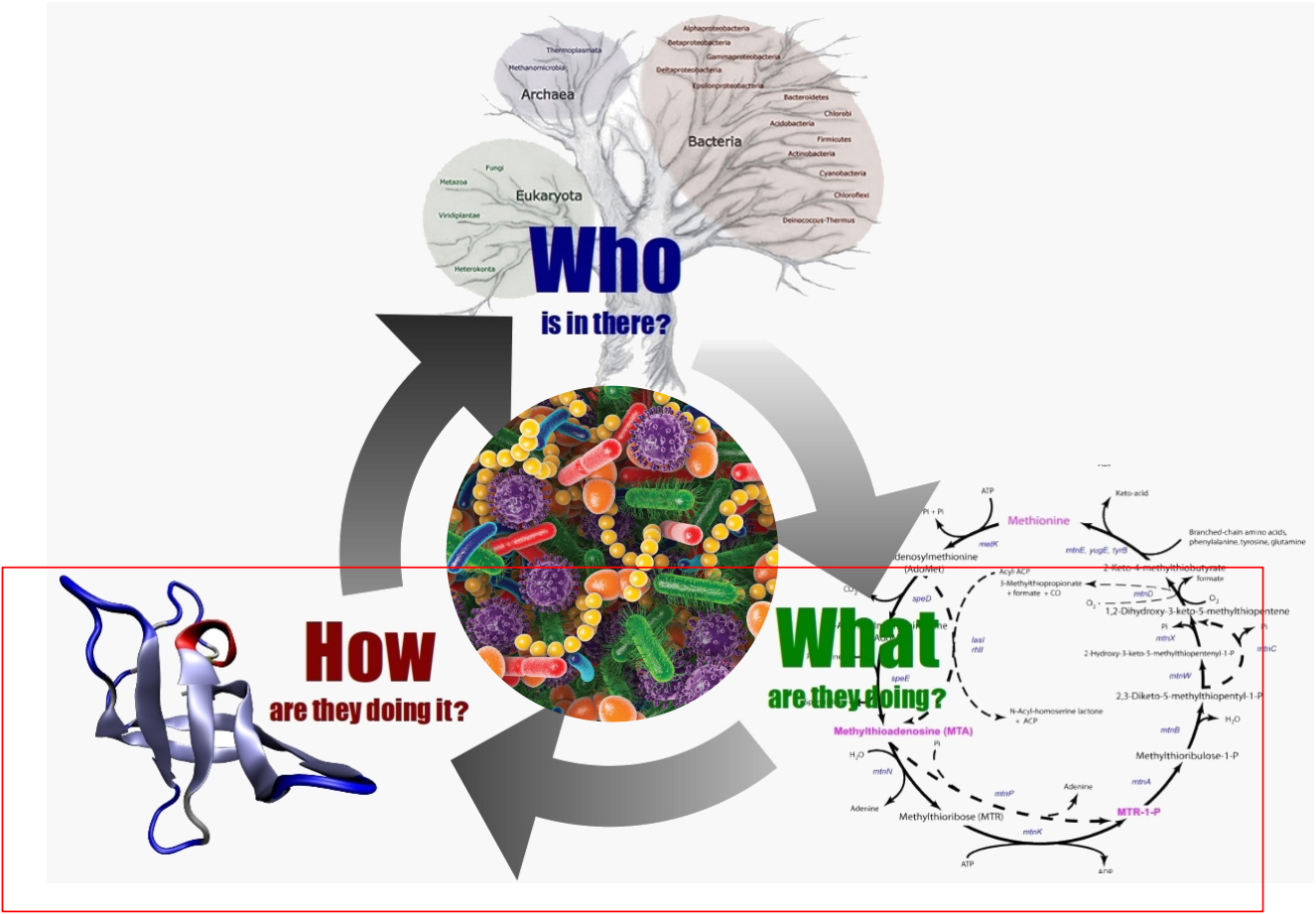


- *Caproiciproducens*
- *Thermoanaerobacterium*
- *Fam. Ruminococcaceae*
- *Lactobacillus*
- *Fonticella*
- *Leuconostoc*
- *Moorella*

Community analysis of long-term operated C1 Ghent reactors



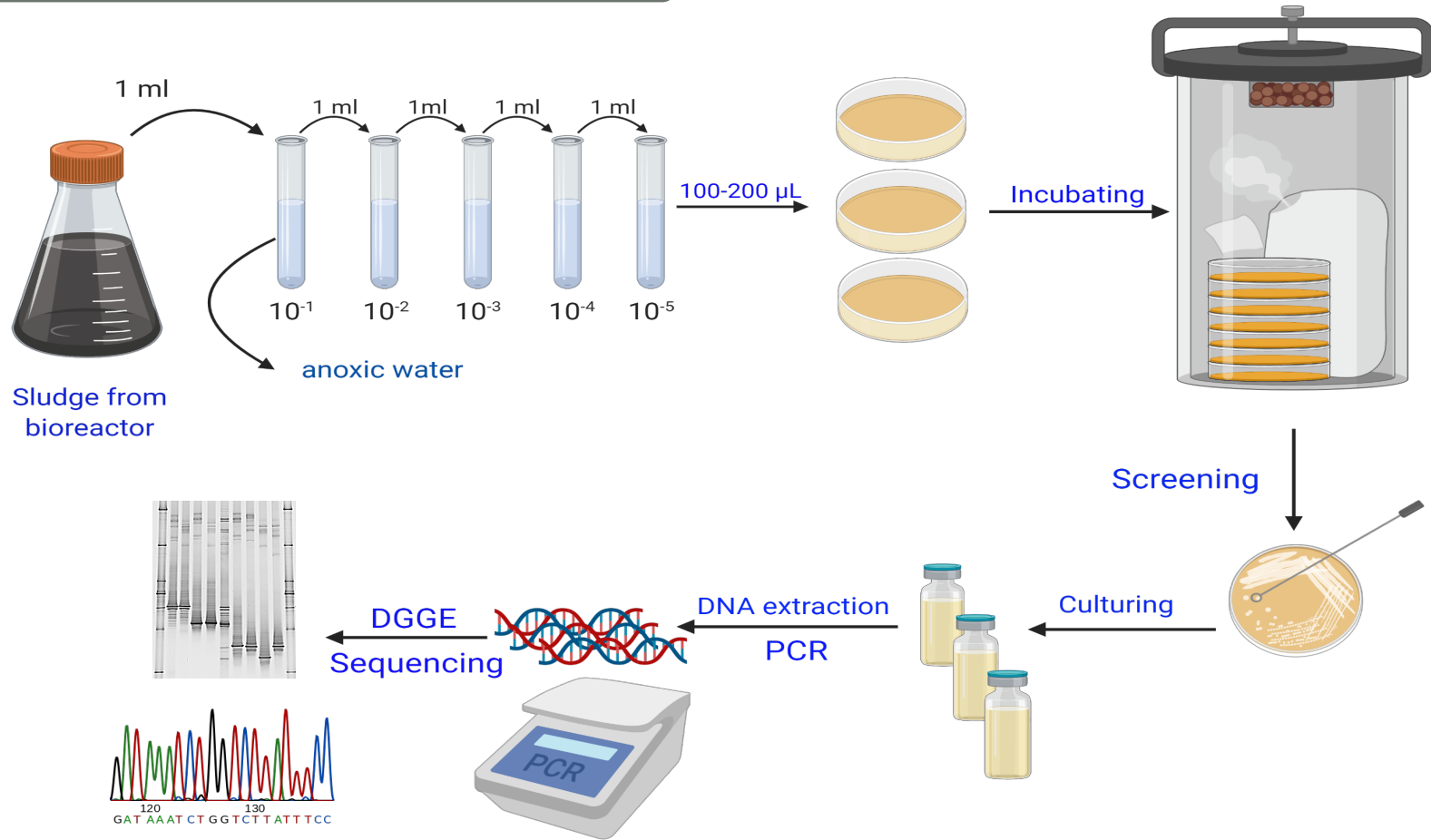
C1 is a **highly stable community** with 4 dominant genera:
Thermoanaerobacterium, *Caproiciproducens*, *Fonticella*, *Leuconostoc*



Isolating dominant/keystone species in C1 community

Isolation of C1 dominant/potentially keystone species

Methods: Isolation of anaerobic bacteria



Isolation of C1 dominant/potentially keystone species

Summary of isolated strains

Isolated strains	Isolated conditions		Reference conditions		Classification
	pH	T (°C)	Optimal pH	Optimal T (°C)	
<i>Thermoanaerobacterium thermosaccharolyticum</i>	5.6; 7.0	55	5.0-6.5	50-60	Strictly
<i>Lactobacillus coryniformis</i>	5.6; 7.0	25; 40	5.5	25-40	Facultative
<i>Lactobacillus harbinensis</i>	5.6	40	...	37	Facultative
<i>Lactobacillus otakiensis</i>	5.6	25	4.0-8.5	30-37	Facultative
<i>Lactobacillus paracasei</i>	5.6; 7.0	40	6.2	37	Facultative
<i>Lactobacillus plantarum</i>	5.6	25	5.0-8.0	25-37	Facultative
<i>Bacillus paralicheniformis</i>	7.0	40	7.0-8.0	37	Facultative
<i>Bacillus coagulans</i>	5.5	37	5.5-6.5	35-50	Facultative
<i>Caproiciproducens sp.</i>	5.5	55	30	5.5/7.0	Strictly

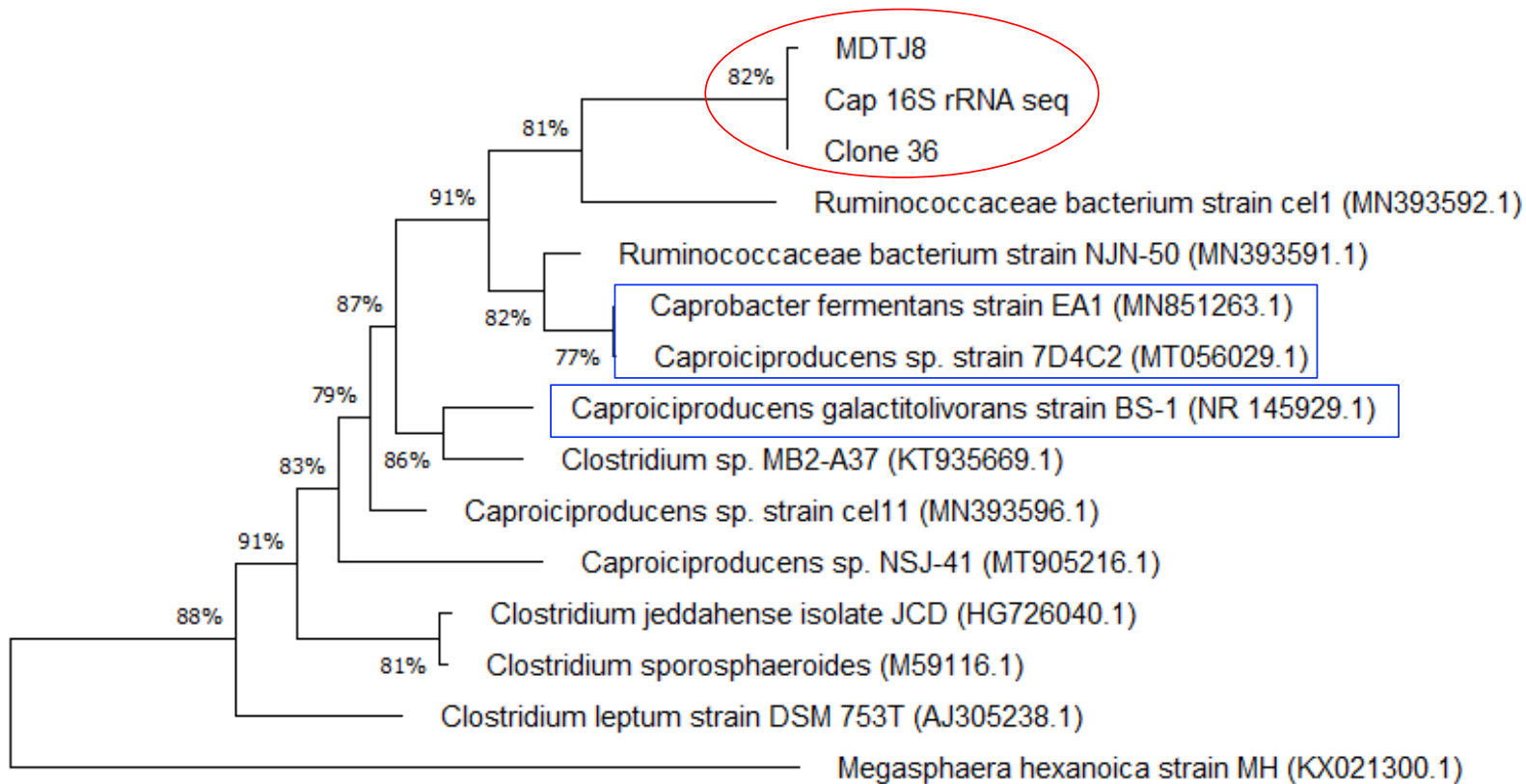
Isolation of C1 dominant/potentially keystone species

16S rRNA gene analysis of *Caproiciproducens* isolate MDTJ8

□ 16S rRNA gene of *Caproiciproducens* isolate MDTJ8

- **100%** identity with 1 **cloned** 16S rRNA gene and extracted sequence from **16S sequencing**
- **94.8%** identity with
 - *Caprocibacter fermentans* strain EA1 (Flaiz et al., 2020)
 - *Caproiciproducens* sp. 7D4C2 (Esquivel-Elizondo et al., 2020)
- **93.6%** identity with *Caproiciproducens galactitolivorans* strain BS-1 (Kim et al., 2015)

16S rRNA gene analysis of *Caproiciproducens* isolate MDTJ8



0,050

IMDTJ8: a new species of *Caproiciproducens* or a new genus of Ruminococcaceae

Isolation of C1 dominant/potentially keystone species

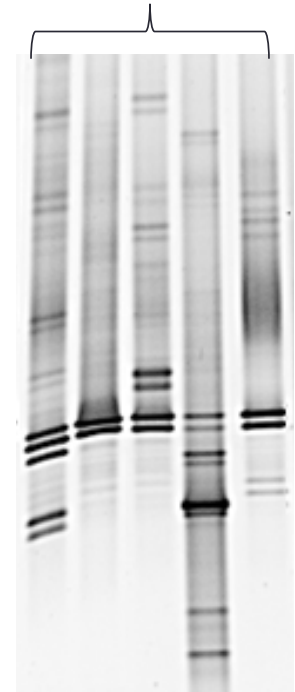
Analysis of *Thermoanaerobacterium* isolates

- *T. thermosaccharolyticum* is the most dominant bacterium in the C1
- Different DNA profiles of *T. thermosaccharolyticum* on DGGE
 - Different strains?
 - The same strain: more than one copy of 16S rRNA gene?



Further investigation: whole genome sequencing analysis

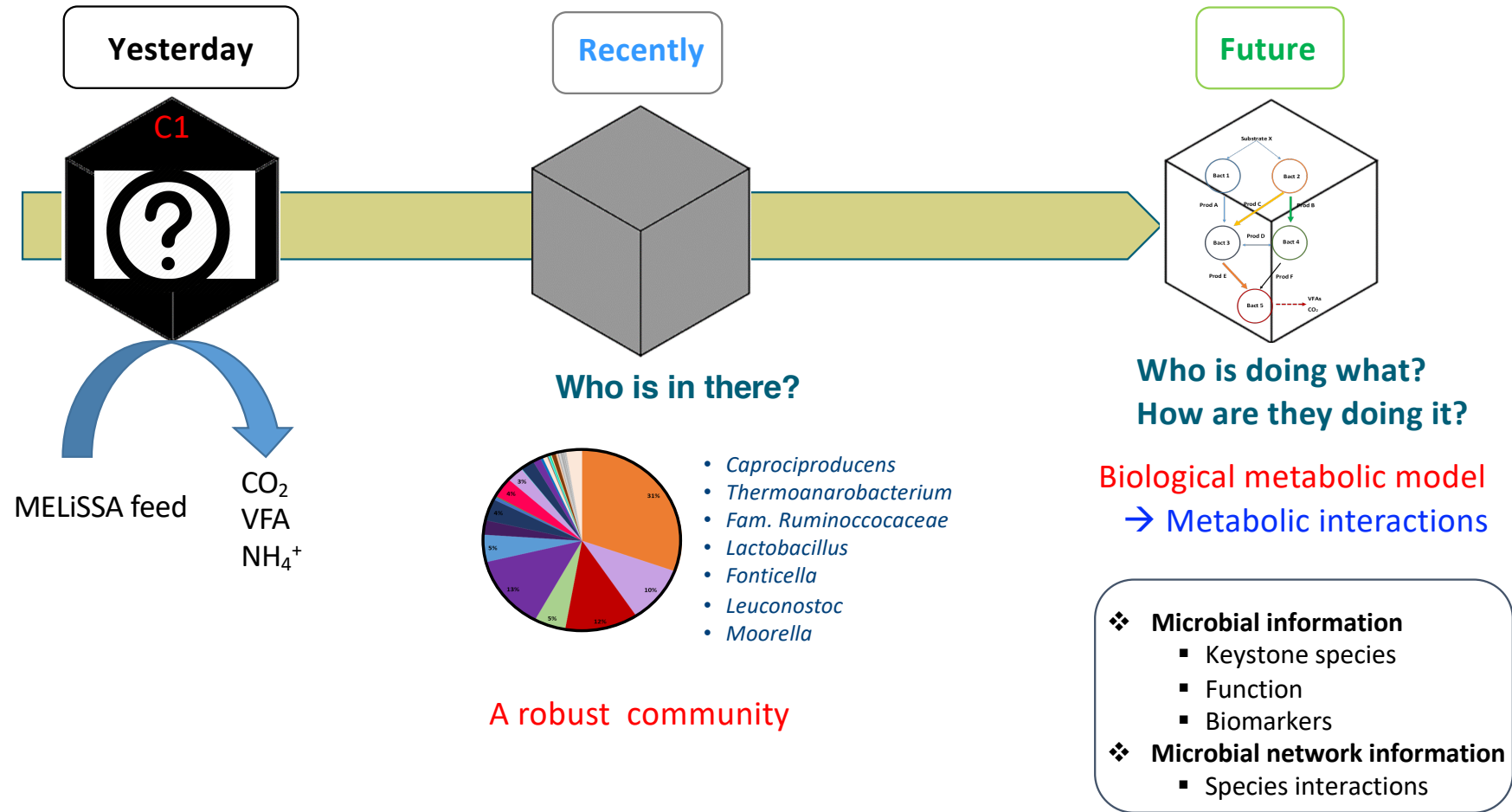
T. thermosaccharolyticum



Conclusions

- ❑ C1 core microbiome: ***Caproiciproducens*** , ***Thermoanerobacterium***,
uncl. Ruminococcaceae, ***Lactobacillus***, ***Fonticella***, ***Leuconostoc***,
Moorella
- ❑ C1 is a **highly stable community**
- ❑ Three dominant anaerobic bacteria were isolated
 - ***Thermoanerobacterium***
 - ***Caproiciproducens***
 - ***Lactobacillus***

C1 study progress



Thanks for your attention!



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