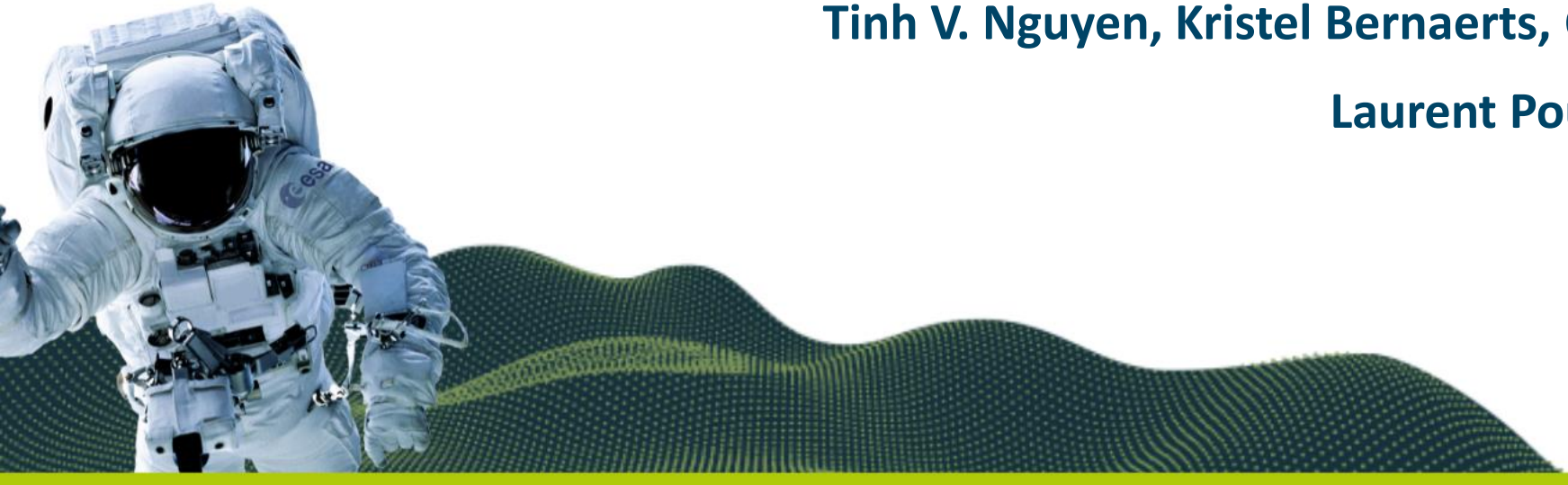
MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

2022 MELISSA CONFERENCE
8-9-10 NOVEMBER 2022

CREATING A CIRCULAR FUTURE

Characterization of a promising thermophilic chain elongating- bacterium isolated from a MELiSSA waste compartment reactor, *Thermocaproicibacter melissae* gen. nov., sp. nov. for *n*-caproate production utilizing polymeric carbohydrates.

Tinh V. Nguyen, Kristel Bernaerts, Claude-Gilles Dussap, Laurent Poughon, Dirk Springael



KU LEUVEN

UNIVERSITÉ Clermont Auvergne

Sustainable recycling organic wastes

- Medium chain carboxylic acid (MCCA): C5-C8
  - Example: Caproate (C6), Octanoate (C8)
  - Value-added chemicals with various applications
  - Produced by chain elongating bacteria





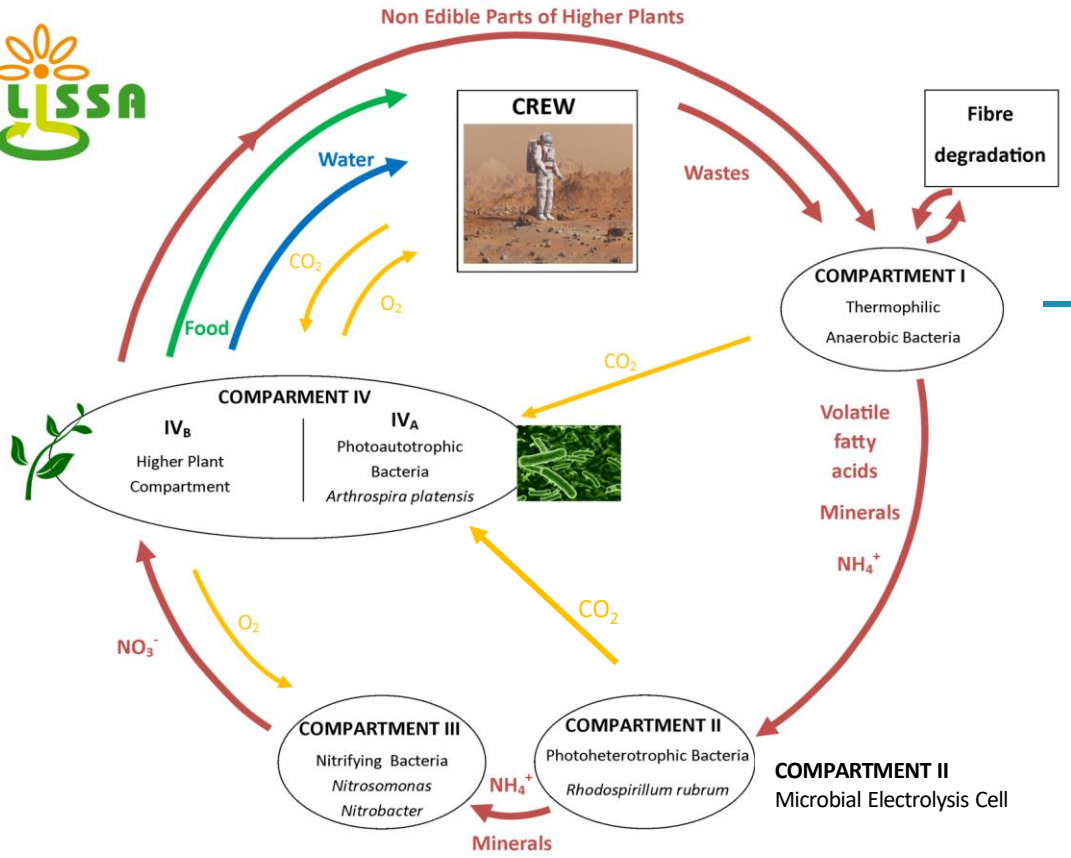

Microbial MCCA production

Anaerobic digestion by Mixed culture/Pure culture

Solid waste is about 1.3 billion/ year

46% organic contents

Thermophilic acidogenic C1 bioreactor

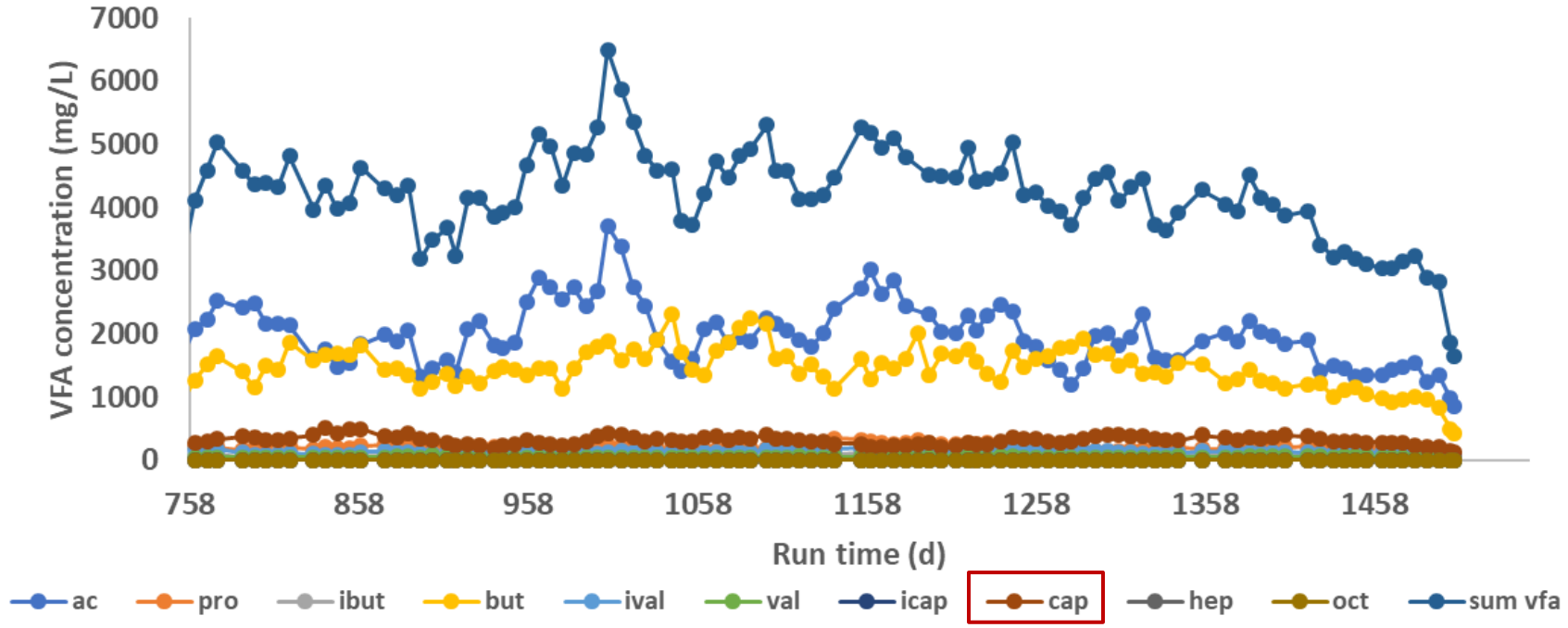


My research

C1 reactor

- Inoculum
  - MELiSSA Pilot Plant (Barcelona)
  - BELISSIMA (VITO) (Stored)
  - KUL C1 (Fresh)
  - DRANCO (Fresh)
  - Thermophillic AD (Fresh)
  - Lignocellulosic AD (Fresh)
- Feed:
  - beetroot,
  - wheat straw,
  - toilet paper,
  - feces,
  - lettuce
- 55°C; pH 5.5

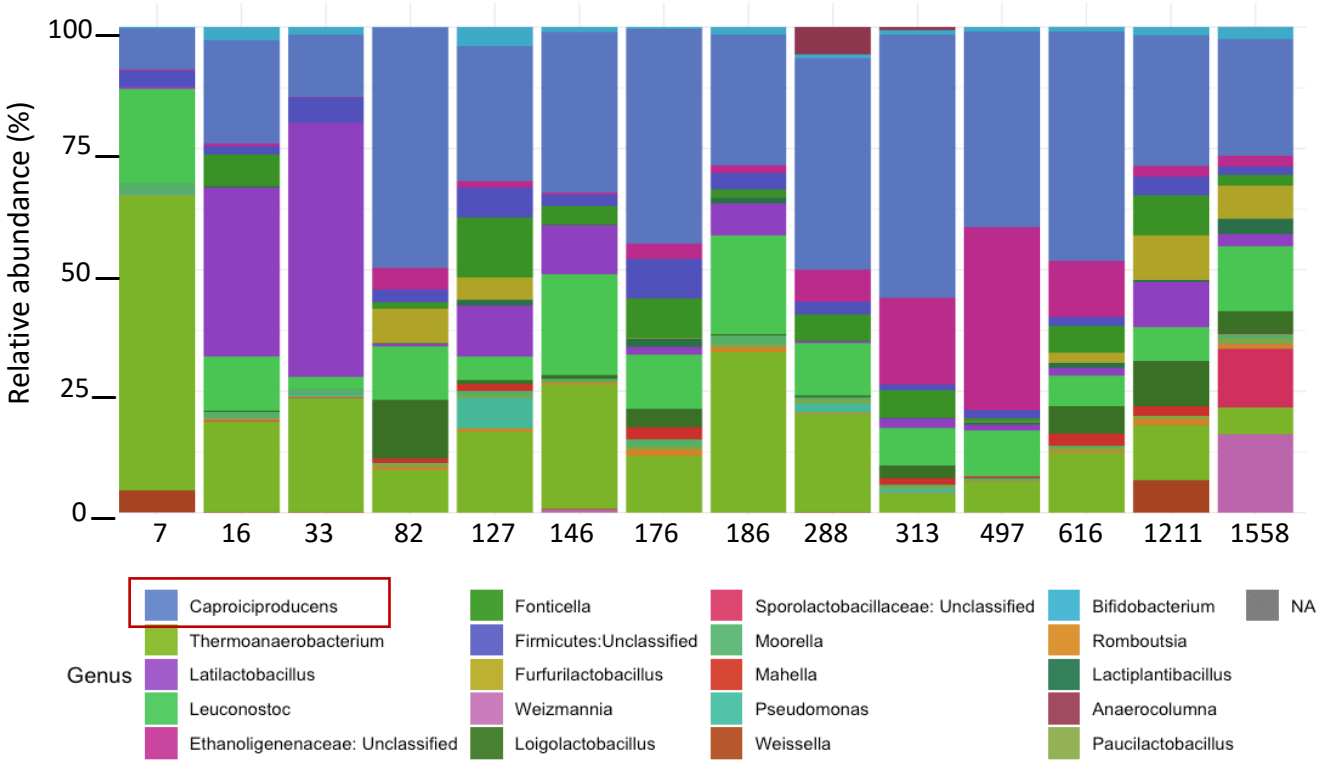
Caproate- one of the most dominant fermentation products



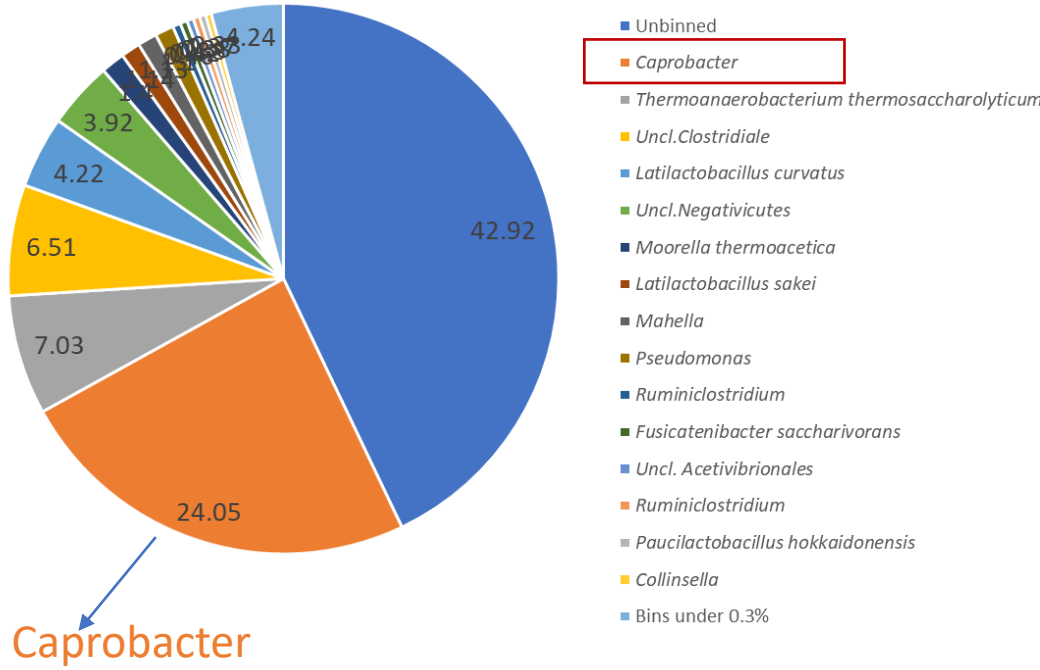
What are bacteria responsible for caproate production?

Caproiciproducens (Caprobacter), the most dominant bacteria

16S rRNA amplicon sequencing (V3)



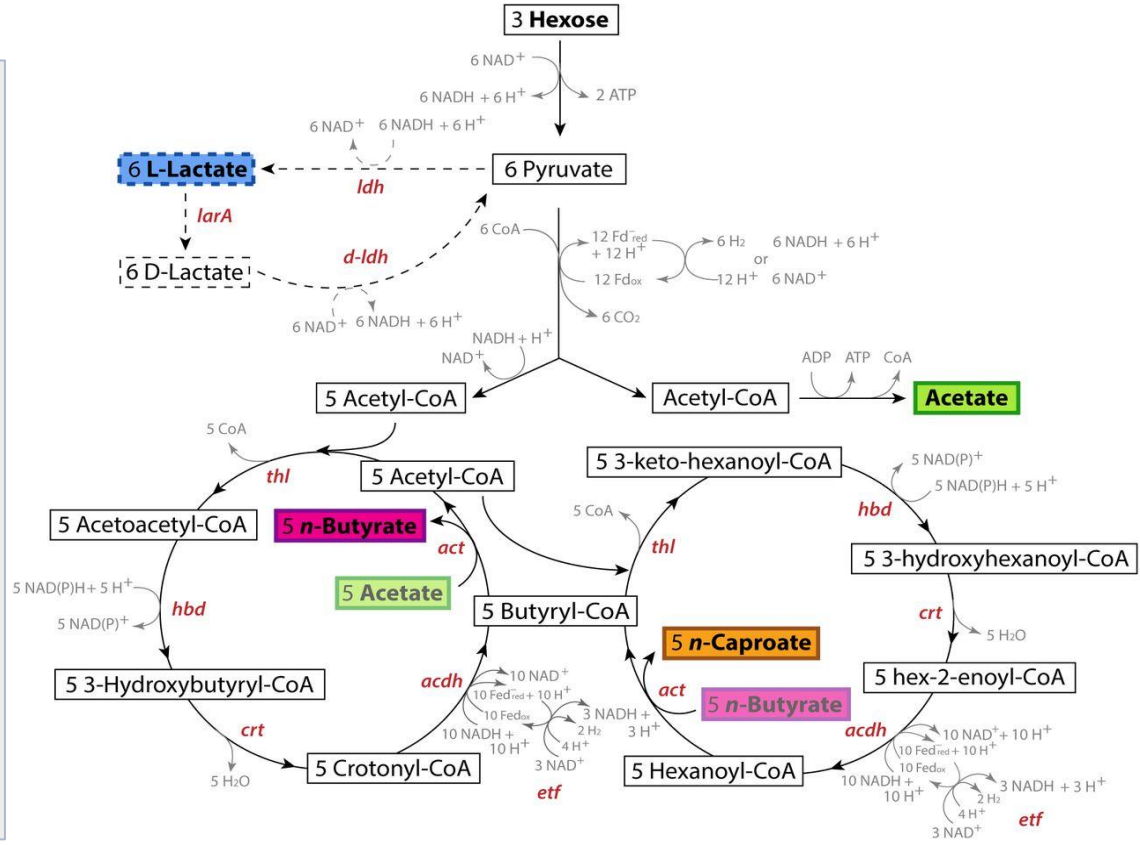
Metagenome



Do *Caproiciproducens*-like organisms really produce Caproate in C1 reactor?

Chain elongators of the family *Oscillospiraceae*

- Chain elongation via **reverse β-oxidation (RBO)**
- Published species:
  - *Caproiciproducens galactitolivorans* BS-1^T (Kim *et al.*, 2015)
  - *Caproicibacter fermentans* EA1^T (Flaiz et al., 2020)
  - *Caproicibacterium amylolyticum* LBM18003^T (Gu et al., 2021)
  - *Caproicibacterium lactatifermentans* LBM19010^T (Wang et al., 2022)
- Mostly ferment **sugars** to acetate, butyrate, caproate, CO_2, H_2
- They are mesophilic chain elongators

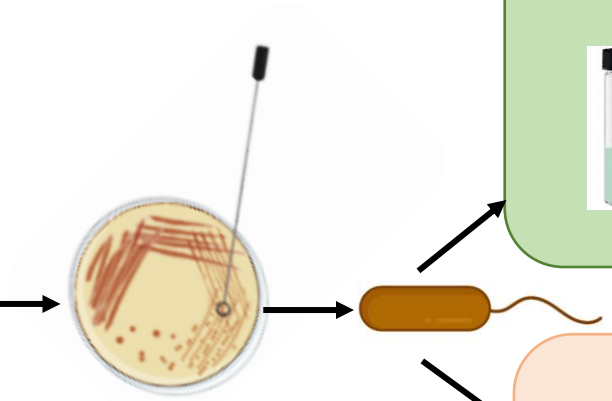


Esquivel-Elizondo et al., (2021)

Are *Caprociproducens*-like organisms thermophilic chain elongators within *Oscillospiraceae*?

Do thermophilic chain elongators perform chain elongation by RBO?

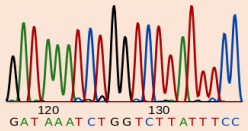
Methodology



RCM; pH 5.5, 55°C

Phenotypic characteristics

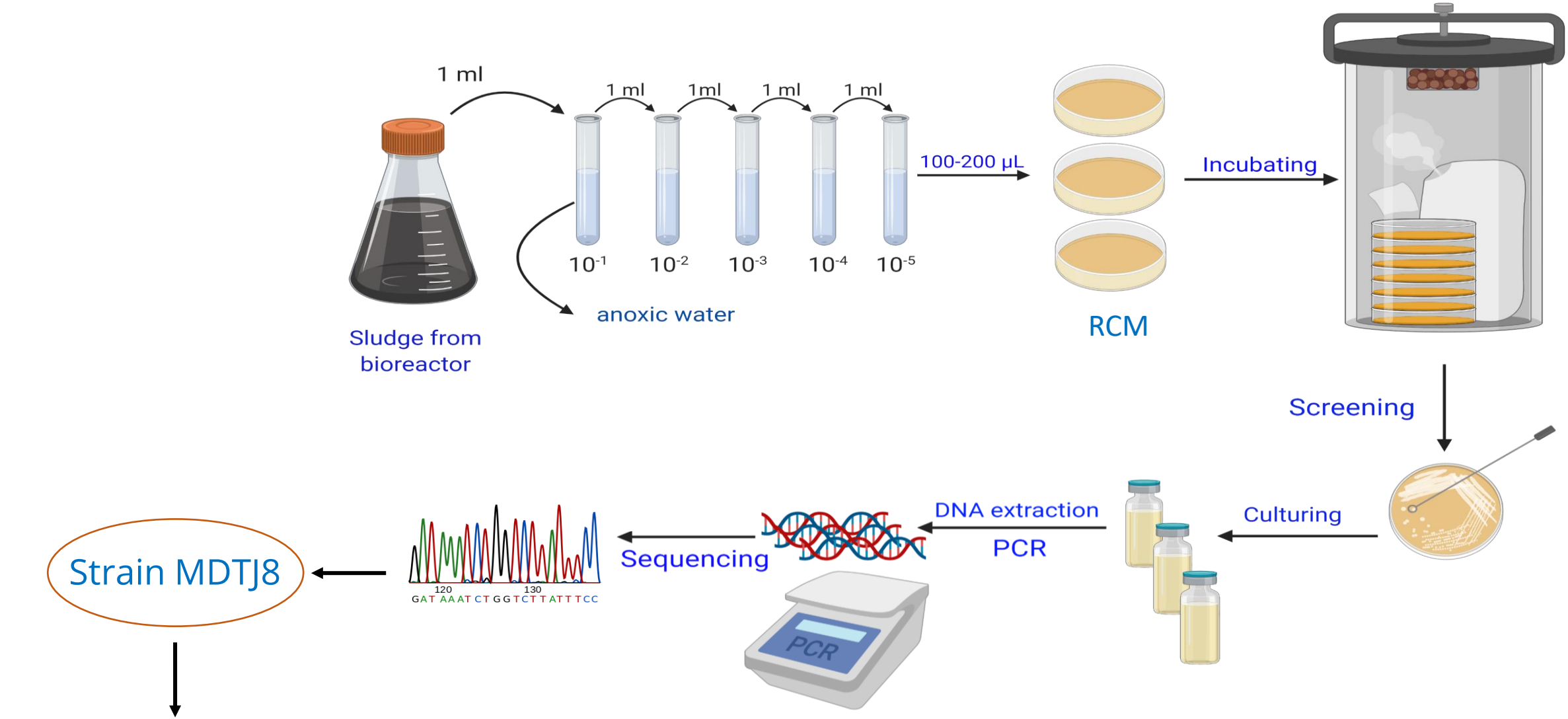
- pH, Temperature
- Carbon sources
- Metabolites



- Phylogeny, ANI, DDH
- Genome annotation
- Energy conservation
- Metabolic pathways

Genomic characteristics

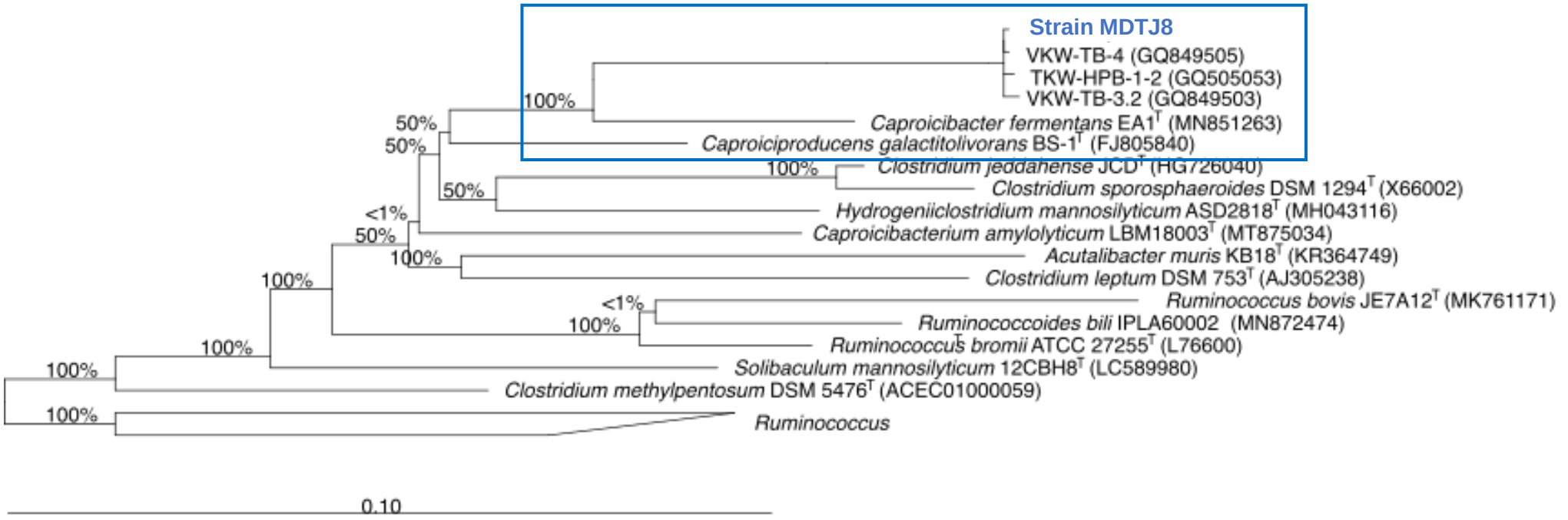
Direct isolation without enrichment step



Share 100% similarity with 16S rRNA sequence (V3) (amplicon sequencing)

Strain MDTJ8: closely related to caproate producing bacteria

- Phylogeny based on 16S rRNA gene



Strain MDTJ8 belongs to a novel genus of the family *Oscillospiraceae*

Strain	ANIb	DDH	AAI	POCP
Acutalibacter muris KB18^T	66.00%	26.90±2.45%	65.23%	47.40%
Caproicibacter fermentans EA1^{T*}	69.82%	20.20±2.35%	63.58%	30.90%
Caproicibacterium amylolyticum LBM18003^{T*}	68.21%	26.70±2.40%	67.55%	48.20%
Caproiciproducens galactitolivorans BS-1^{T*}	69.68%	21.30±2.35%	65.04%	56.30%
Clostridium jeddahense JCD^T	67.99%	20.20±2.30%	64.61%	45.40%
Clostridium leptum DSM 753^T	67.14%	29.30±2.45%	60.34%	40.60%
Clostridium methylpentosum DSM 5476^T	65.82%	22.30±2.35%	63.47%	34.40%
Clostridium sporosphaeroides DSM 1294^T	68.20%	19.60±2.30%	64.98%	48.00%
Hydrogeniiclostridium mannosilyticum ASD2818^T	68.10%	21.80±2.35%	59.53%	44.10%
Ruminococcus albus ATCC 27210^T	64.31%	28.00±2.40%	61.47%	25.30%
Ruminococcus bovis JE7A12^T	64.75%	34.50±2.50%	62.01%	36.70%
Ruminococcus bromii ATCC 27255^T	66.07%	31.60±2.45%	58.80%	39.70%
Ruminococcus callidus ATCC 27760^T	65.56%	22.90±2.40%	59.07%	28.60%
Ruminococcus flavefaciens ATCC 19208^T	64.73%	27.60±2.40%	61.97%	28.70%

New species

Species: >96.5% for ANI and >70% for DDH

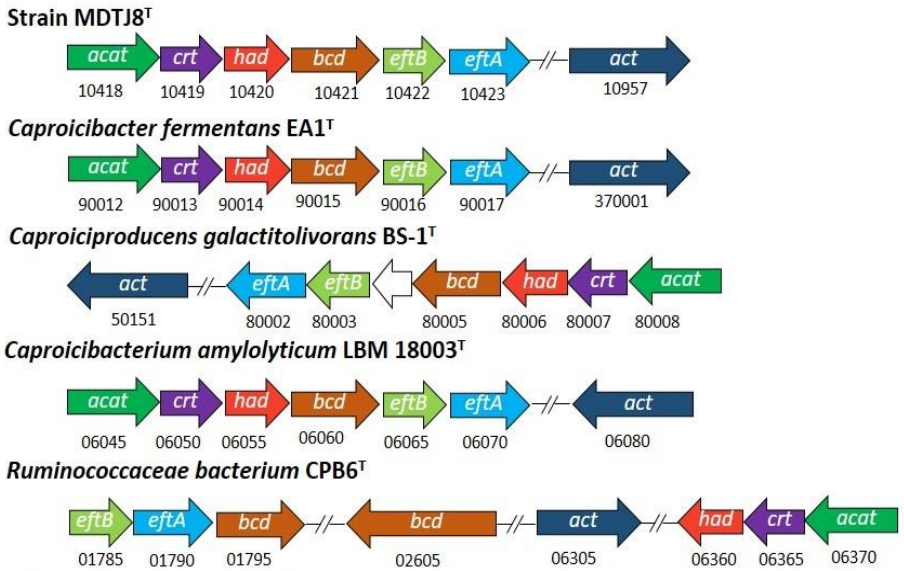
New genus

Genus <70% for AAI, and <50% for POCP

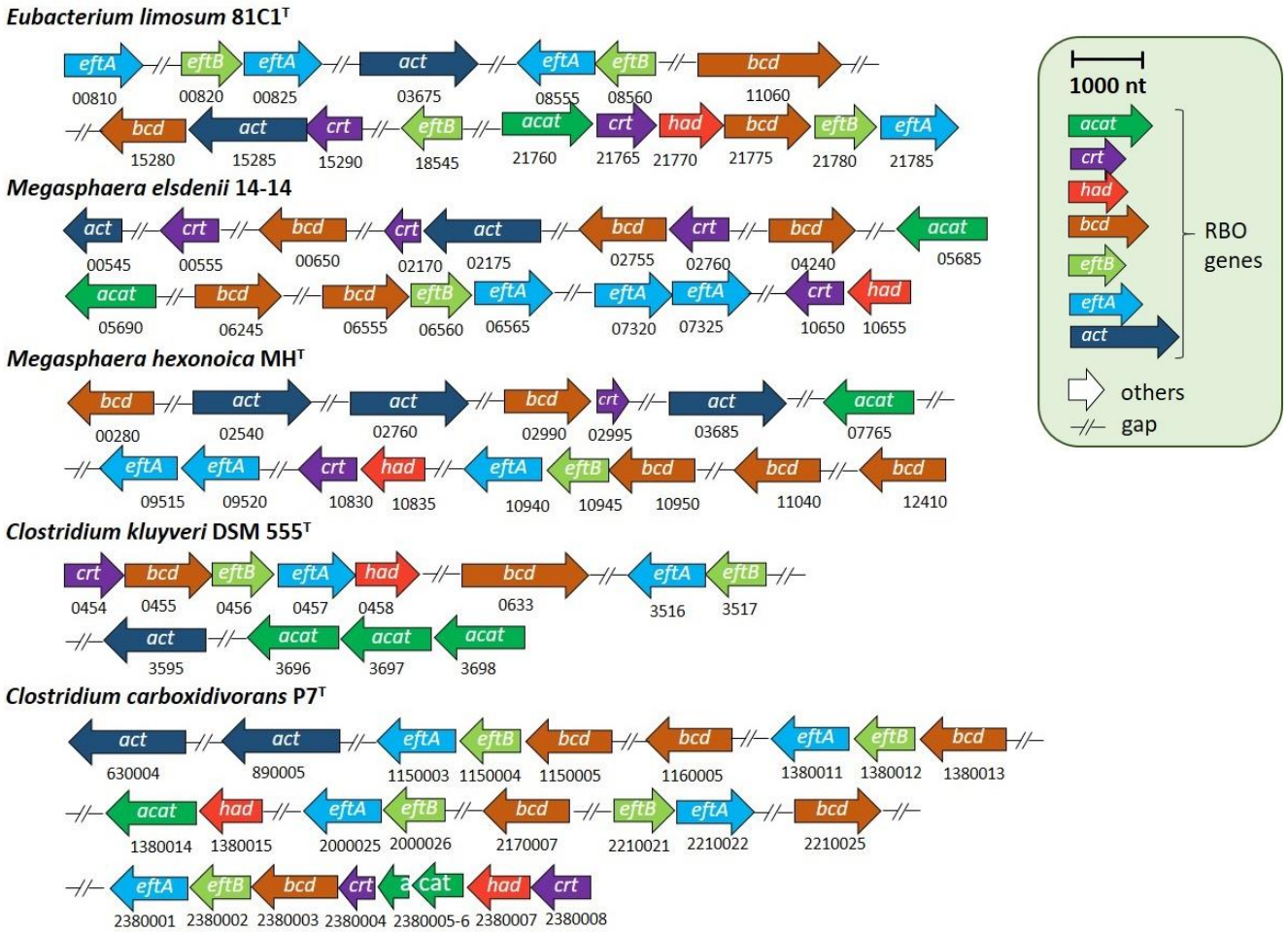
→ *Thermocaproicibacter melissae* gen. nov., sp. nov

Lineage: Bacteria; Terrabacteria group; Firmicutes; Clostridia; Eubacteriales; Oscillospiraceae;

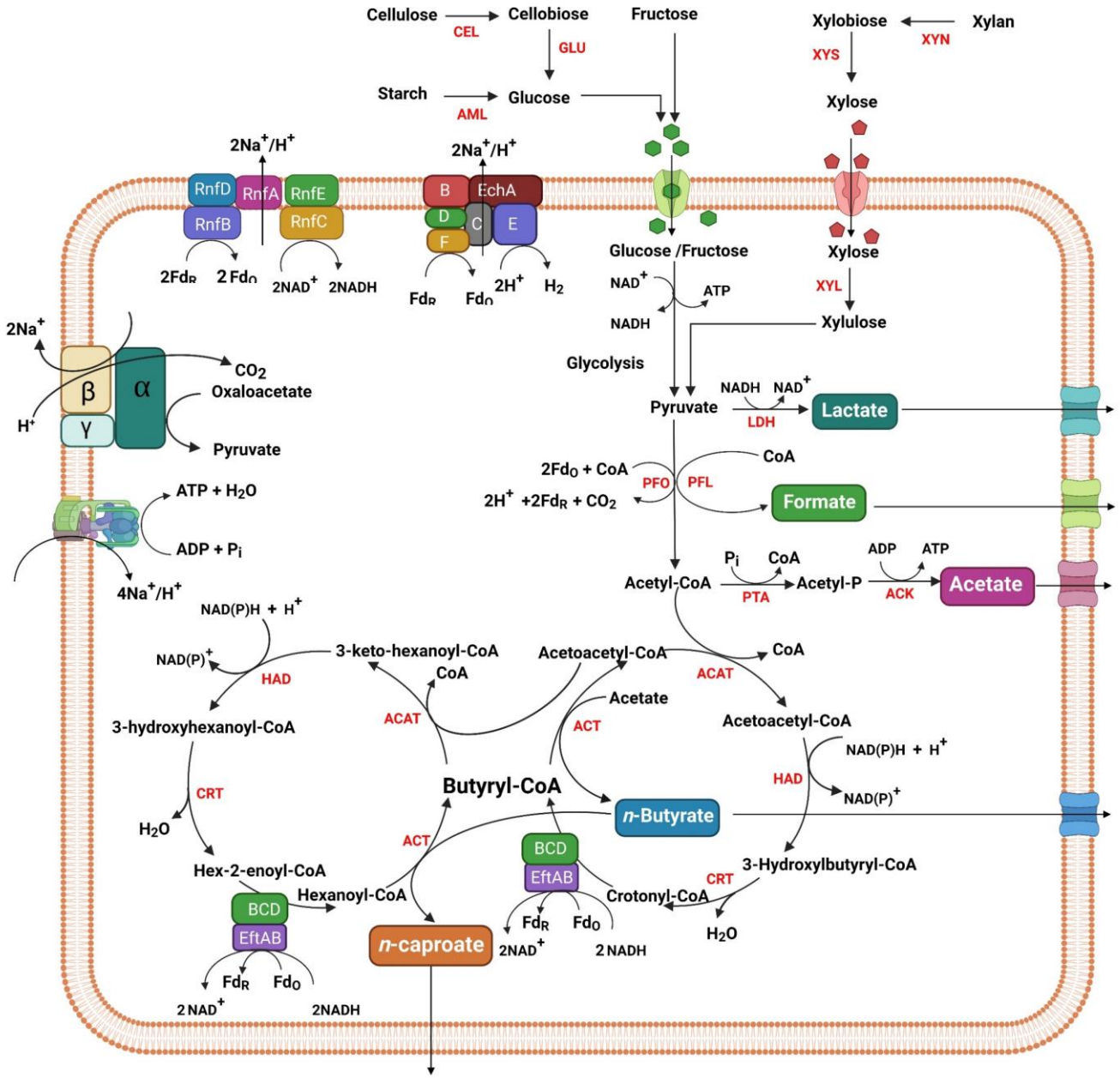
Strain MDTJ8^{T} is a chain elongating bacterium



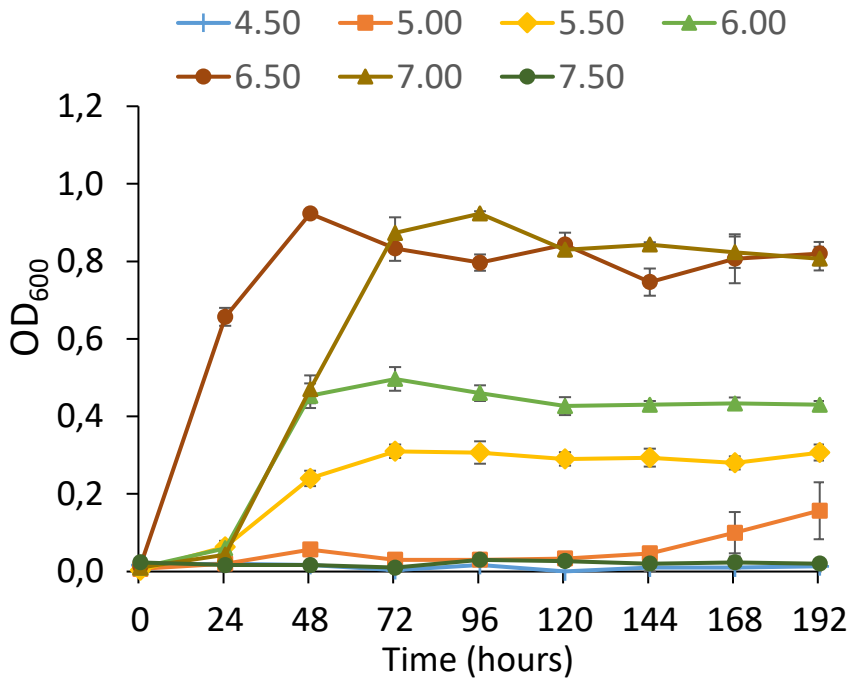
Family *Oscillospiraceae*

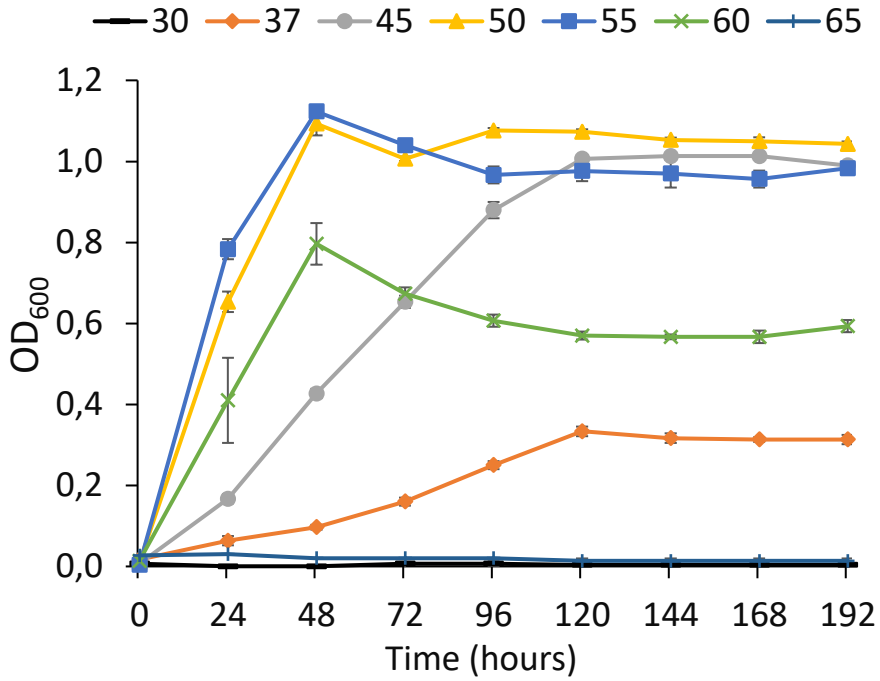


Predicted metabolic pathways in strain MDTJ8^{T}



Optimal culture conditions: pH 6.5 and 50-55°C



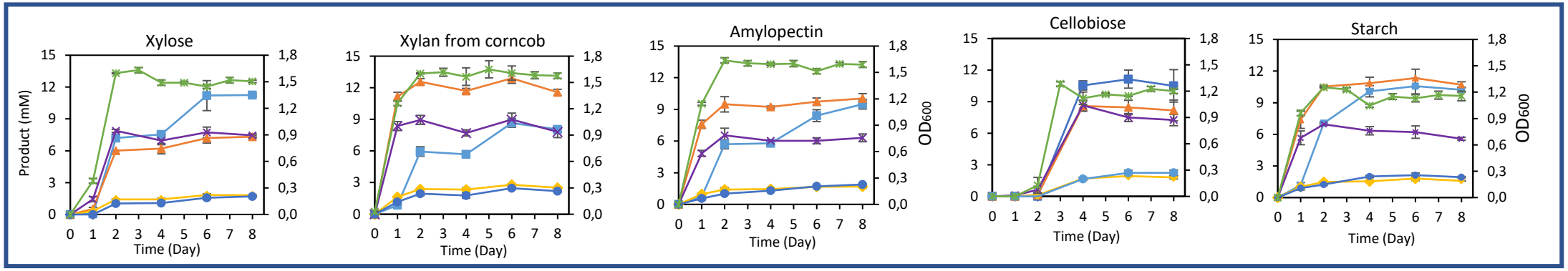


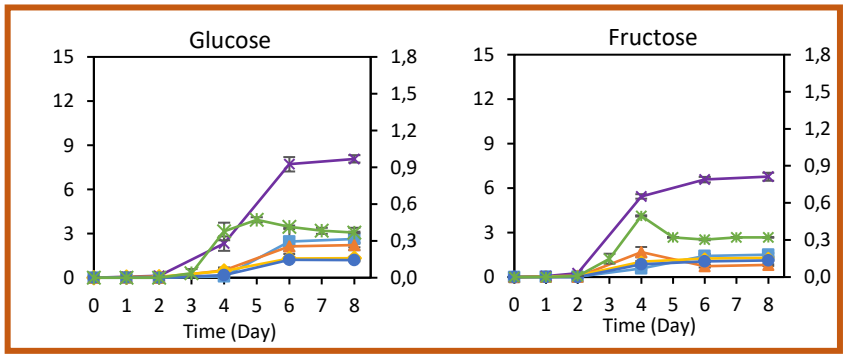
Strain MDTJ8 is a thermophilic chain elongator within *Oscillospiraceae*

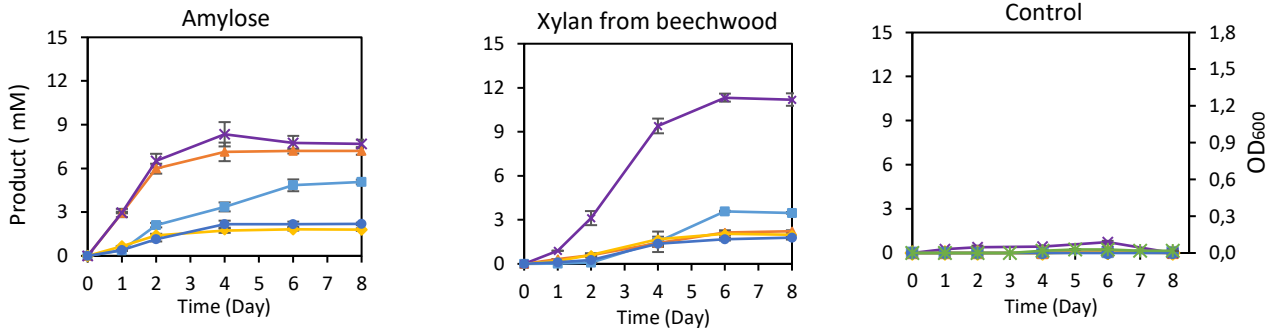
Strain MDTJ8T produces n-caproate from mono-, di-, and polysaccharides at thermophilic conditions

- Medium:
  - Carbohydrate (10g/L)
  - Mineral solution
  - Vitamin solution
- pH 6.5, 55°C

High growth



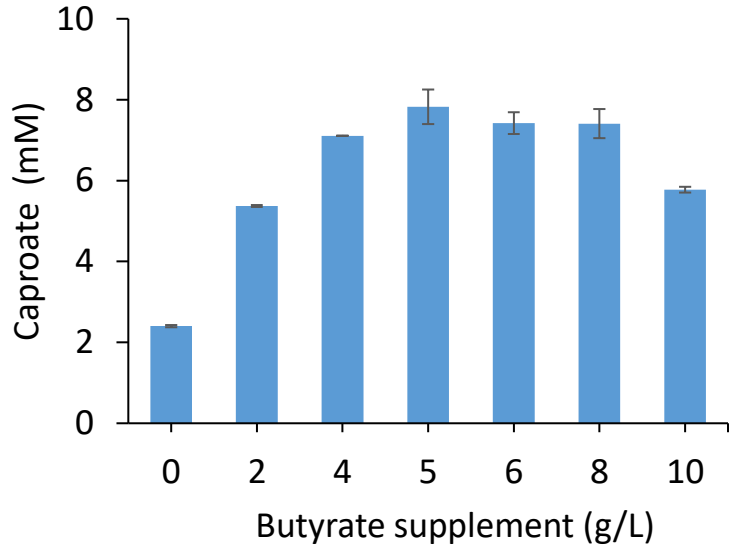




Less growth

Next steps: optimizing operational parameters for higher caproate production

Strain	Carbon Substrate	Substrate consumed (mg/L)	n-caproate produced (mg/L)	Yield mg/L caproate produced per 100 mg/L carbon substrate
MDTJ8^T	Glucose	2763	141	5.1
MDTJ8^T	Fructose	2094	127	6.1
MDTJ8^T	Xylose	5665	195	3.8
LBM18003^T	Glucose	5202	92	1.8
EA1^T	Fructose	3423	345	10.1
CPB6	Lactate	24850	8070	32.5



- Butyrate supplement increases caproate production

- Electron acceptors
- pH
- Carbon source and concentrations

Conclusions and perspectives

- Strain MDTJ8^T is **the first axenic thermophilic chain elongating bacterium**
- MDTJ8^T is a promising candidate for **producing *n*-caproate from low-cost hemicellulose or carbohydrate-rich waste** at thermophilic conditions.
- MDTJ8^T can be a model organism to further understand and explore the metabolic potential of **thermophilic MCCA production** both in single and mixed fermentation.

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Isolation and characterization of a thermophilic chain elongating bacterium that produces the high commodity chemical *n*-caproate from polymeric carbohydrates

Tinh Van Nguyen [a,b], Tomeu Viver [c], Jonah Mortier [a], Bin Liu [d], Ilse Smets [e], Kristel Bernaerts [e], Karoline Faust [d], Rob Lavigne [f], Laurent Poughon [b], Claude-Gilles Dussap [b], Dirk Springael [a,*]

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THANK YOU.

Tinh Van Nguyen

Division of Soil and Water Management, KU Leuven

tintin.nguyenvan@kuleuven.be

www.melissafoundation.org

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UNIVERSITÉ Clermont Auvergne

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