BioRECO2VER

New microbial platforms for CO_2 conversion

GIULIANA D'IPPOLITO, Online webinar, 23 November 2021

Horizon 2020
European Union Funding
for Research & Innovation

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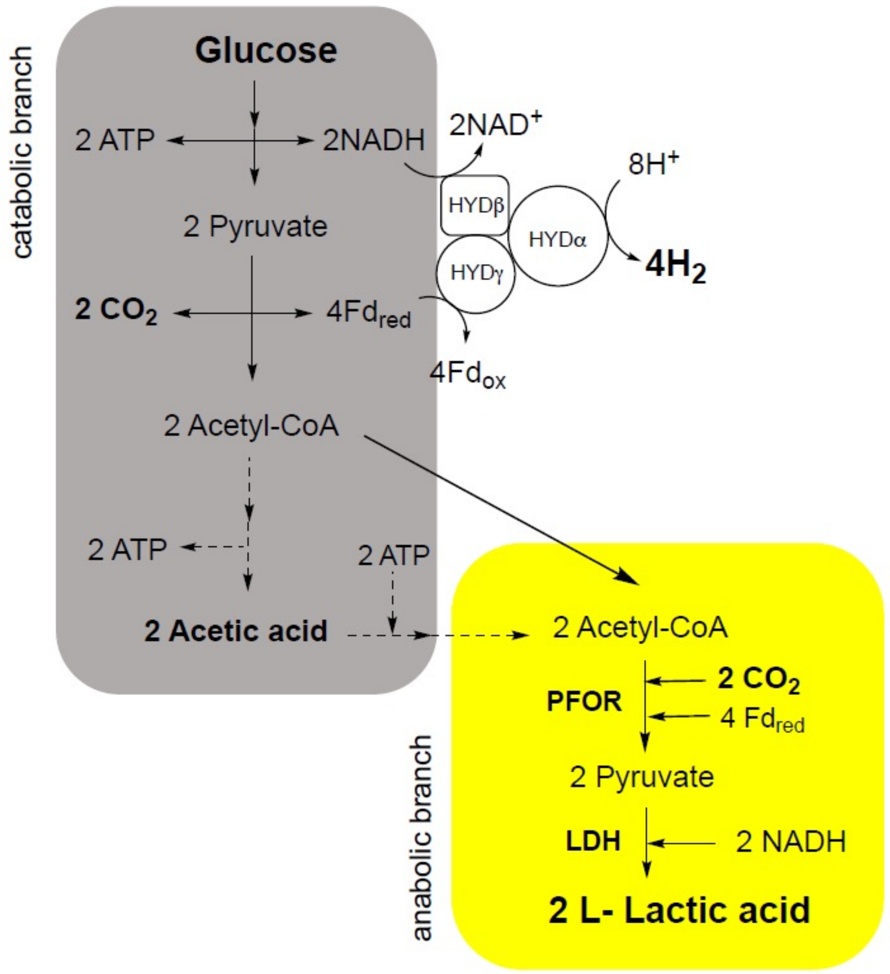
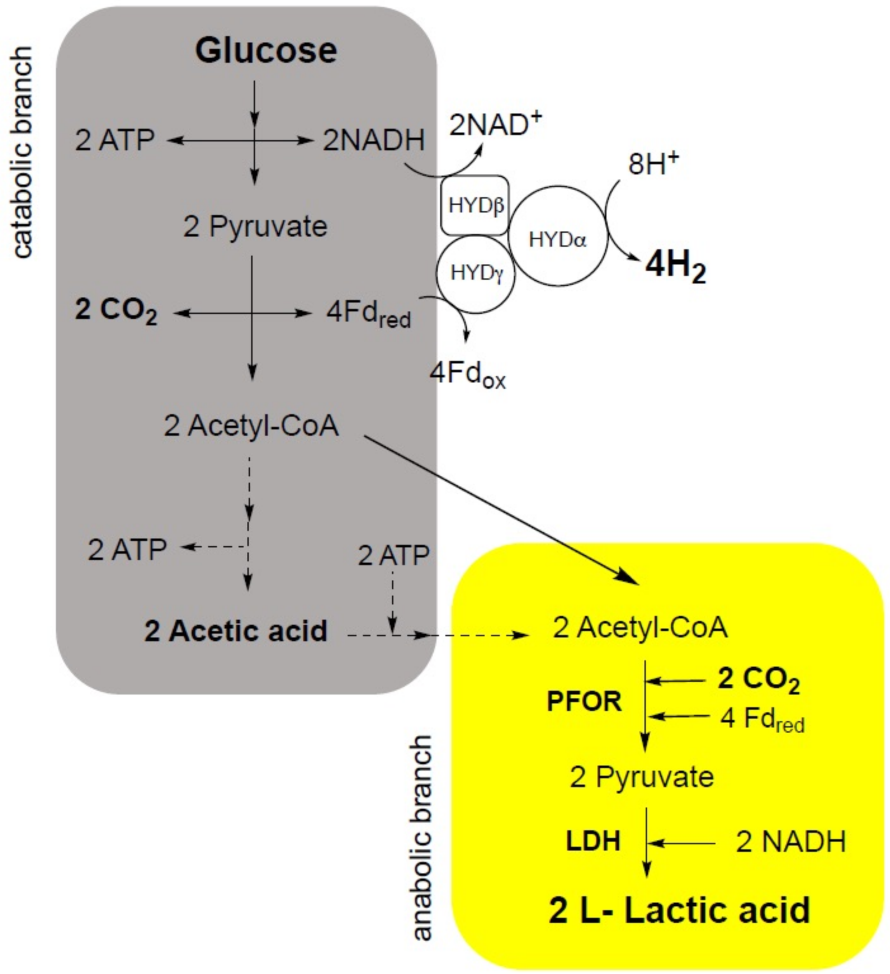
Thermotoga platform
Capnophilic (CO_2 requiring) Lactic Fermentation (CLF)



Thermotoga neapolitana: Strictly anaerobic, hyperthermophilic (80°C) gram-negative bacterium that can ferment sugar to H_2 and L-lactic acid

- Newly discovered pathway (*ChemSusChem*, **2014**, 7, 2678-2683, EU patent EP2948556B1)
- Dissection of anabolic branch of CLF
- Increase of the metabolic flow from CO_2 and acetate to lactic acid
- Proof of concept net CO_2 fixation in lactic acid
- Explore the feeding of exogenous acetate as C2 unit

Thermotoga platform
Capnophilic Lactic Fermentation (CLF) pathway



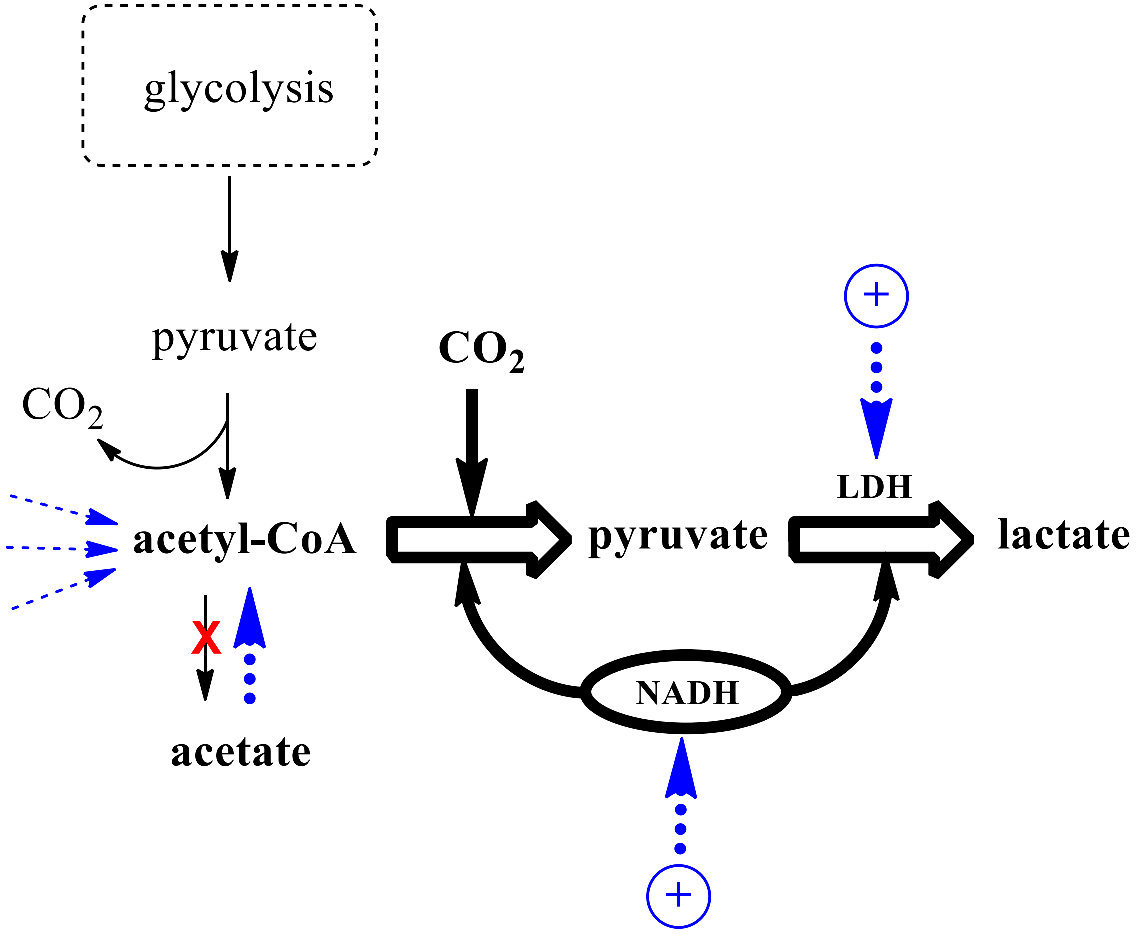
- Metabolic engineering of CLF in T. neapolitana
  Objective: Optimization of CLF
- Off gas impurity tolerance in *T. neapolitana*
  Objective: Test/adapt strains to real off-gas
- Bioprocess optimization of CLF in *T. neapolitana*
  Objective: Technological optimization of operational parameters

Thermotoga platform
Capnophilic Lactic Fermentation (CLF) pathway

- ✓ Selection of a model strains with increased CLF productivity: ***T. neapolitana* subsp. *capnolactica*** (DSM33003)
- ✓ Quantification of acetate up-take and CO_2 absorption in 95% e.e. L-lactic acid
- ✓ Identification of key enzymes in CLF pathway induced by CO_2
- ✓ Assessment of additional source of reductants to sustain CLF
- ✓ Development of genetic tools & transformation method
- ✓ Transformable strains: DSM33003 and RQ7

*Grand Challenges in Marine Biotechnology, Springer Nature **2018**, Ch. 6, pp. 217-235; FERMENTATION **2019**, 5 (2), 34;* FRONTIERS IN MICROBIOLOGY, **2020**, Volume: 11, 171

Thermotoga platform
Capnophilic Lactic Fermentation (CLF) pathway

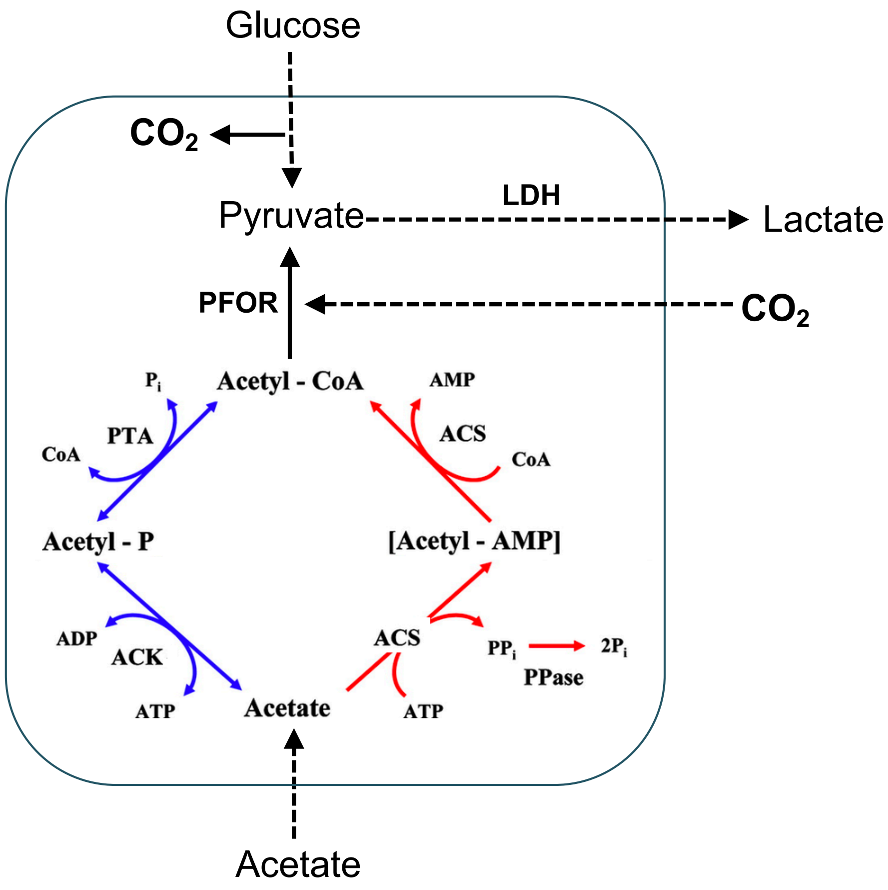


Thermotoga platform

Metabolic engineering of CLF in *T. neapolitana*

✓ **Molecular targets to improve acetate and CO_2 coupling to lactate**

- overexpression of Acetate kinase (acK)
- overexpression of Pantothenate kinase (panK)
- heterologous expression of AcetylCoA synthetase (acS) of *Thermus thermophilus*



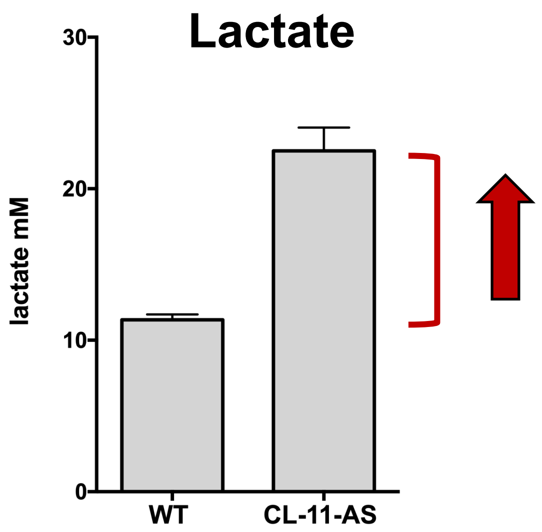
Acetate Dissimilation: the PTA-ACK Pathway
Acetate Assimilation: ACS pathway

Thermotoga platform

Metabolic engineering of CLF in *T. neapolitana*

✓ **Generation of 12 recombinant strains in DSM33003 and RQ7**

- comparable growth curves
- same glucose consumption rate
- decreased H_2 production



➡ Lactate production from CO_2 showed an increase up to 30% in comparison to wt strain

➡ Recombinant strain with ACS showed an increase of lactic acid production of 100%

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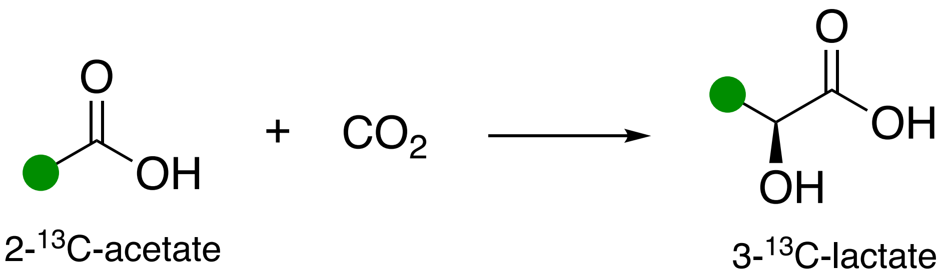
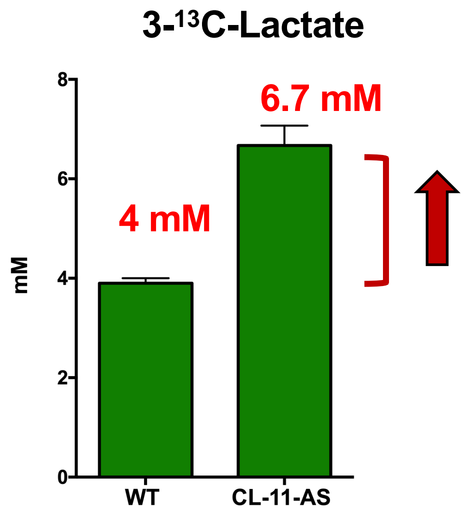
Thermotoga platform

Metabolic engineering of CLF in *T. neapolitana*

✓ Assessment of CO_2 -derived lactate by ^{1}H-NMR





Increase of CO_2-derived lactate of 70%

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Thermotoga platform

Metabolic engineering of CLF in *T. neapolitana*

ACETATE	+	CO_2	→	L-LACTATE	WILD TYPE
228 mg L^{-1}		167 mg L^{-1}		340 mg L^{-1}	
ACETATE	+	CO_2	→	L-LACTATE	ACS recombinant strain
403 mg L^{-1}		295 mg L^{-1}		750 mg L^{-1}	

Thermotoga platform

Bioprocess optimization of CLF in *T. neapolitana*

Serum Bottle 120 ml
Oven, static condition

Serum Bottle 250 ml
Shaker

3L CSTR Fermenter

- Set-up of mini bioreactor systems (100-250 ml) to explore in parallel different culture conditions
- Design of experimental programme and model relationships between different input and process response.

Thermotoga platform

Bioprocess optimization of CLF in *T. neapolitana*

Objective: Technological optimization of operational parameters

Process improvements concern the following culture parameters:

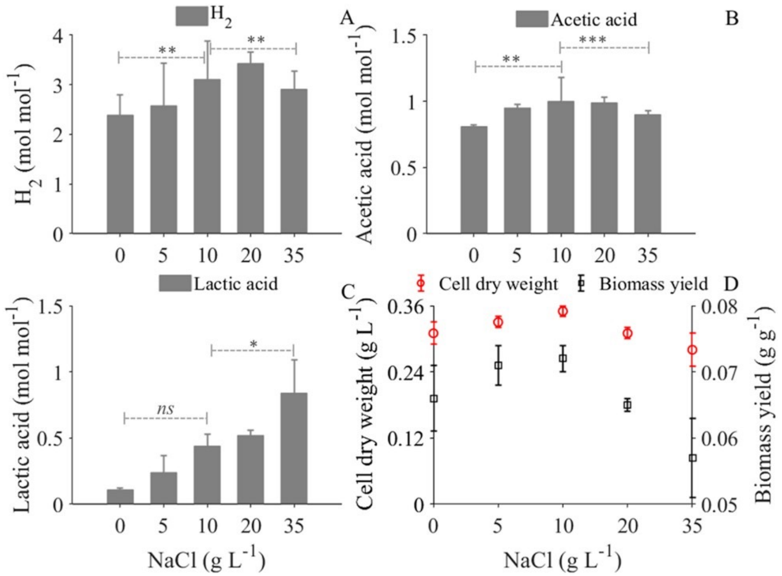
- Substrate input
- CO_2 supply
- Nutrient supply
- Acetate source

***Thermotoga* platform**

Bioprocess optimization of CLF in *T. neapolitana*

✓Assessment of **salinity level**, **buffering agent** and **carbon source** on H_2 and lactic acid synthesis under CLF conditions

- Increased concentrations of NaCl from 5 to 35 g/L showed proportional increase of LA productions from 0.27 g L^{-1} to 2 g L^{-1}
- Monosaccharides (e.g. xylose, arabinose), disaccharides (e.g. sucrose), polysaccharides (e.g. starch) can feed the process
- pH control is fundamental to increase lactate production
- Sulfur sources with different S redox state affect production of CO_2-derived lactate



MICRORGANISMS, 2021, 9(8), 1688
BIOMASS & BIOENERGY, 2019, 125, 17

Thermotoga platform

Bioprocess optimization of CLF in *T. neapolitana*

✓ Generation of adapted strains to minimal media

- New adapted strain able to growth on glucose as only organic source (CL-A5)
- New adapted strain able to growth on acetate, CO_2 , Na_2S and yeast extract (CL-A6)

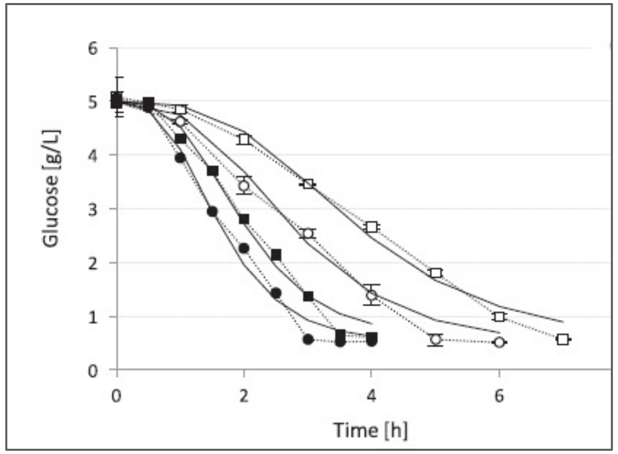
Current production:
100 mg L^{-1} of CO_2-derived sodium lactate
140 mL/L hydrogen

Thermotoga platform

Bioprocess optimization of CLF in *T. neapolitana*

✓Effect of high cell density approach

- ➢Increase of the initial biomass concentration from 0.46 to 1.74 g cell dry weight/L led to general acceleration of the fermentation process down to 3 h



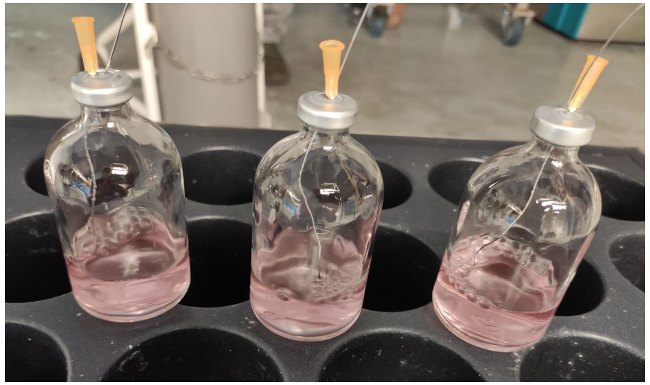
Feasible approach to increase lactate productivity of 100%

INTERNATIONAL JOURNAL OF HYDROGEN ENERGY, **2019**, 44, 36, 19698
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY, **2018**, 43, 29, 13072

Thermotoga platform

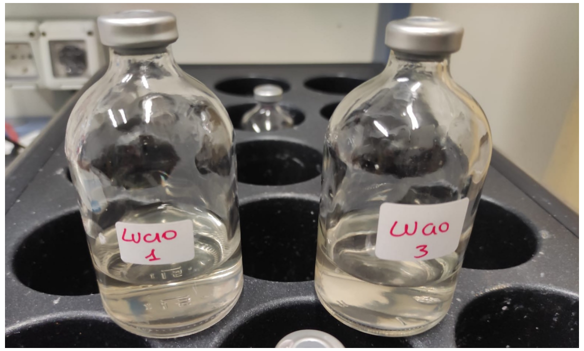
Off-gas impurity tolerance in *T. neapolitana*

- ✓ Insufflation of real off-gas



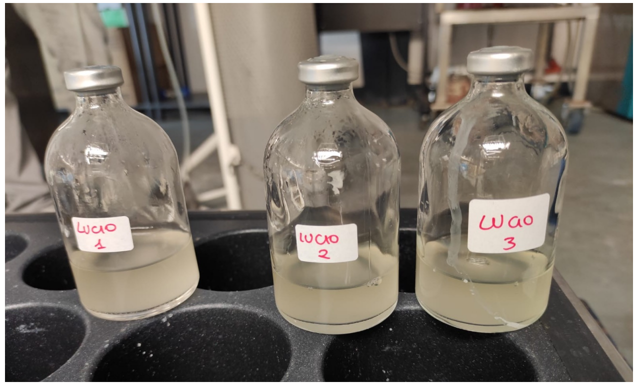
30’

80°



48 h

80°



→ *Thermotoga neapolitana* is tolerant to O_2 contained in real off-gas

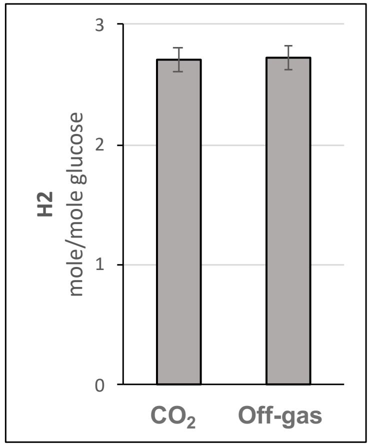
Thermotoga platform

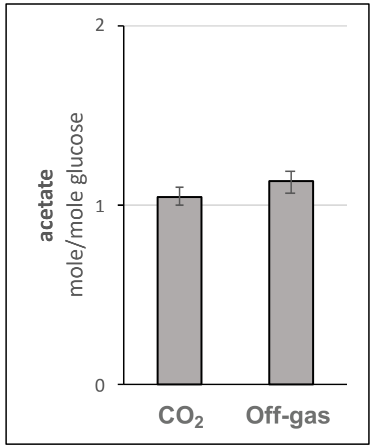
Off-gas impurity tolerance in *T. neapolitana*

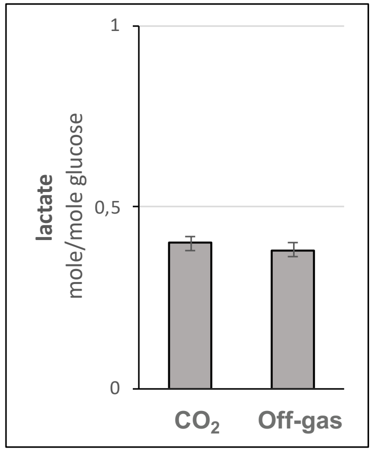
✓ Validation Test on 1L scale with real off-gas

Same performances in comparison to insufflation with pure CO_2

- Growth curves
- Glucose consumption rate
- H_2 production
- Acetate and Lactate production





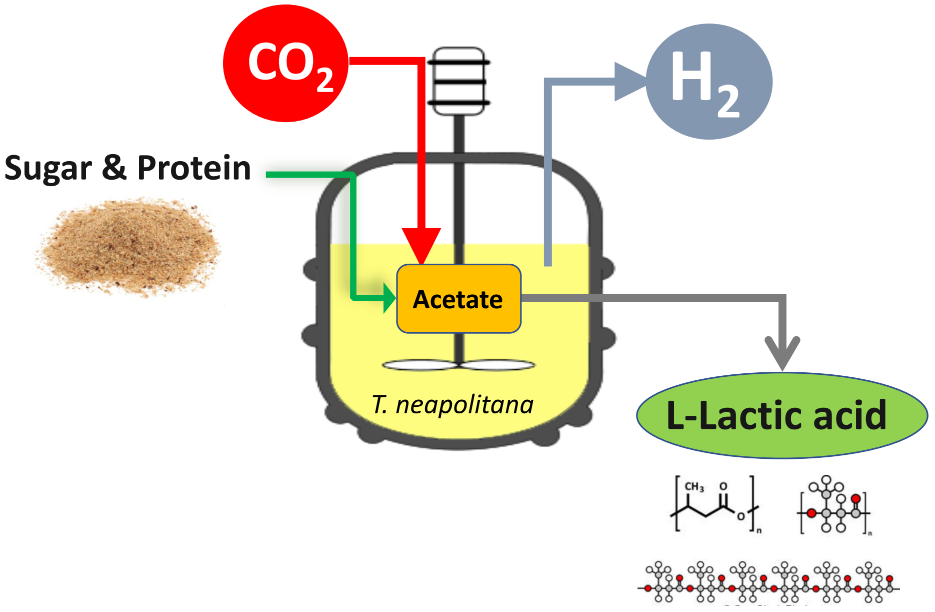


Thermotoga neapolitana is tolerant to off-gas impurities without pretreatments

Capnophilic Lactic Fermentation (CLF)

(European patent EP 2 948 556 B1)

- ✓Bio-based thermophilic process at TRL4
- ✓Recovery and valorization of CO_2
- ✓Production of hydrogen and L-lactic acid and from agro-food industry: monosaccharides (e.g. xylose, arabionose), disaccharydes (e.g. sucrose), polysaccharides (e.g. starch)
- ✓Scale-up until 50L fermenter
- ✓No technological bottleneck
- ✓Industrial partnership in R&D



Poly-lactic acid (PLA) polymers

Esercizio et al., Microrganisms **2021**, 9(8), 1688; Pradhan et al., Bioresource Technology **2021**, 332, 125127; Esercizio et al., **2021**, International Journal of Molecular Sciences, 9,8, 1688; d'Ippolito et al., **2021**, Bioresource Technology, 319; Esercizio et al., **2021**, Resources, 10, 4; Lanzilli et al., **2021**, International Journal of Molecular Sciences, 22, 1:341; d'Ippolito et al., **2020** Frontiers in Microbiology, 11, Article Number: 17; Nuzzo et al., **2019** Fermentation, Volume: 5 Issue: 2, Article Number: 34. Dreschke et al., **2019** International Journal of Hydrogen Energy 44(36), 19698; Pradhan et al., **2019** Biomass and Bioenergy 17; Dreschke et al., **2018** International Journal of Hydrogen Energy 43(29), pp. 13072-13080; Dipasquale et al., **2018** Grand Challenges in Marine Biotechnology 217-235; Pradhan et al., **2017** International Journal of Hydrogen Energy 42(25), pp. 16023-16030; Pradhan et al., **2017** Energies,10, 665; Pradhan et al., **2016** Water Research 99, 225; Pradhan et al., **2016** International Journal of Hydrogen Energy 41(9), 4931; Dipasquale et al., **2014** International Journal of Hydrogen Energy 39(10), pp. 4857-4862; d'Ippolito et al., **2014** ChemSusChem 7(9), pp. 2678-2683; Dipasquale et al., **2012** International Journal of Hydrogen Energy 37(17), pp. 12250-12257; d'Ippolito et al., **2010** International Journal of Hydrogen Energy 35(6), pp. 2290.

Thank you for your attention!

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