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Syngas fermentation by biotechnologically interesting clostridia

09/09/2016 Sabrina Hoffmeister

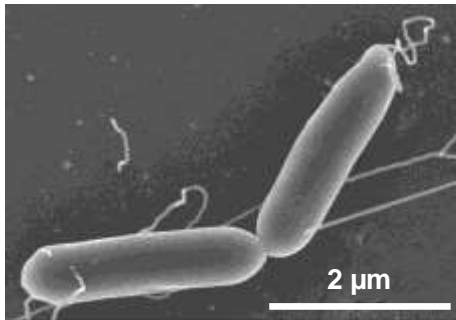
Institute of Microbiology and Biotechnology

Agenda

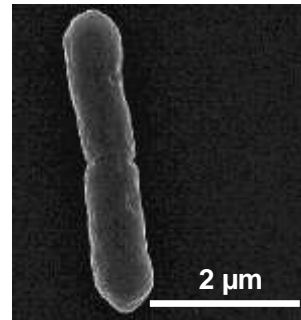
- Genus *Clostridium*
 - Historical background
- How can clostridia utilize syngas?
- How can we take advantage of syngas-utilizing clostridia?
 - Industrial applications

Genus *Clostridium*

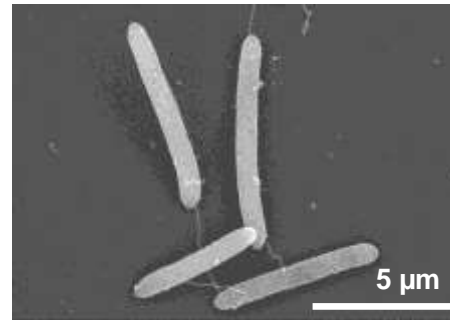
- mostly Gram-positive
- rod-shaped
- anaerobic to aerotolerant
- spore-forming
- **several clostridia can grow autotrophically** (CO, H₂+CO₂ energy and carbon source)
 - reductive acetyl-CoA pathway



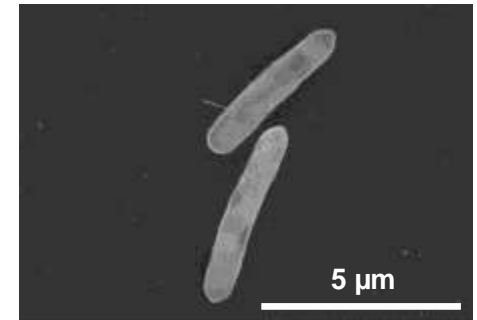
Clostridium ljungdahlii



Clostridium autoethanogenum



Clostridium aceticum



Clostridium formicoaceticum

Isolation of 1st autotrophic acetogenic bacterium - *Clostridium aceticum*



Klaas T. Wieringa



Original spore isolation from *C. aceticum*

1936



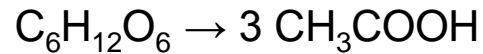
Isolation of 1st autotrophic acetogenic bacterium - ***C. aceticum***

Isolation of 2nd autotrophic acetogenic bacterium

Clostridium thermoaceticum

(re-classified: *Moorella thermoacetica* (Collins et al., 1994))

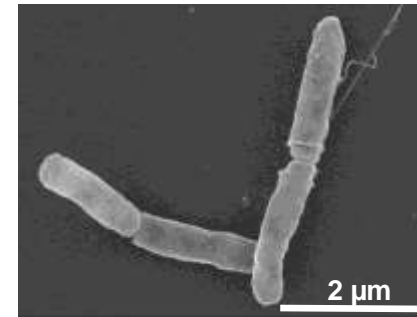
- isolated under heterotrophic conditions



- investigation of **reductive acetyl-CoA pathway** begins
- autotrophic growth (Daniell et al., 1990)



Francis E. Fontaine



M. thermoacetica

1936

1942

Isolation of 1st autotrophic acetogenic bacterium - ***C. acetium***

Isolation of 2nd autotrophic acetogenic bacterium - ***C. thermoaceticum***

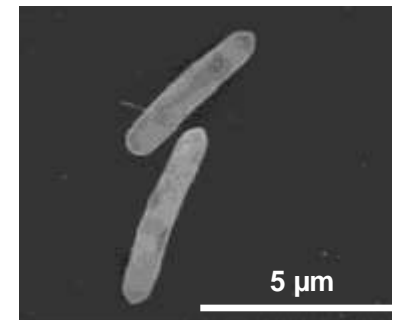
(re-classified: *Moorella thermoacetica* (Collins *et al.*, 1994))

Isolation of 3rd autotrophic acetogenic bacterium - ***Clostridium formicoaceticum***

- produced formate and acetate (glucose-dependent fermentation)
- CO₂ was incorporated into acetate formation
- autotrophic growth (Lux and Drake, 1992)



Jan R. Andreesen



C. formicoaceticum

1936 1942 1970

A large teal arrow pointing to the right, starting from the year 1936 and ending at 1970, indicating the time period covered by the events on the timeline.

Isolation of 1st autotrophic acetogenic bacterium - ***C. aceticum***

Isolation of 2nd autotrophic acetogenic bacterium - ***C. thermoaceticum***

(re-classified: *Moorella thermoacetica* (Collins *et al.*, 1994))

Isolation of 3rd autotrophic acetogenic bacterium - ***C. formicoaceticum***

Elucidation of enzymology of reductive acetyl-CoA pathway
(Wood-Ljungdahl pathway)



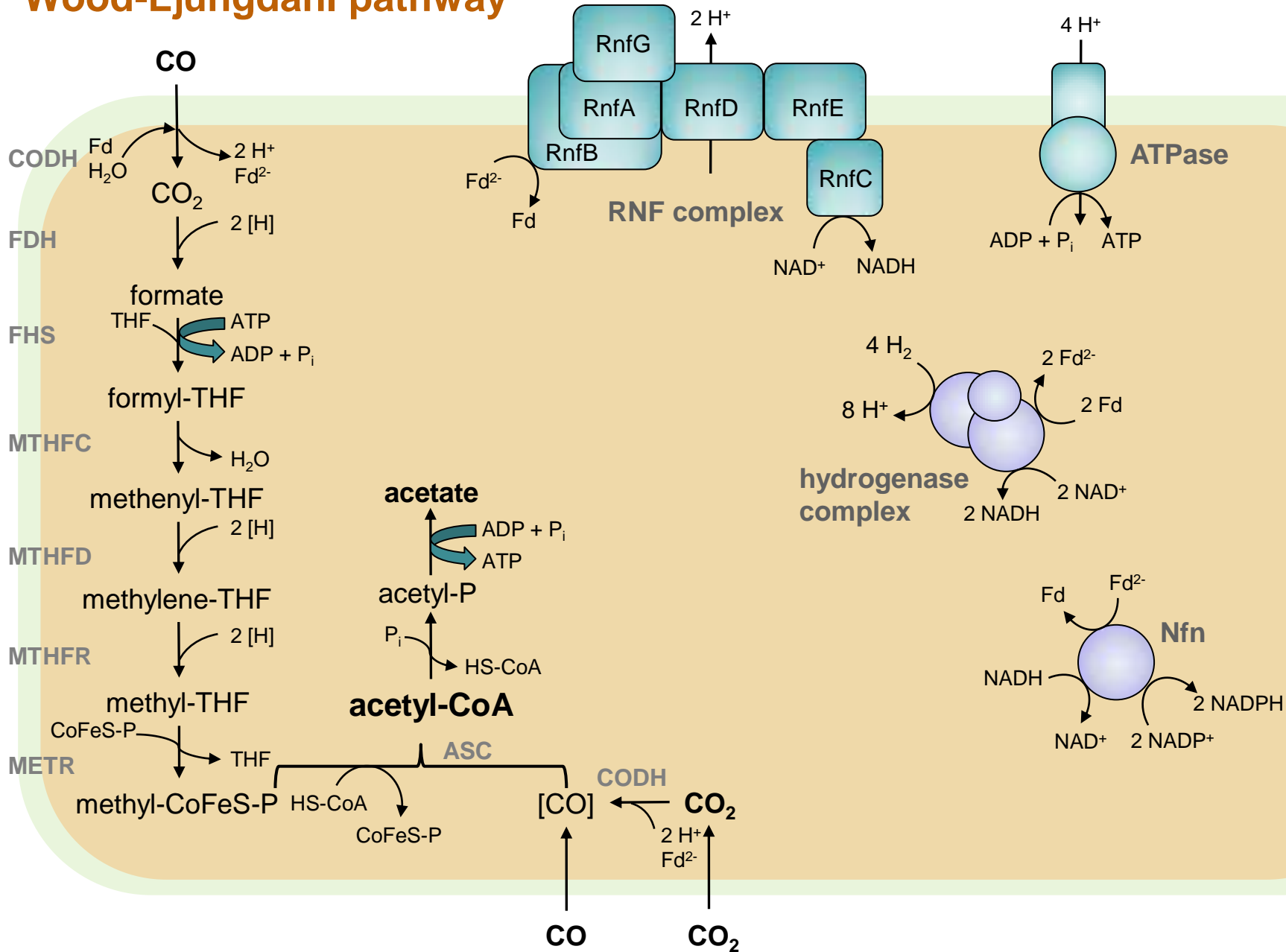
Lars G. Ljungdahl

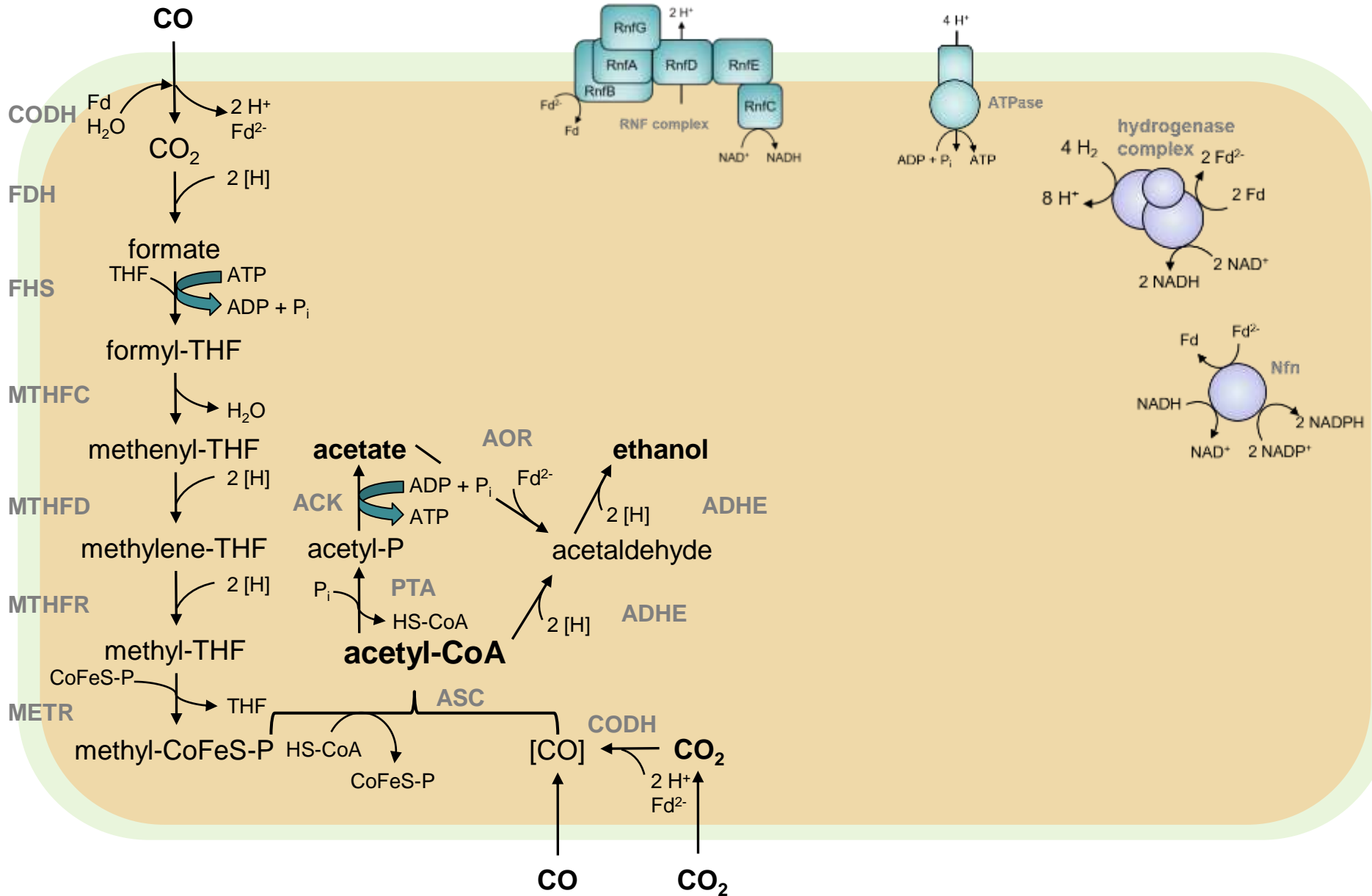


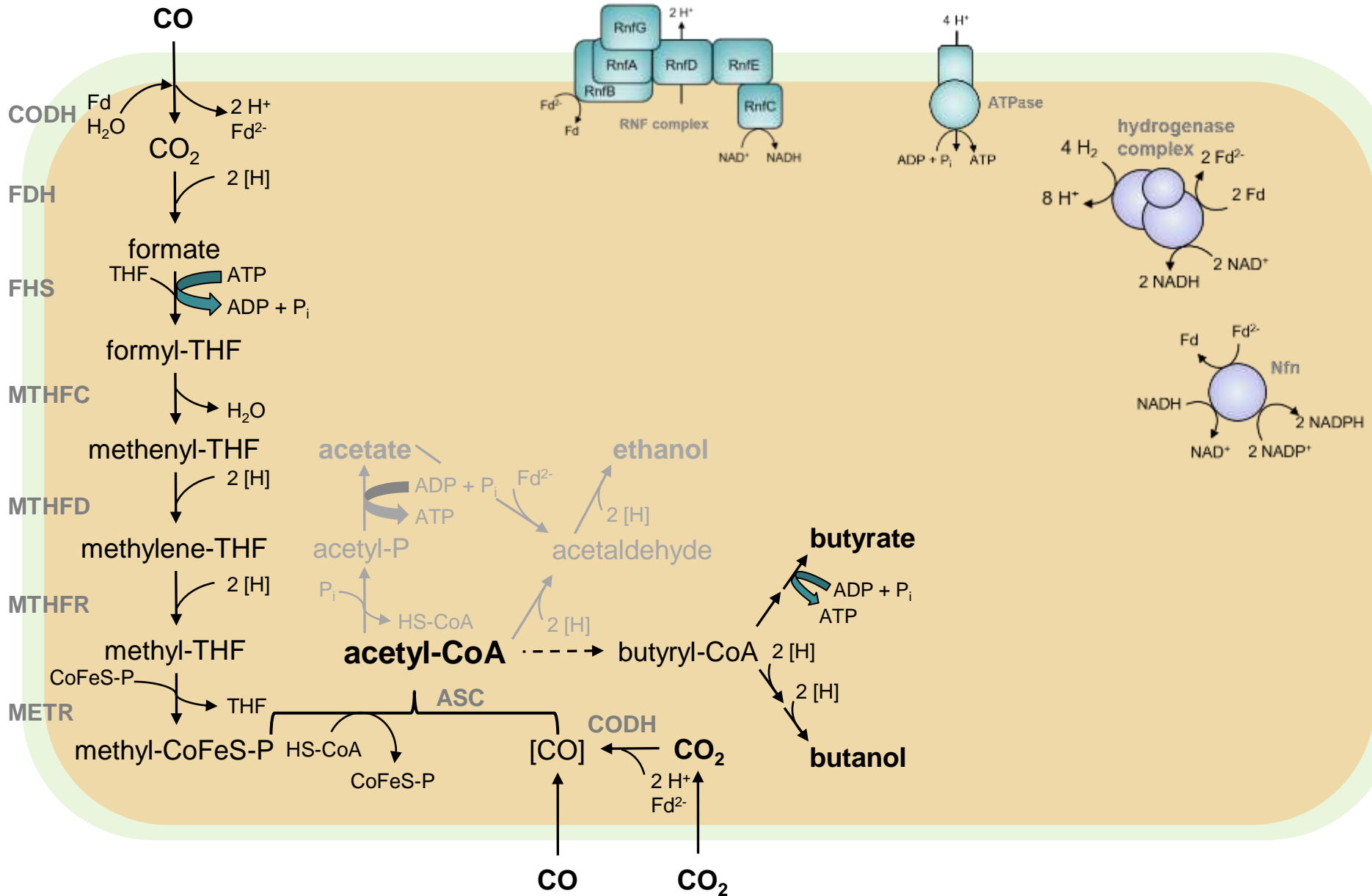
Harland G. Wood

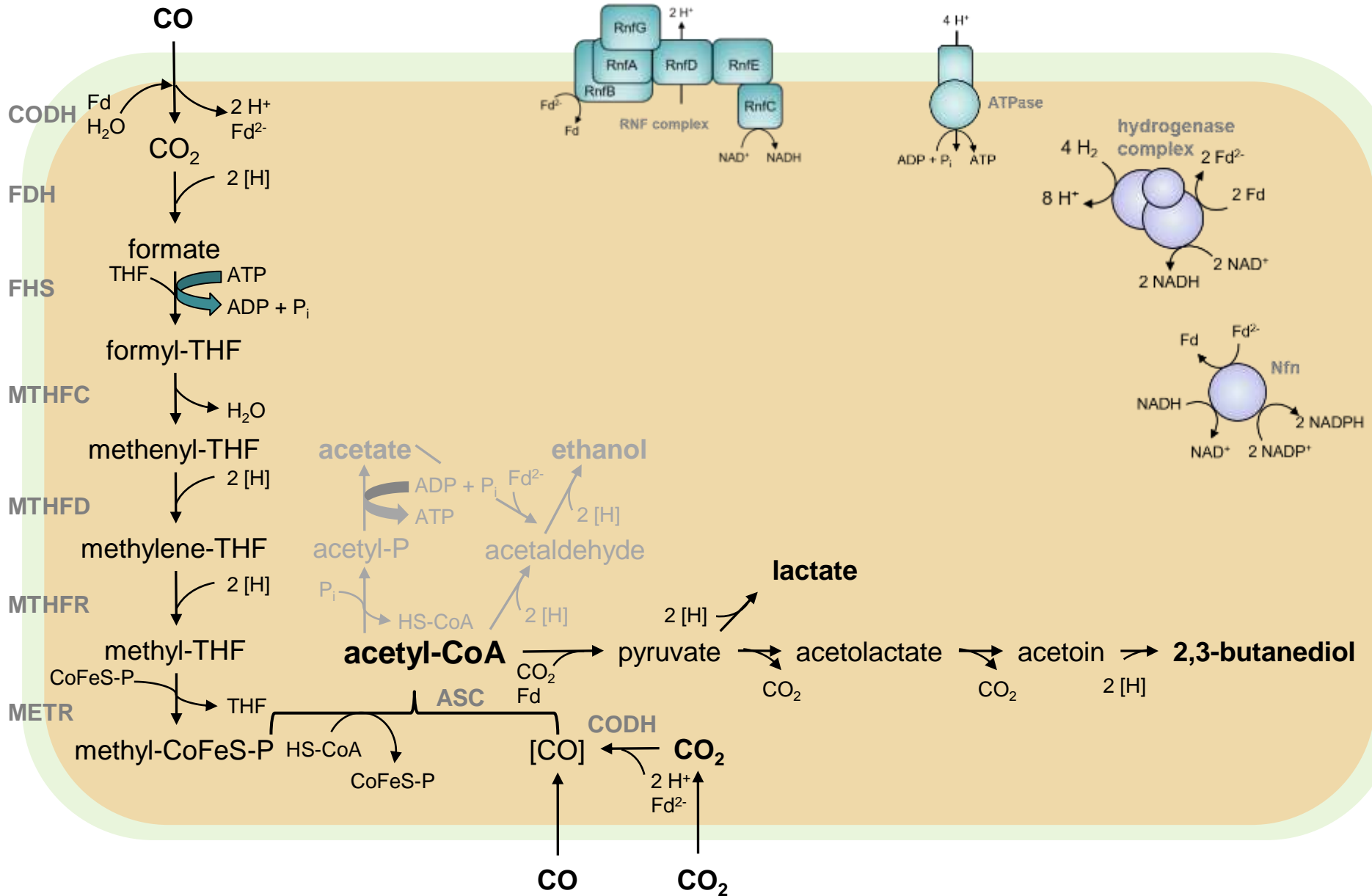
1936 1942 1970
1990

Wood-Ljungdahl pathway







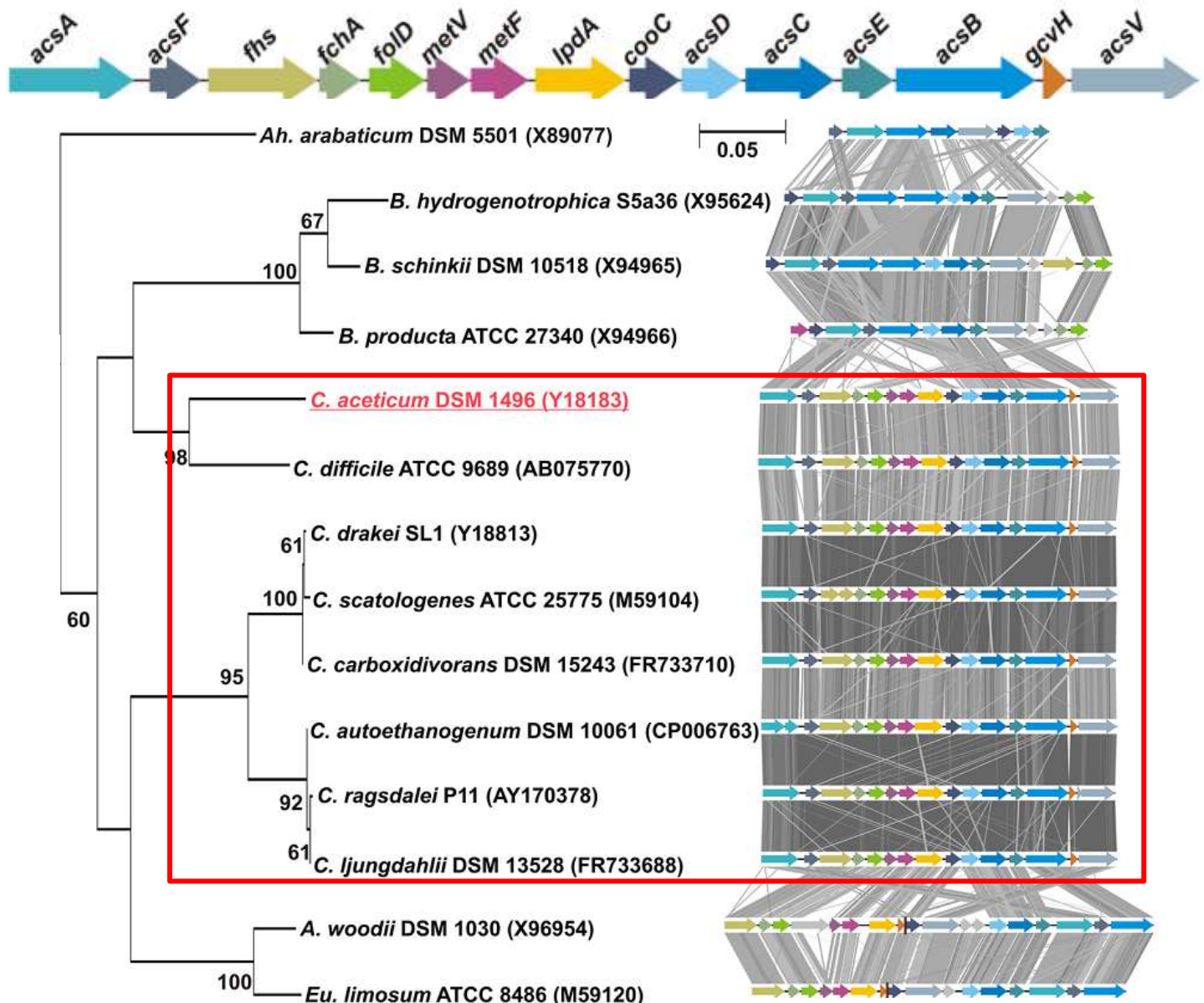


List of biotechnologically promising acetogenic clostridia

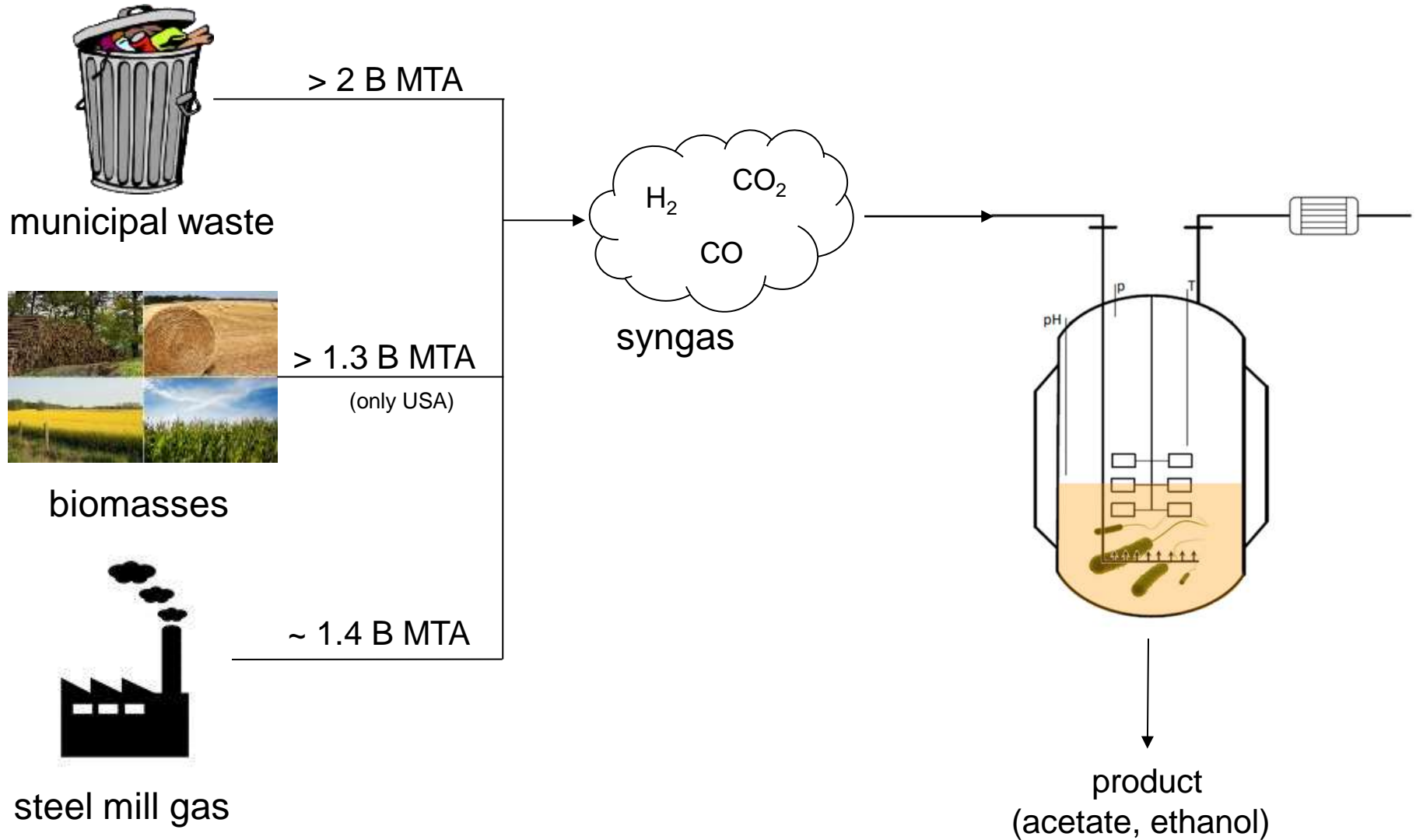
Organism	Substrate	Product	Genetically accessible	Genome sequence available	Reference
<i>C. ljungdahlii</i>	H ₂ +CO ₂ , CO	acetate, ethanol, 2,3-BD, lactate	yes*	yes	Vega <i>et al.</i> , 1989, Tanner <i>et al.</i> , 1993, Köpke <i>et al.</i> , 2010
<i>C. autoethanogenum</i>	H ₂ +CO ₂ , CO	acetate, ethanol, 2,3-BD, lactate	yes*	yes	Abrini <i>et al.</i> , 1994, Köpke <i>et al.</i> , 2011 Brown <i>et al.</i> , 2014 Liew <i>et al.</i> , 2016
<i>C. aceticum</i>	H ₂ +CO ₂ , CO	acetate		yes	Wieringa, 1940 Braun <i>et al.</i> , 1981 Poehlein <i>et al.</i> , 2015
<i>C. carboxidivorans</i>	H ₂ +CO ₂ , CO	acetate, ethanol, butyrate, butanol, lactate, (hexanol)	yes*	yes	Worden <i>et al.</i> , 1991, Liou <i>et al.</i> , 2005 Ukpong, 2014
<i>C. coskatii</i>	H ₂ +CO ₂ , CO	acetate, (ethanol)		yes	Zahn and Saxena, 2012
<i>C. ragsdalei</i>	H ₂ +CO ₂ , CO	acetate, ethanol, 2,3-BD, lactate		yes	Huhnke <i>et al.</i> , 2008

- transformation protocols published

genes of
Wood-
Ljungdahl
pathway



Industrial application



Market and usage of autotrophic fermentation products

- **ACETIC ACID**

- global acetic acid: 12,125 kilo metric tons in 2014
- vinyl acetate monomer (VAM) / polyvinyl acetate (PVA)
- solvent in production of terephthalic acid (TPA)



Market and usage of autotrophic fermentation products

- **ETHANOL**

- global production of 80 billion liters/a
- biofuel (gasoline substitute)



Industrial application

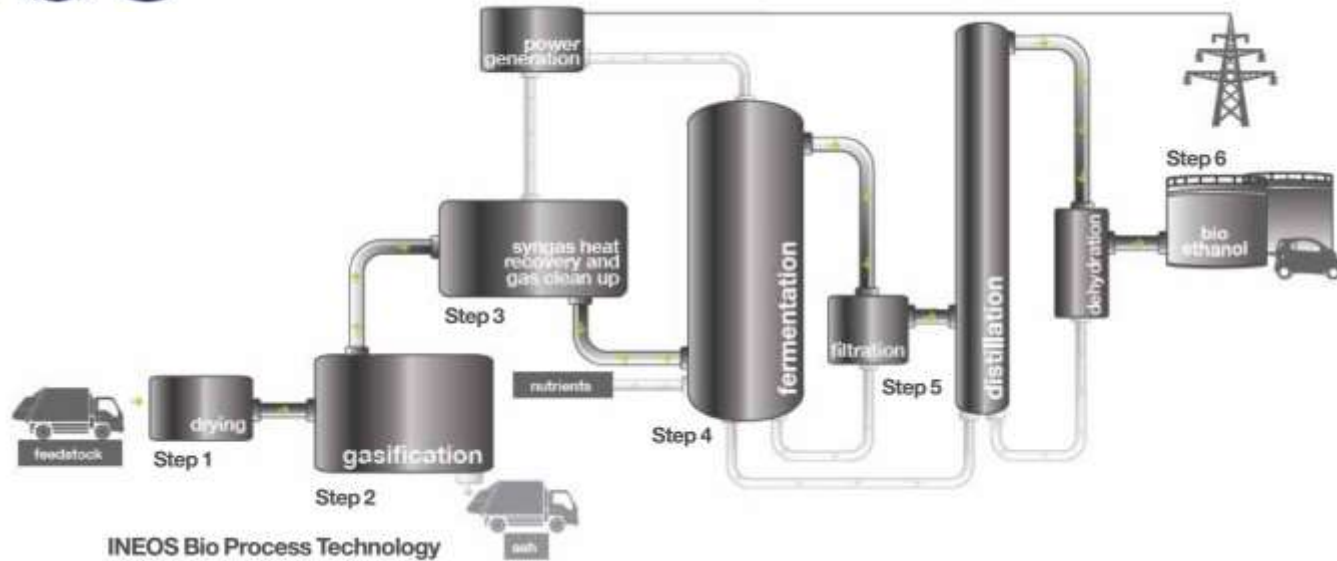


INEOS Bio



- founded 2008
- business unit of INEOS (multinational chemicals company, founded 1998)
 - ***C. ljungdahlii*** (proprietary strain)
 - production of ethanol

INEOS Bio



Step 1:
Feed reception and drying



Step 2:
Gasification



Step 3:
Gas clean up and syngas heat recovery (to generate renewable power)



Step 4:
Fermentation (bioethanol production from syngas through biological process)



Step 5:
Filtration, distillation and dehydration (bioethanol recovery and purification)



Step 6:
Renewable power generation

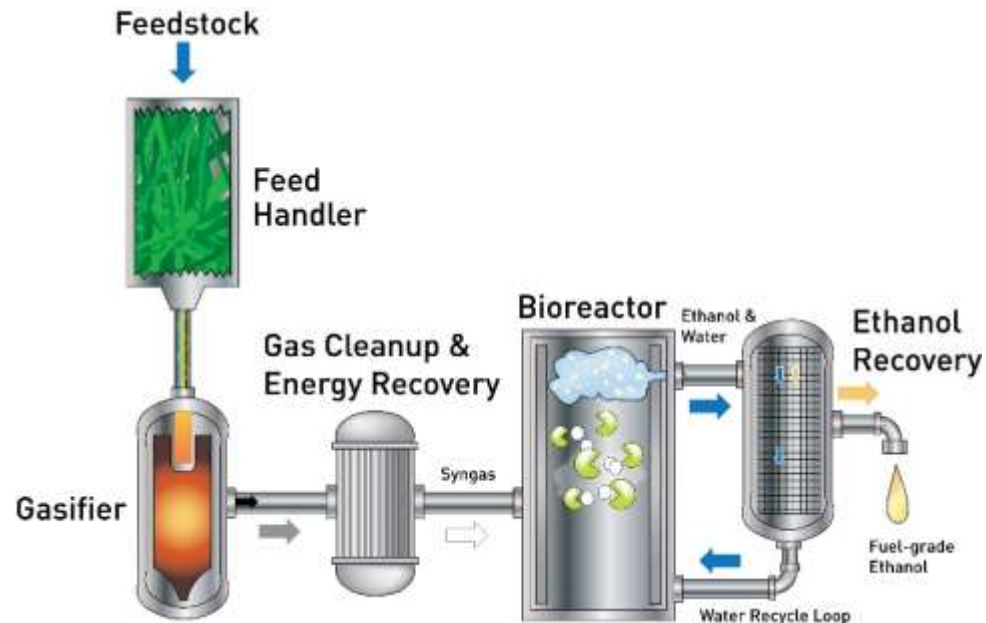


- **2016:** industrial scale plant not on-line
 - HCN: by-product of gasification
 - toxic for *C. ljundahlii*
 - installation of HCN scrubber system



- founded 2006
 - ***C. coskatii*** and ***C. ragsdalei*** (proprietary strains)
 - development of proprietary high temperature plasma gasification process

- gasification of wood and municipal wastes
- production of ethanol





Coskata's semi-commercial FlexEthanol™ facility

- **2009:** opened small-scale/semi-production facility in Madison, Pennsylvania
 - capable of producing 50,000 gallons/a ethanol
 - successfully running for 2 years
- **2016:** technology re-emerged as new company Synata Bio



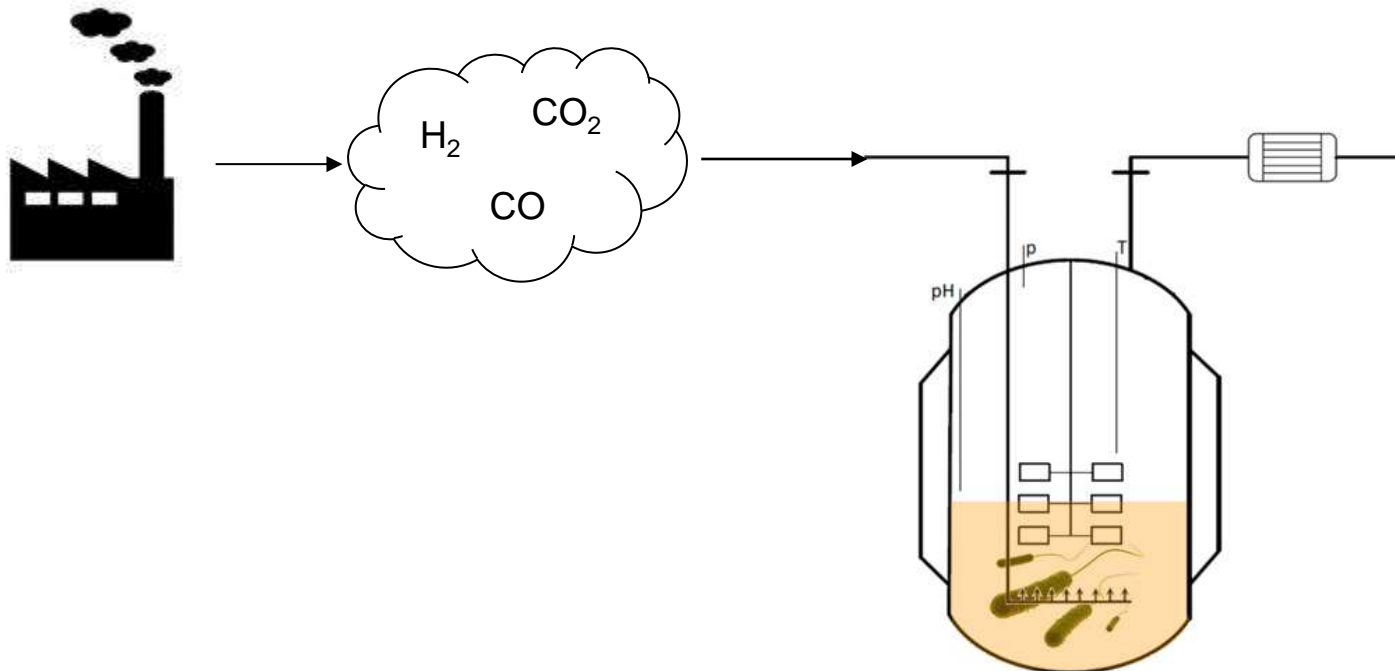
- founded 2005
- technology developed in New Zealand
 - *C. autoethanogenum* (proprietary strain)
 - prototype plant: 16,000 gallons/a ethanol
- expansion to USA and China (2008-2010)
- **2011**: pre-commercial plant 100,000 gallons/a ethanol (Shanghai)
- **2012**: second pre-commercial plant (100,000 gallons/a ethanol)
- **2014**: two commercial plants (China) - under construction



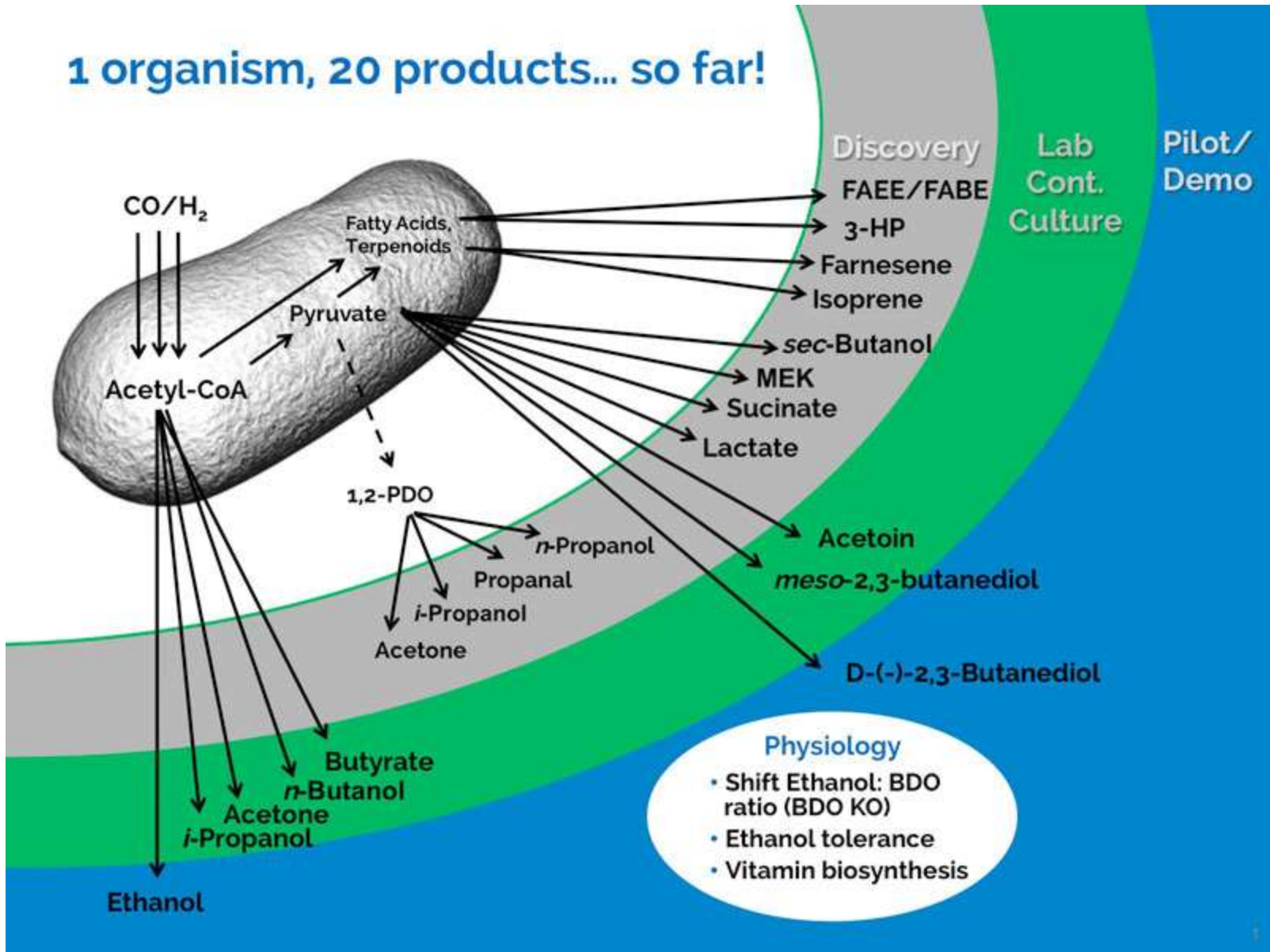
pre-commercial plant, Shanghai



- **2016:** construction of Europe's first-ever commercial scale production facility together with ArcelorMittal and Primetals Technologies (Ghent, Belgium).



1 organism, 20 products... so far!



Thank you for your attention