

Biobased Economy workshop

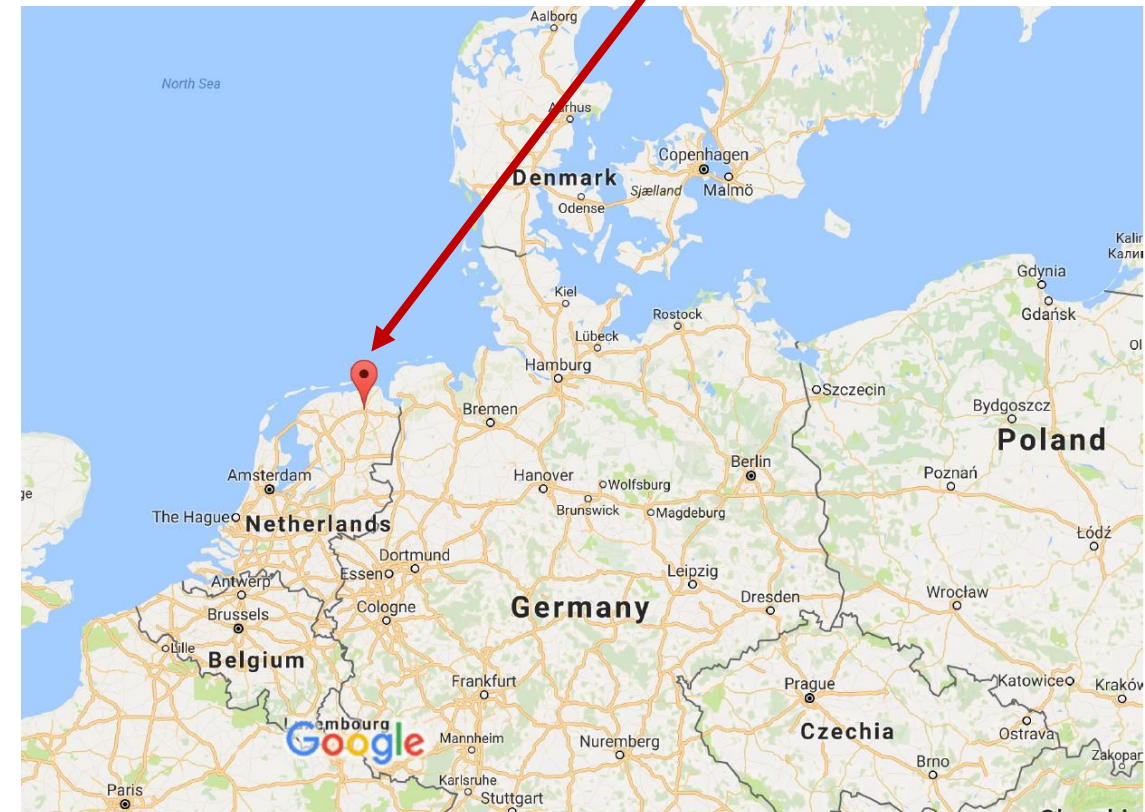
Brenda Bley Folly
Prof. Dr. Marco Fraaije



Biobased Economy Workshop
April, 5 and 6, 2017 - FAPESP Auditorium

Who are we?

- Molecular Enzymology department at the University of Groningen
- Brenda Bley Folly – Post-doc
- Head of the department:
Prof. Dr. Marco Fraaije

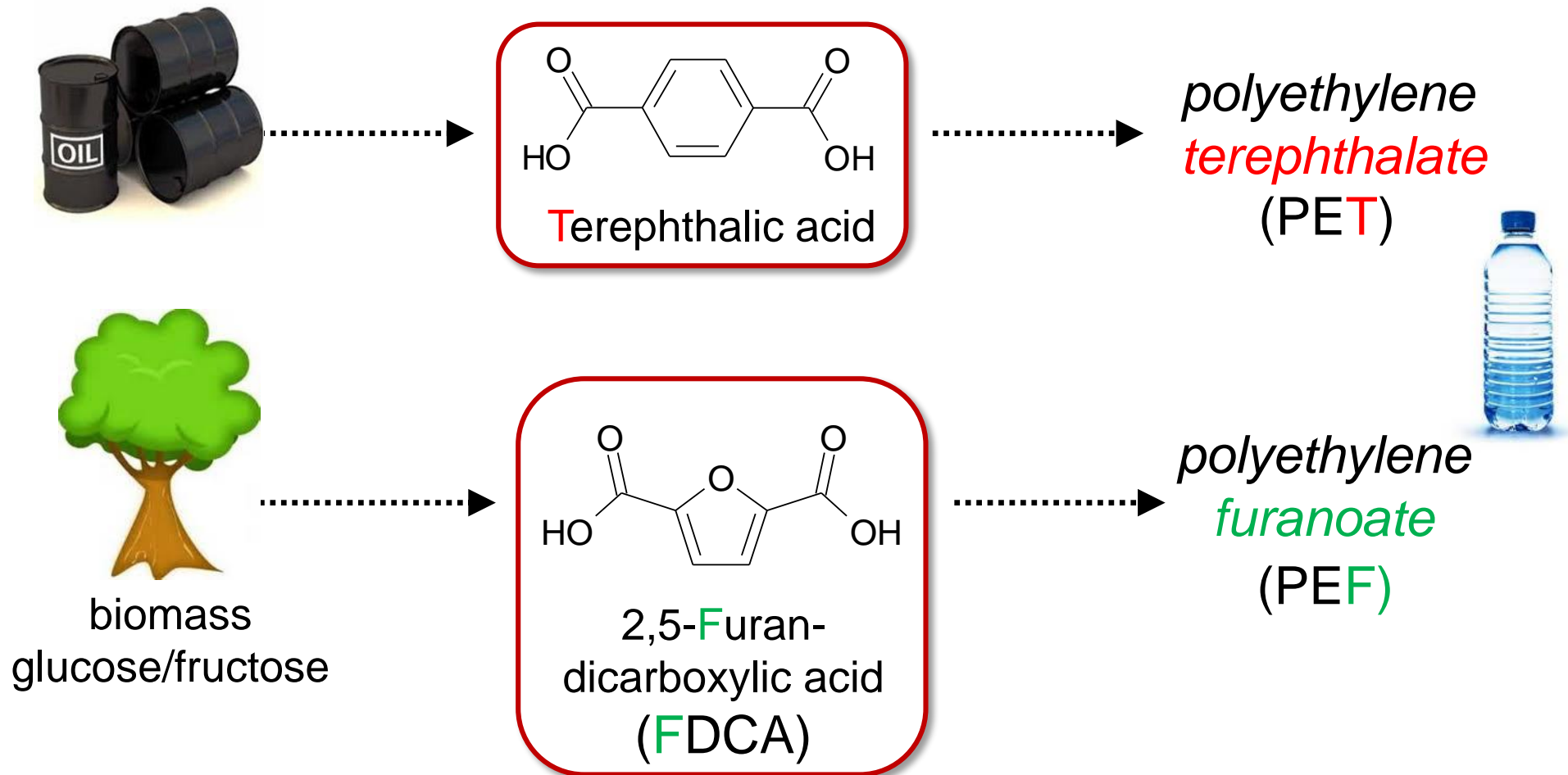


What are we interested in?

- Our research focus on the discovery, engineering and applications of redox enzymes.
- Our goal is to unravel the molecular basis by which cofactor-dependent redox enzymes perform catalysis.
- The generated knowledge is used for mechanism- and structure-inspired enzyme redesign approaches.

One example...

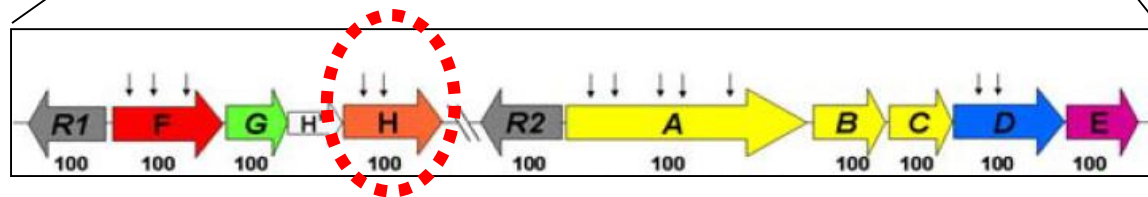
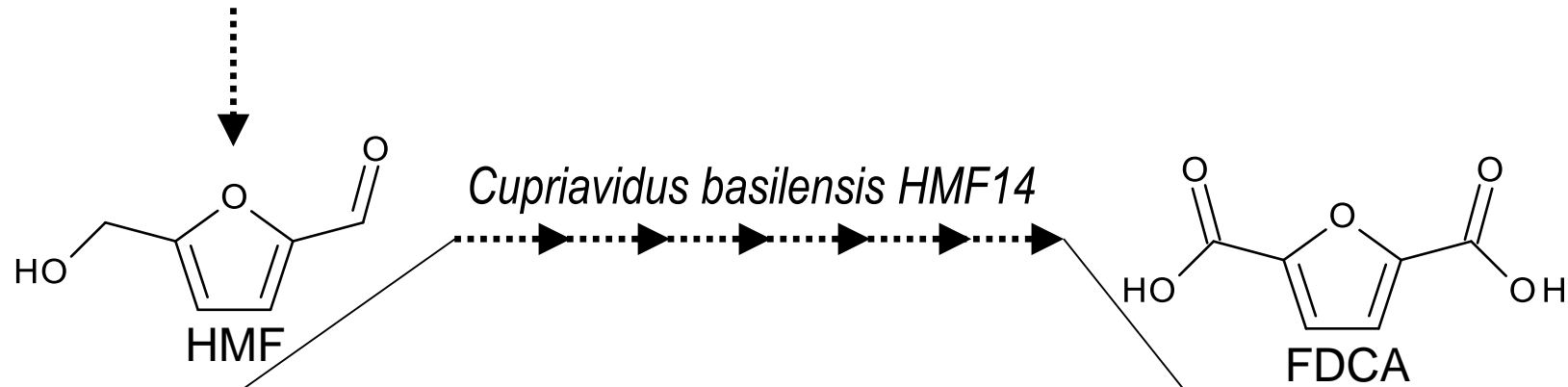
- Recently discovered oxidase: 5-hydroxymethyl-furfural oxidase (HMFO)



HMFO



biomass/fructose



predicted
flavoprotein oxidase

polymers

Getting the enzyme...

organism



- *Cupriavidus basilensis* HMF14
- Gram-negative, rod-shaped, obligate aerobic
- Isolated for its capacity to degrade HMF



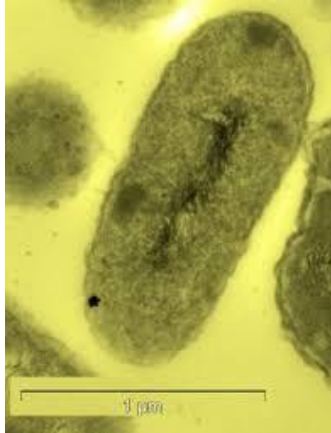
HmfH gene

- Original gene & *E. coli* & plasmids & conditions



Getting the enzyme...



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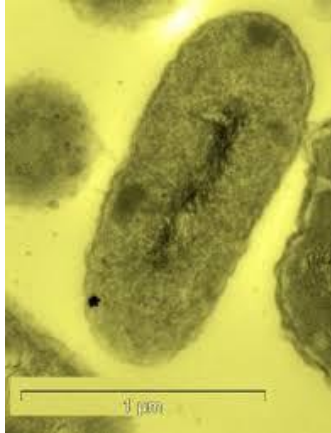
HmfH gene

- Original gene & *E. coli* & plasmids & conditions 
- Synthetic genes (!) & *E. coli* & plasmids & conditions 



Getting the enzyme...

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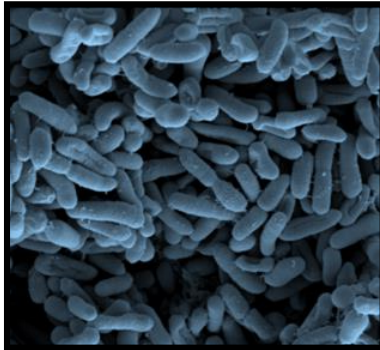
HmfH gene



- Original gene & *E. coli* & plasmids & conditions 
- Synthetic genes (!) & *E. coli* & plasmids & conditions 
- Expression in other hosts 

Getting and testing the enzyme...

organism

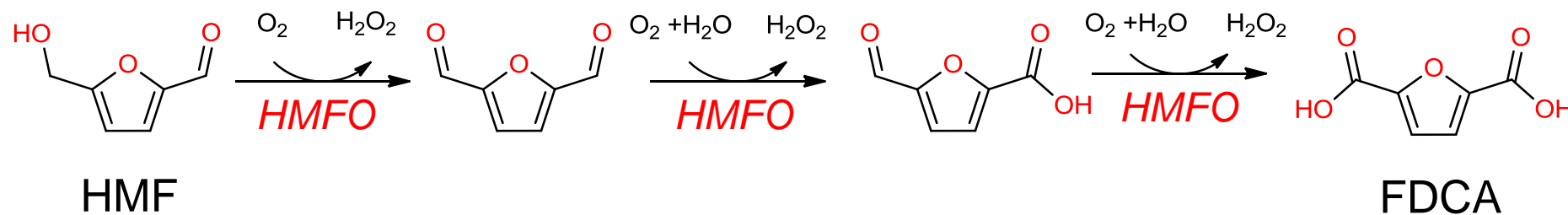


- *Methylovorus* sp. strain MP688: methylotroph
- genome: 2.9 Mb (55 % GC), 2719 ORFs



HmfH homolog (46 % protein seq. ident.)

- overexpression in *Escherichia coli* (100 mg/L culture!)
- soluble and stable monomeric protein
- FAD cofactor as prosthetic group



NWO-Fapesp

- New oxidative enzymes related to biomass degradation
- Microbiology group in Brazil: to isolate microbes and look for enzyme activities (Dr. Suzan Pantaroto, Unifesp)
- Our group in Groningen: identify the respective genes, produce and study these enzymes.
- Main interests:
 - HMFOs,
 - Peroxidase activities (related to lignin degradation),
 - Lytic polysaccharide monooxygenases (LPMOs),
 - Carbohydrate/alcohol oxidases.

Thank you!

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 - Suzan Pantaroto: suzan.pantaroto@unifesp.br