

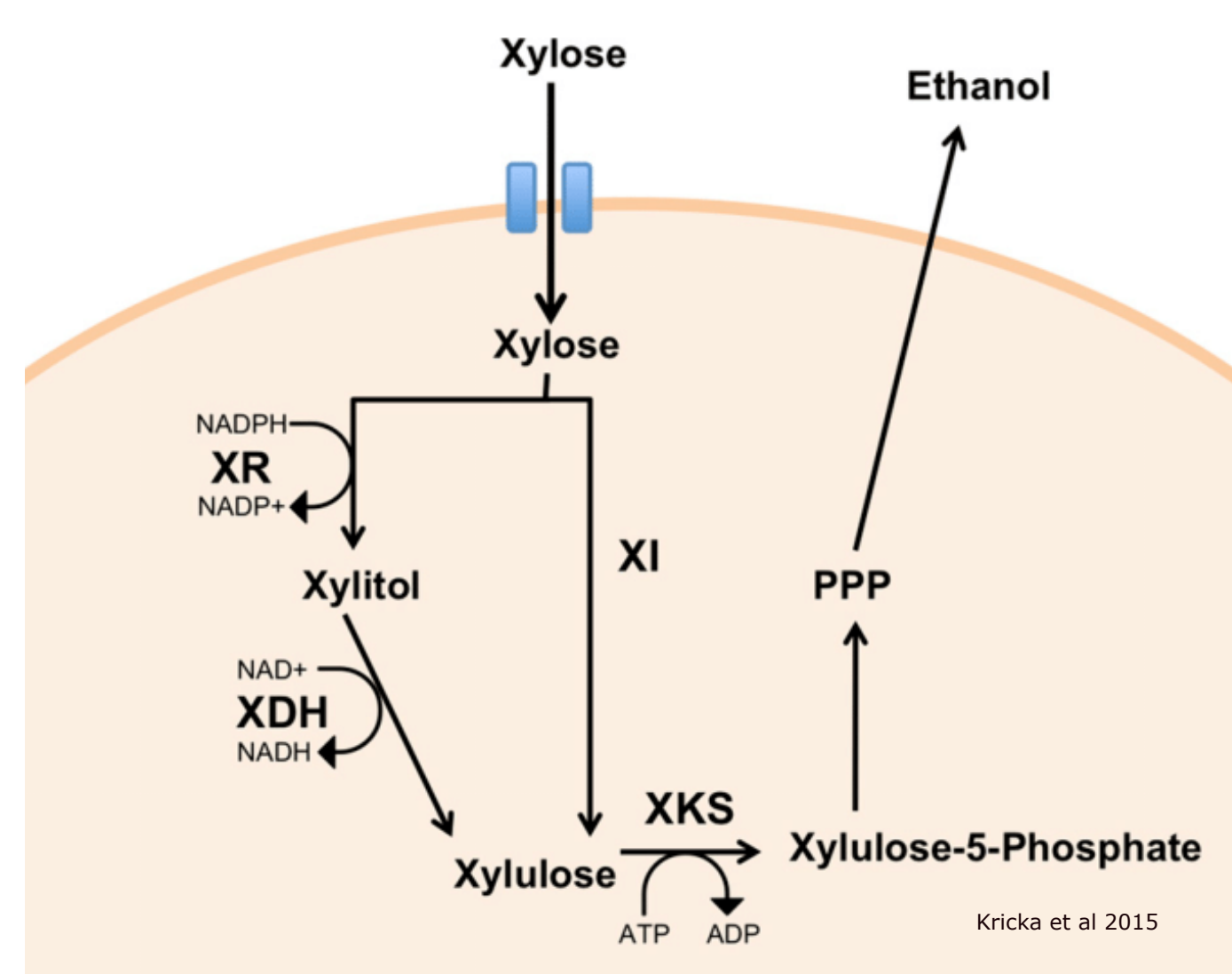
A novel D-xylose isomerase from the gut of the wood feeding patent-leather beetle *Odontotaenius disjunctus*

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Background

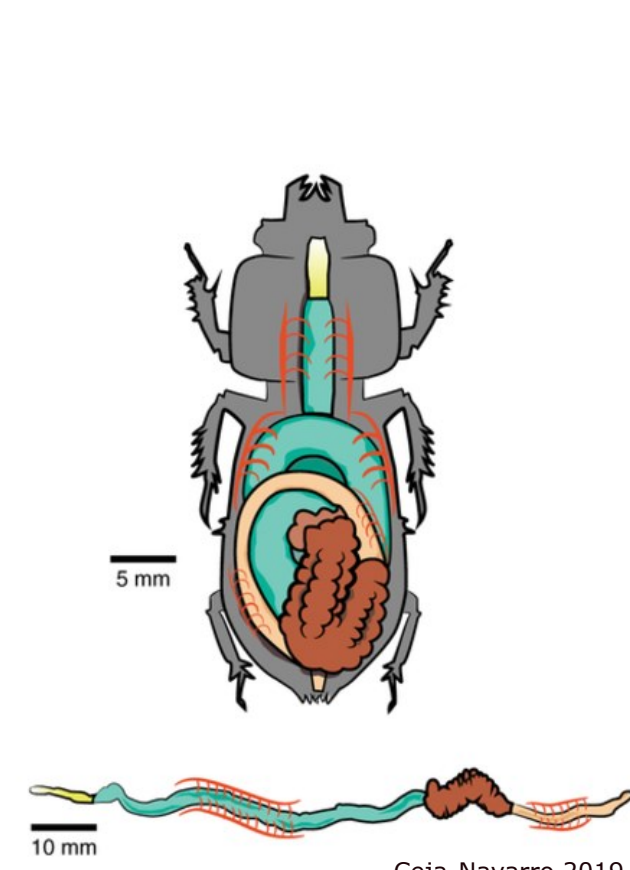
- ⇒ Xylan, the second most abundant biopolymer on earth, contains mostly the pentose sugar D-xylose
- ⇒ *Saccharomyces cerevisiae* is the preferred organism for industrial fermentation of lignocellulose-derived sugars
- ⇒ Expression of heterologous pathways are necessary for D-xylose as it is not metabolized naturally by *S. cerevisiae*



- ⇒ D-Xylose Reductase (XR)/Xylitol Dehydrogenase (XDH) pathway suffers from a NAD(P)H cofactor imbalance
- ⇒ Only 13 different Xylose Isomerases (XI) have been expressed in *S. cerevisiae*
- ⇒ XI's suffer from low capacity and inhibition by xylitol (Brat et al 2009)

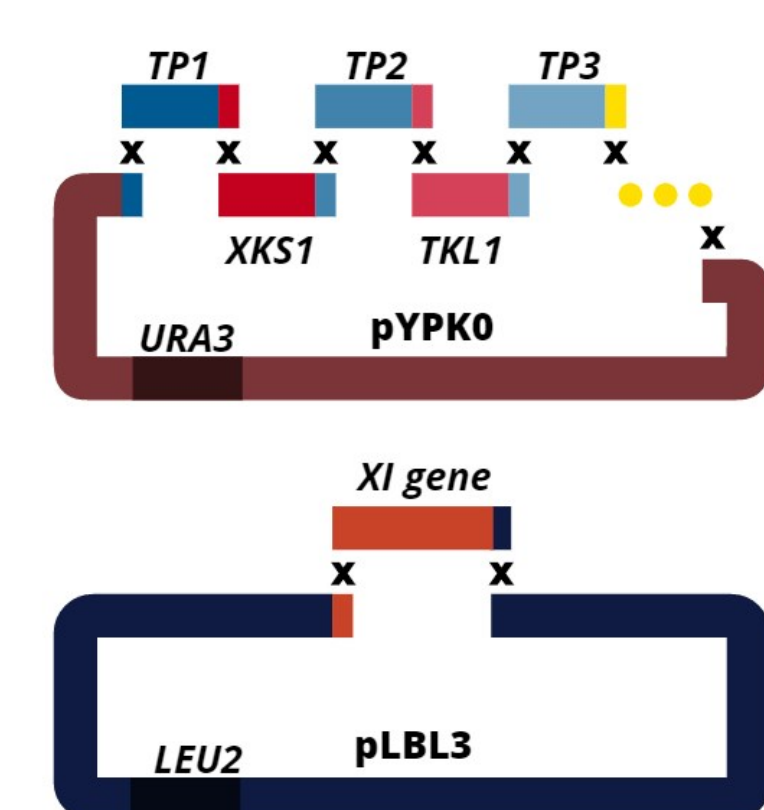
The aim of this work was to express actively a new Xylose Isomerase in *S. cerevisiae*

Methodology

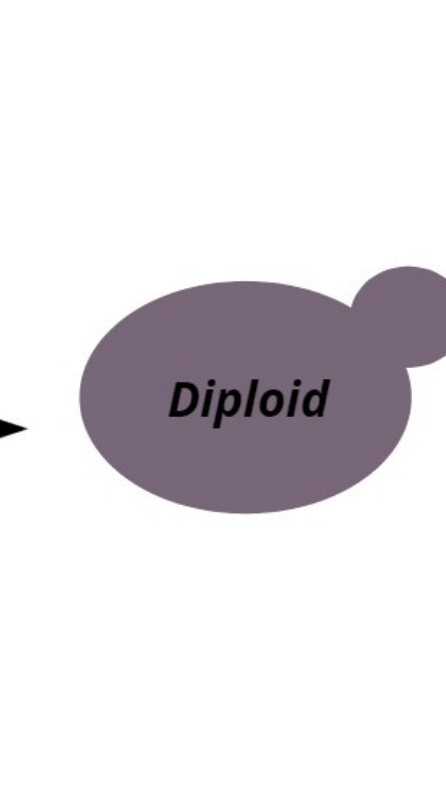


Metagenome sequencing of gut microbiota from the wood feeding beetle *Odontotaenius disjunctus*

Metagenome assemblies with XI functional predictions



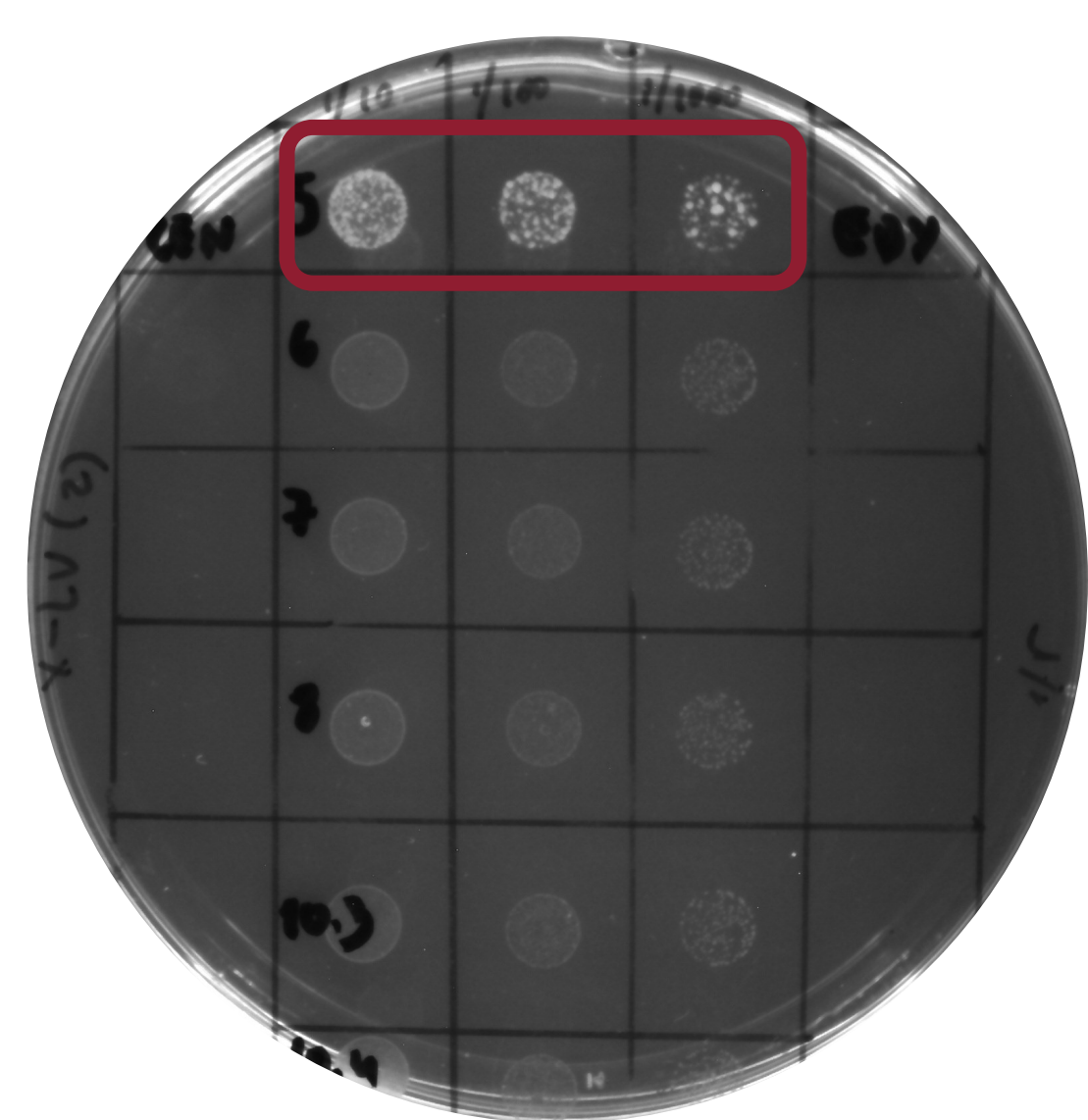
Mating of a yeast strain containing a partial xylose utilization pathway with a strain expressing a synthesized XI gene



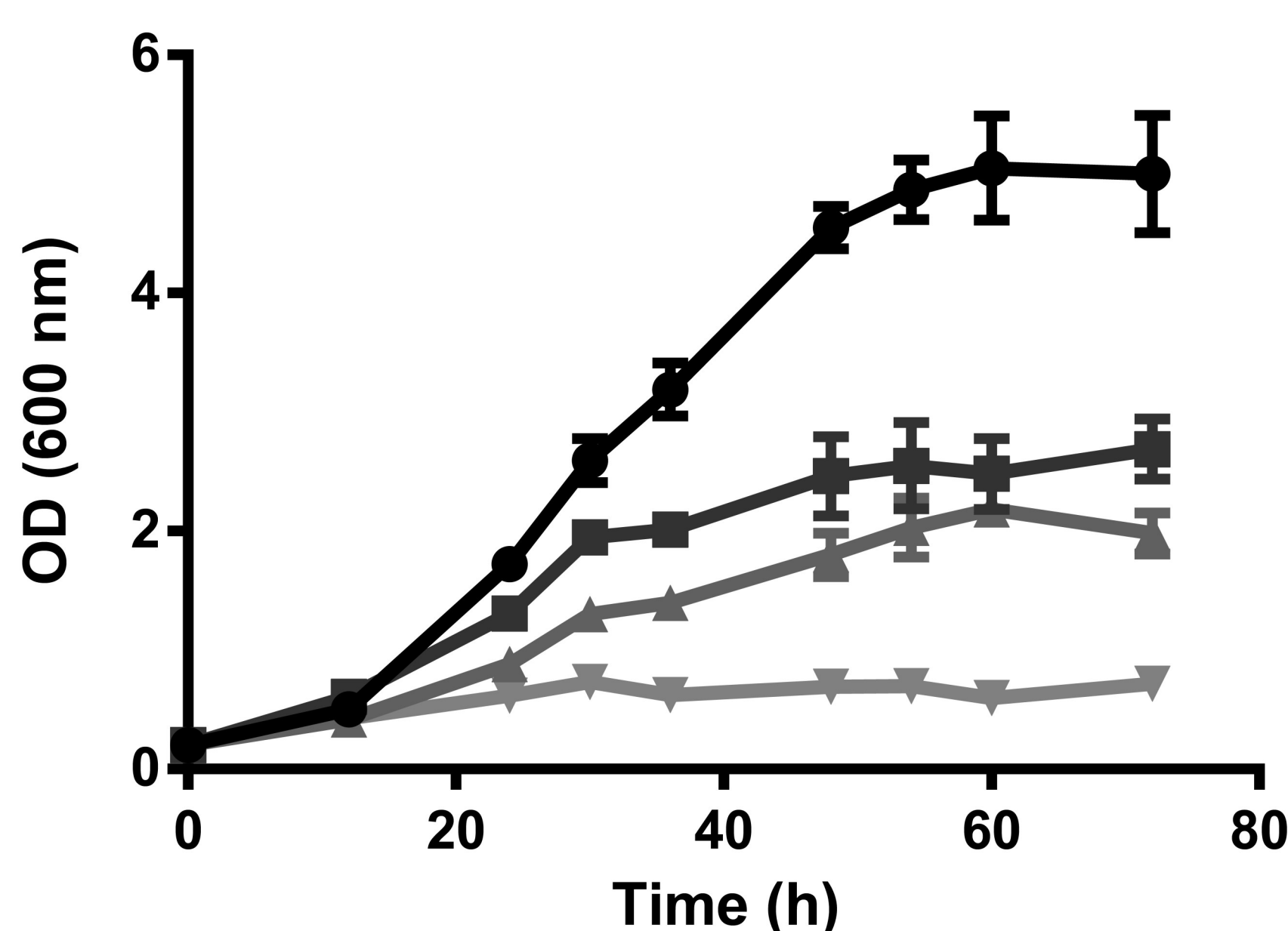
Functional screening by scoring growth on solid medium



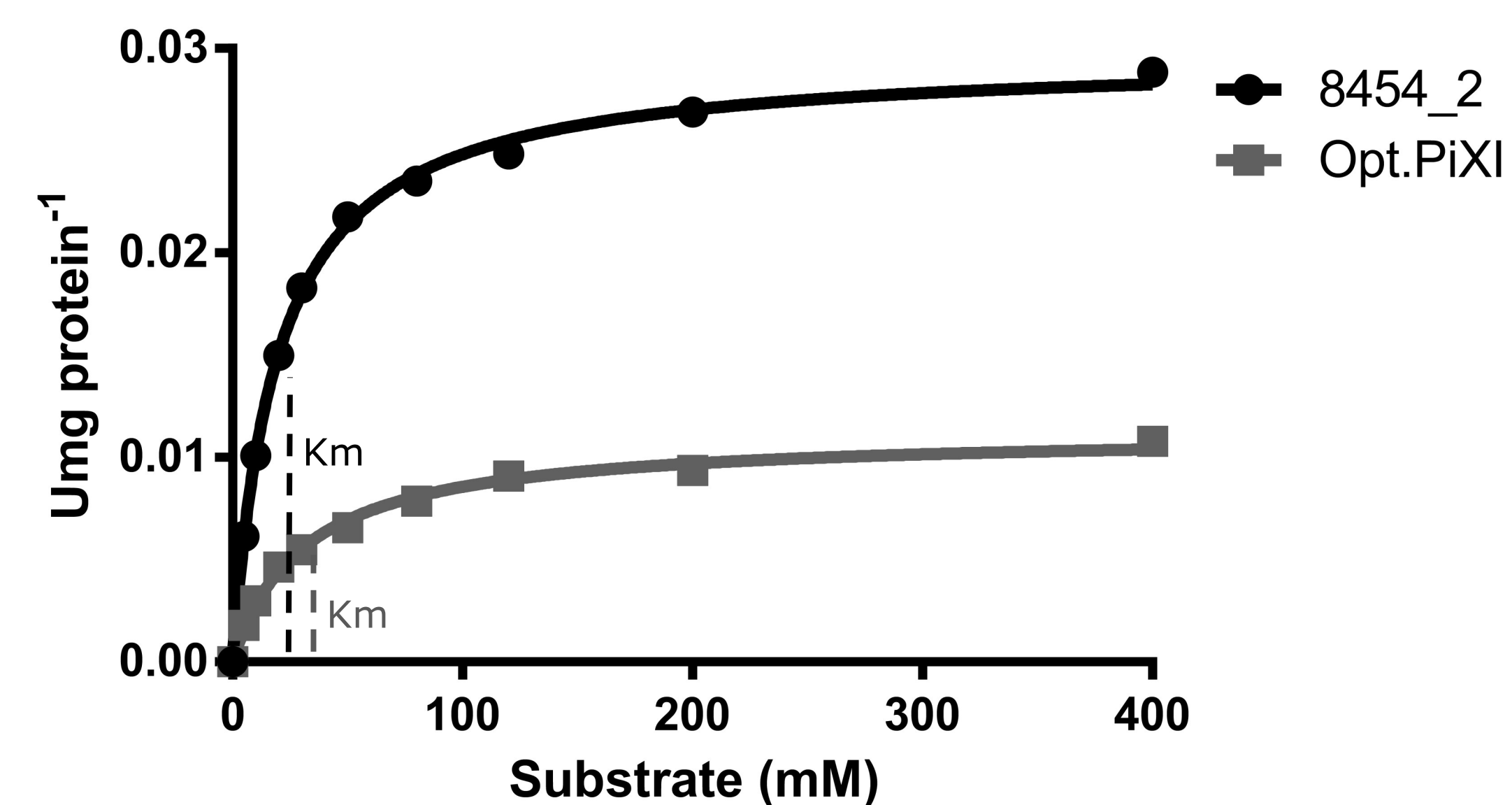
Results



- ⇒ 1 in 3 synthetic XI genes conferred active growth of yeast on solid medium; this XI was identified as "8454_2"
- ⇒ A clone expressing a codon-optimized XI from *Piromyces sp.* (opt.PiXI) grew poorly on this medium



- ⇒ *S. cerevisiae* cells expressing different D-xylose utilization pathways were cultivated in liquid media containing D-xylose as the sole carbon source
- ⇒ Growth rates without evolutionary adaptation to xylose:
XR/XDH: 0.15 h⁻¹; 8454_2: 0.11 h⁻¹; opt.PiXI: 0.07 h⁻¹



- ⇒ 8454 XI shows higher Vmax (~3x) and affinity to D-xylose than XI from *Piromyces sp.*
- ⇒ Correlation between superior yeast growth capacity and more efficient kinetic parameters of 8454_2 XI

Conclusions

- ⇒ This strategy amenable to high-throughput analysis is a viable option for the identification of novel XI genes for *S. cerevisiae*
- ⇒ XI identified in this work confers higher growth rate than the widely studied XI from *Piromyces sp.*, although lower than XR/XDH pathway
- ⇒ The novel XI enzyme has superior Vmax and higher affinity to the substrate than XI from *Piromyces sp.*

References

- Kricka, William, Tharappel C. James, James Fitzpatrick, and Ursula Bond. 2015. "Engineering *Saccharomyces Pastorianus* for the Co-Utilisation of Xylose and Cellulose from Biomass." *Microbial Cell Factories* 14 (April): 61.
- Brat, Dawid, Eckhard Boles, and Beate Wiedemann. 2009. "Functional Expression of a Bacterial Xylose Isomerase in *Saccharomyces Cerevisiae*." *Applied and Environmental Microbiology* 75 (8): 2304–11.
- Ceja-Navarro, Javier A., Ulas Karaoz, Markus Bill, Zhao Hao, Richard A. White 3rd, Abelardo Arellano, Leila Ramanculova, et al. 2019. "Gut Anatomical Properties and Microbial Functional Assembly Promote Lignocellulose Deconstruction and Colony Subsistence of a Wood-Feeding Beetle." *Nature Microbiology* 4 (5): 864–75.

Acknowledgments



