



**University of Natural Resources
and Life Sciences, Vienna**
Department of Biotechnology

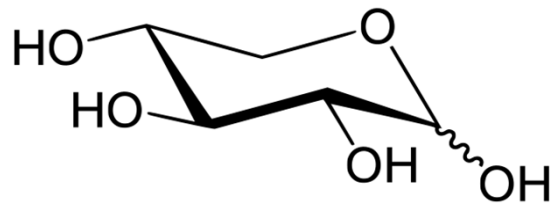
Candida lignohabitans

for biorefinery applications

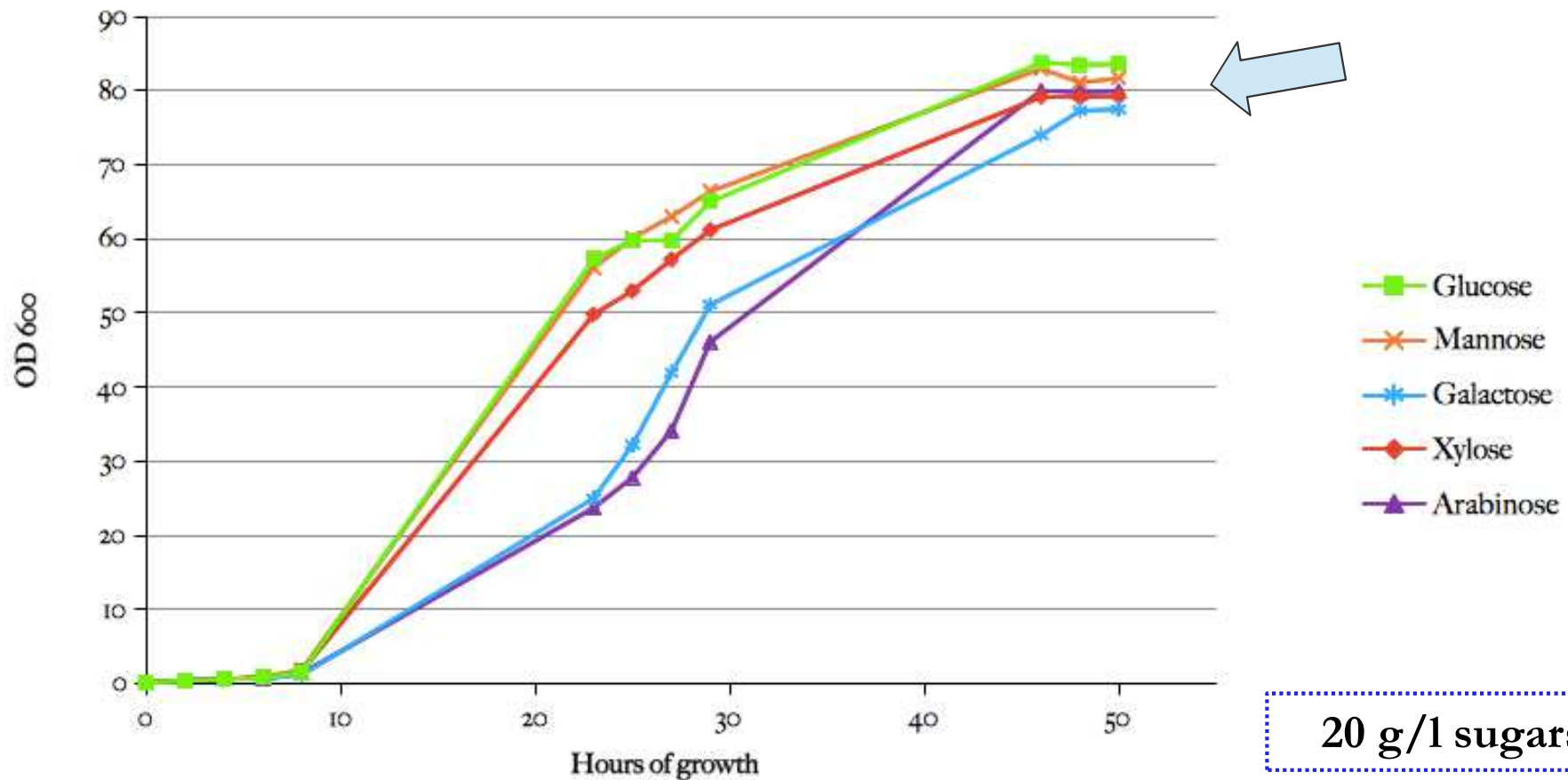
Bellasio Martina, MSc

Introducing *Candida lignohabitans*

- ✓ Non-conventional yeast, belonging to the *Sugiyamaella* clade
- ✓ Isolated from a decayed tree log (*Kurtzman, 2007*)
- ✓ Natural capability to ferment xylose



Growth on different carbon sources



- ✓ Ethanol production: 5 g/l on Glucose, 2,5 g/l on Mannose, 0,9 g/l on Galactose, 0,4 g/l on Xylose, 0,1 g/l on Arabinose

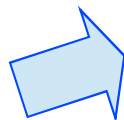
Hydrolysis of lignocellulosic material



6 different feedstocks



Steam explosion treatment



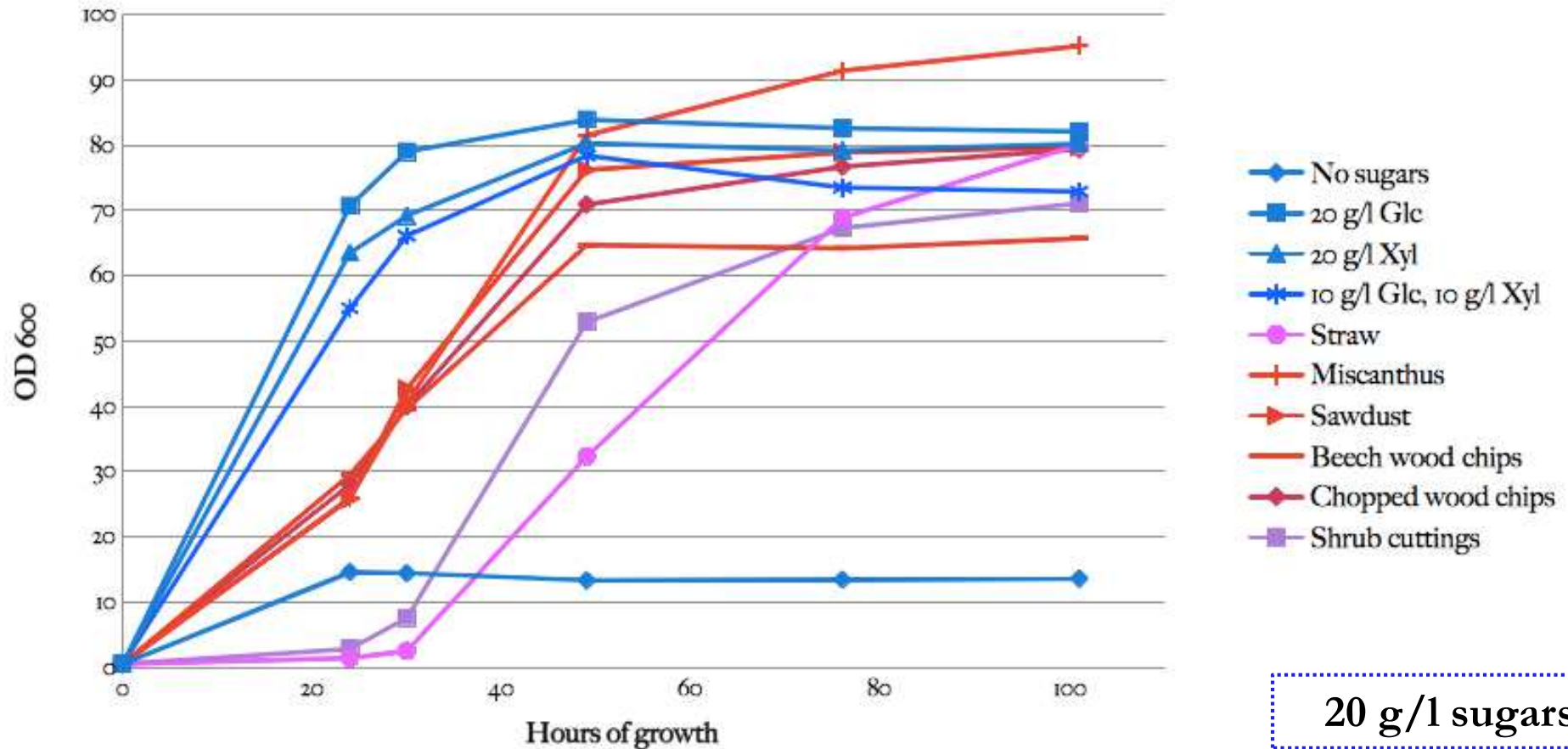
Soluble sugars:
C6: Glucose, Galactose,
Mannose, ...
C5: Xylose, Arabinose, ...



Enzymatic digestions:
Cellic Ctec 2 (Novozymes)
Accelerase 1500 (Genencor)

50°C
pH not regulated (3,7-3,5)
180 rpm
20% (w dry/v)
No accessory enzyme
Up to 3 days of incubation

Growth on hydrolysed lignocellulosic materials

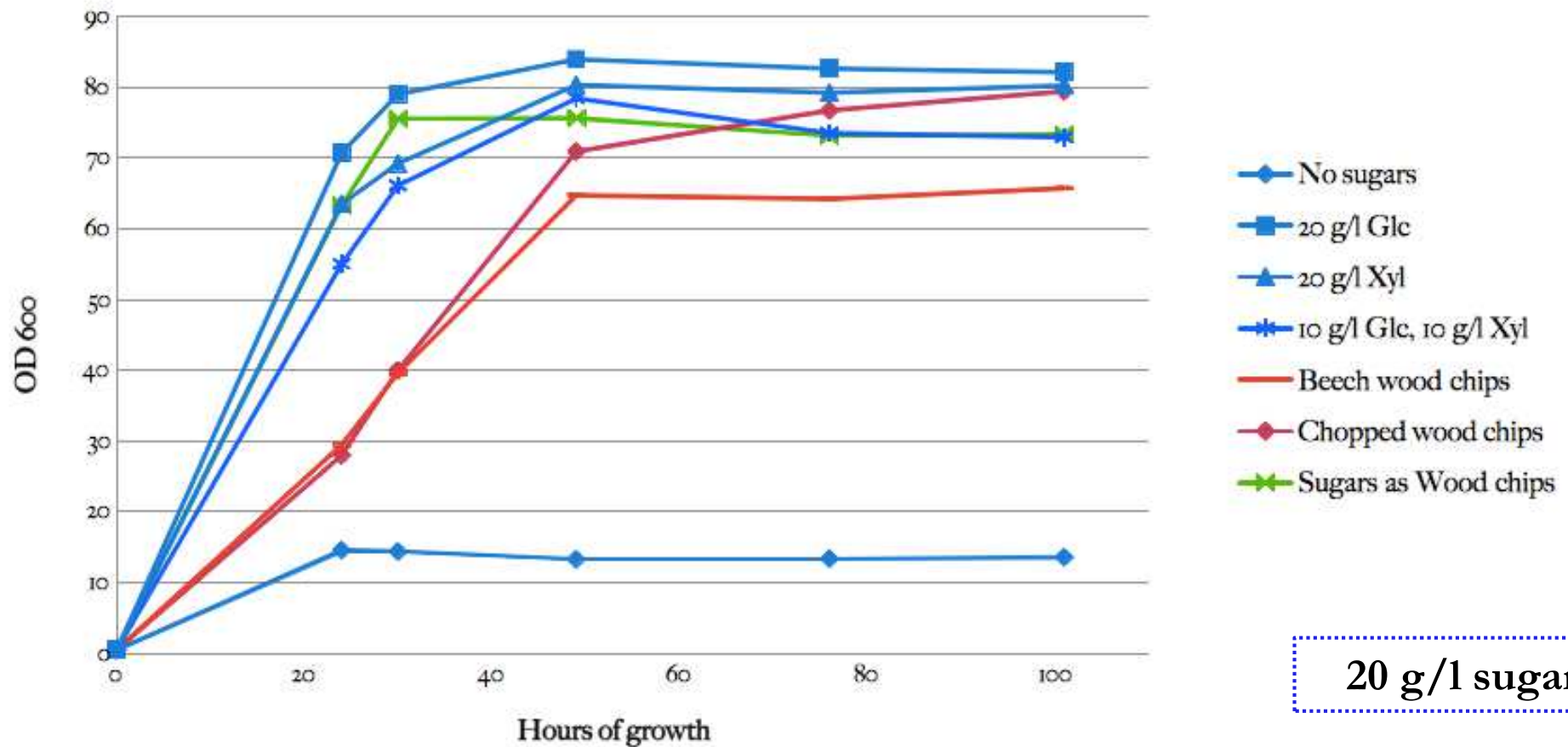


20 g/l sugars

- ✓ The strain grows on sugars present in all the types of hydrolysed lignocellulosic material

Growth on hydrolyzed lignocellulosic materials

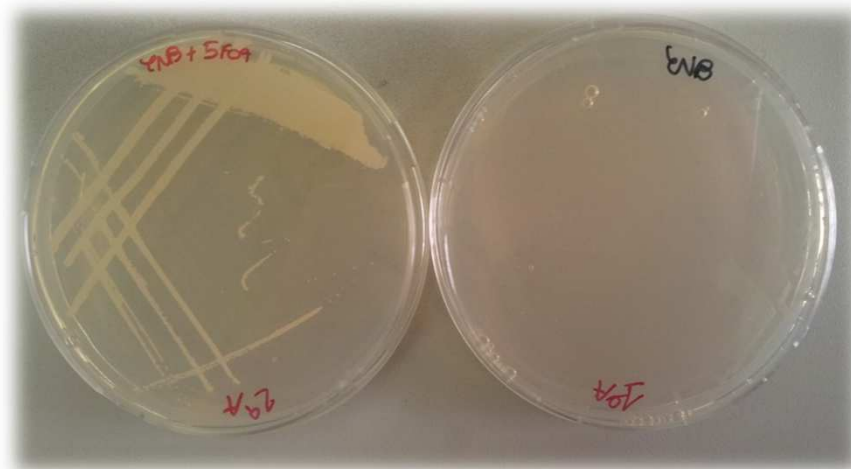
✓ Tolerance to inhibitors in digested steam explosion material



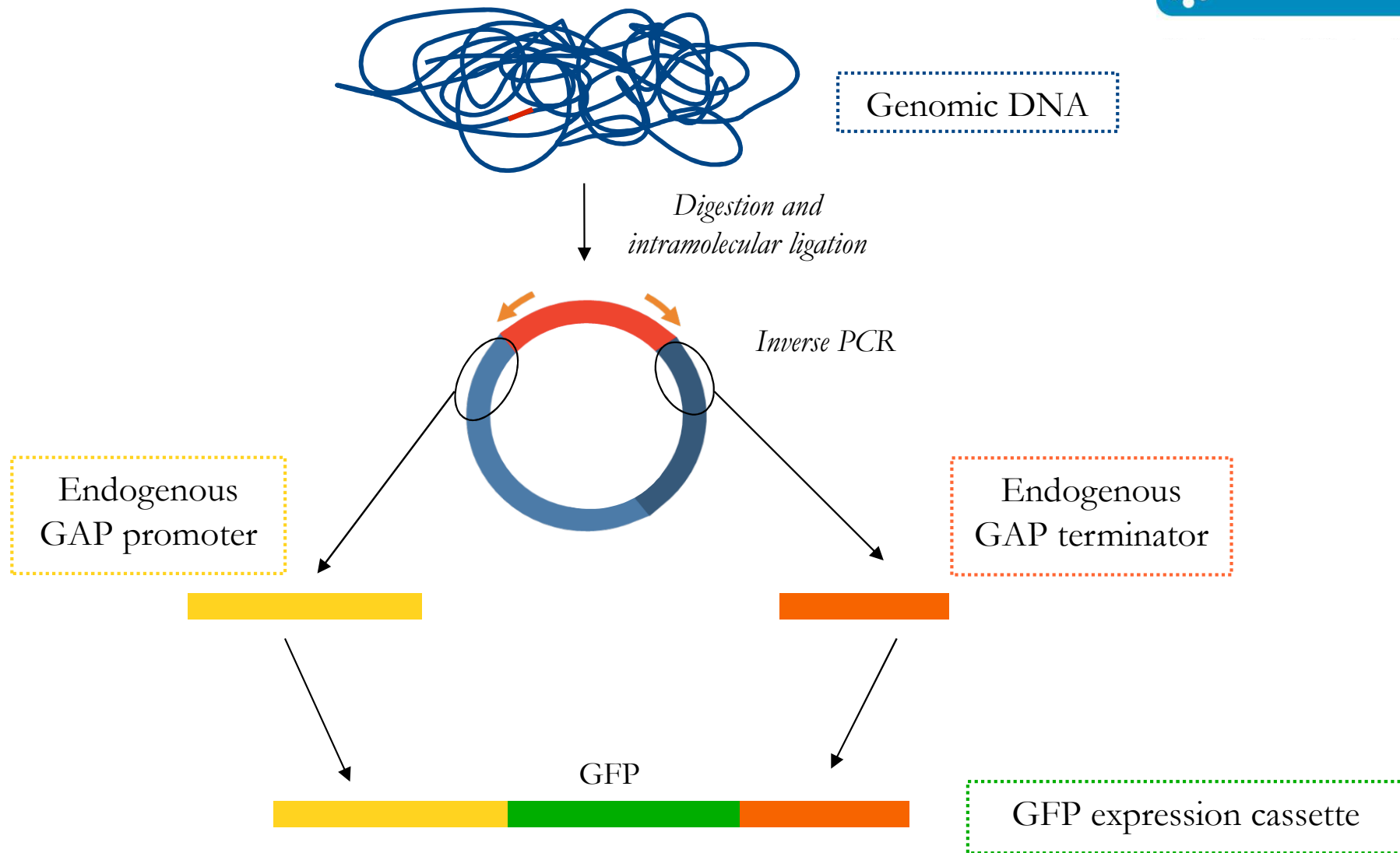
20 g/l sugars

Tools for molecular engineering

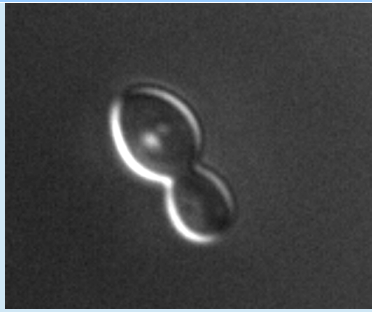
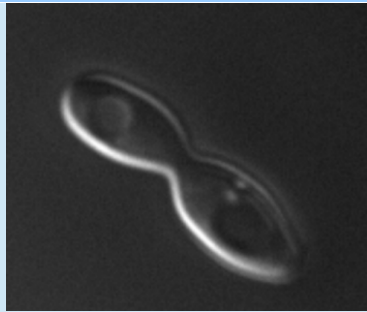

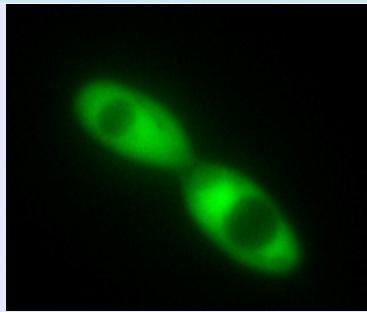
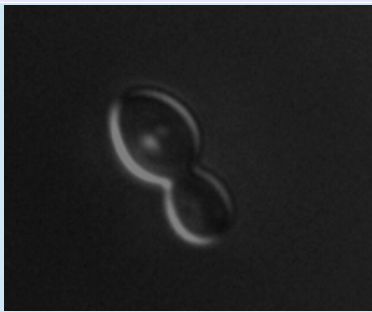
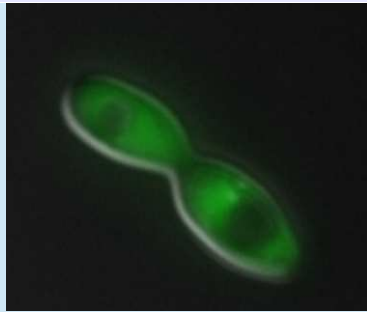
- 1) **Transformation protocol:** *C.lignohabitans* is transformable with a standard electroporation protocol
- 2) **Codon usage** → *C.lignohabitans* is not included in the CTG clade
- 3) *ura3*- mutants were selected on 5-FOA



Endogenous GAP promoter and terminator



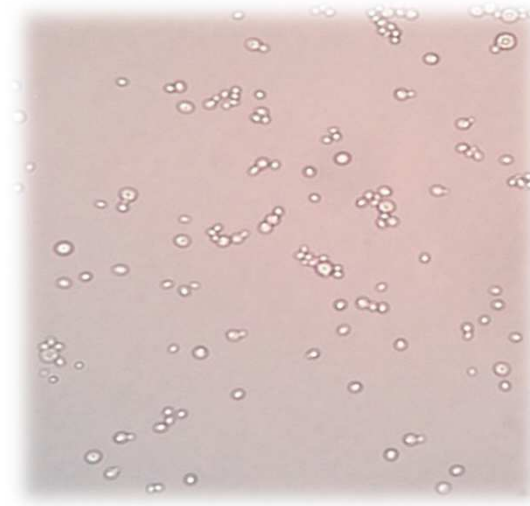
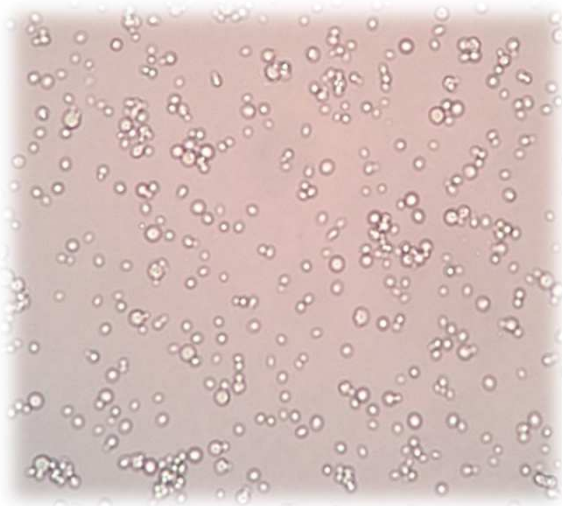
GFP expression

	WT	GFP
Visible	 A brightfield micrograph of a wild-type (WT) yeast cell, showing its characteristic oval shape and budding structure.	 A brightfield micrograph of a yeast cell expressing GFP, showing its characteristic oval shape and budding structure.
Fluorescence	 A fluorescence micrograph of a WT yeast cell, showing no fluorescence (black background).	 A fluorescence micrograph of a yeast cell expressing GFP, showing bright green fluorescence throughout the cell.
Merge	 A merged image of a WT yeast cell, showing the brightfield image overlaid on a black background.	 A merged image of a yeast cell expressing GFP, showing the brightfield image overlaid with green fluorescence.

Perspectives

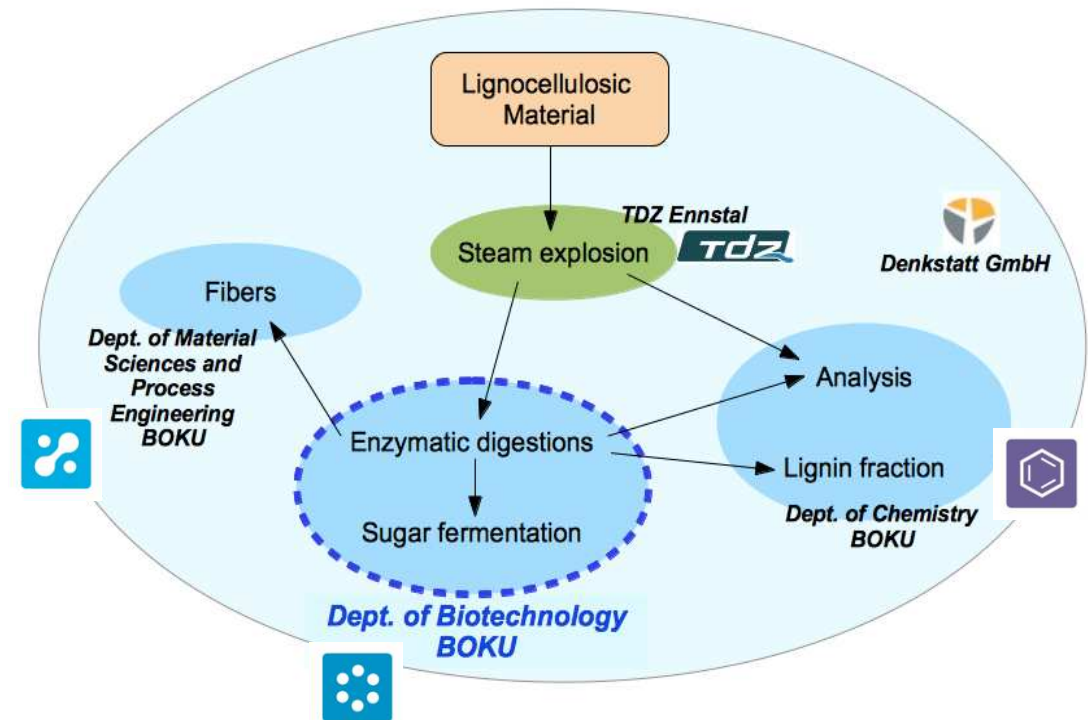
➤ **Metabolic engineering of *C. lignohabitans*:**

Making the strain a useful microbial cell factory for the production of chemical building blocks from lignocellulosic material



Acknowledgments

- ❖ *Michael Sauer*
- ❖ *Hans Marx*
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Q & A