Functional screening for novel D-xylose isomerases from the gut of a wood feeding beetle reveals efficient expression in *Saccharomyces cerevisiae*

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Objectives

⇒ Functional screening of new D-xylose isomerases (XI) from the gut metagenome of the wood feeding beetle *Odontotaenius disjunctus.*

⇒ One of three D-xylose isomerases, codon-optimized and synthesized *in-vitro,* expressed efficiently in *Saccharomyces cerevisiae.*

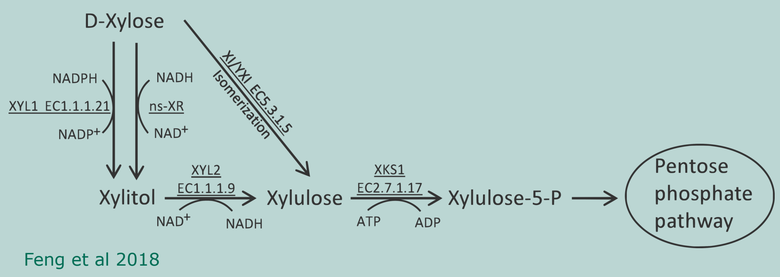
⇒ The new enzyme, **8054_2**, was characterized and compared to the codon-optimized gold-standard of *Piromyces* sp. E2.

Background

Lignocellulosic material continues to be the most promising renewable raw material for the production of sustainable fuels and fine chemicals (Arevalo-Gallegos 2017).

Xylan is the second most abundant biopolymer on earth which contains mostly the pentose sugar D-xylose.

Expression of heterologous pathways are necessary for D-xylose utilization as it is not metabolized naturally by *S. cerevisiae.*



Feng et al 2018

The XR/XDH pathway suffers from a NAD(P)H cofactor imbalance that has proven hard to remedy (Kötter et al 1990).

Only fourteen different XIs with characterized kinetic parameters have been reported to actively express in *S. cerevisiae.*

References

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Acknowledgements

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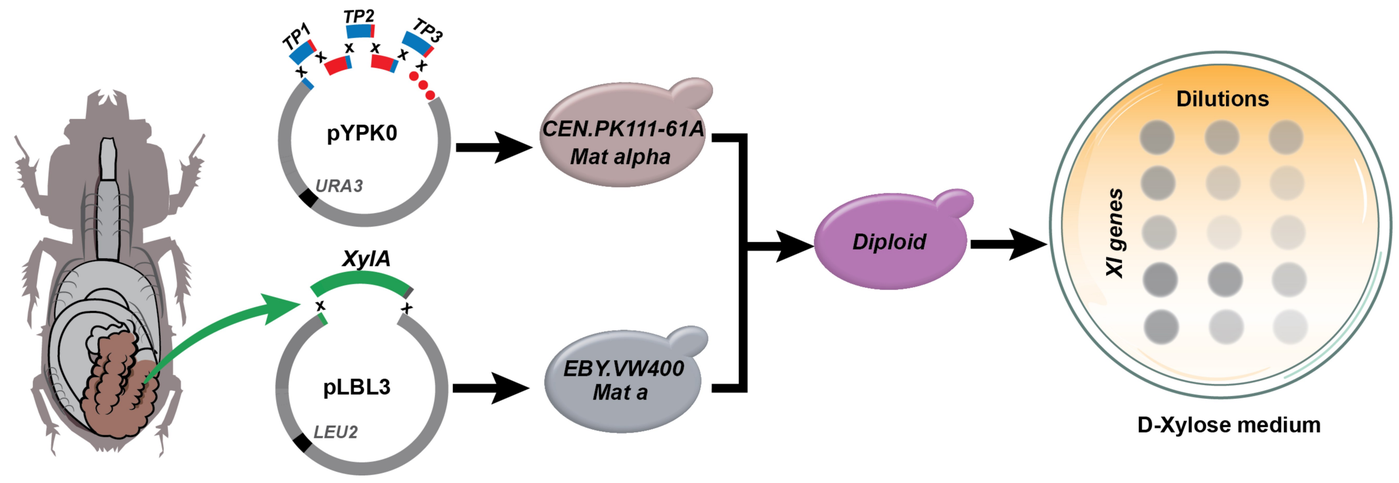
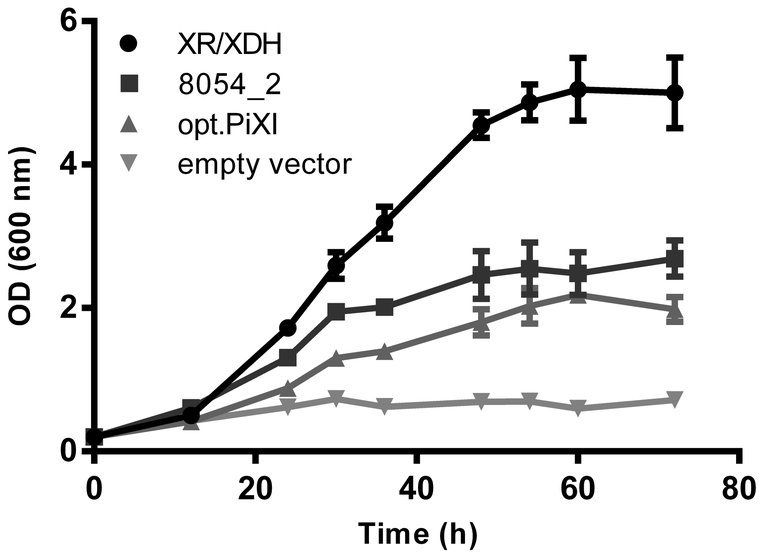
Functional screening in yeast



Putative *xylA* genes (XIs) from the microbial metagenome in the gut of *Odontotaenius disjunctus* were codon-optimized, synthesized and cloned in the plasmid pLBL3. The partial D-xylose pathway (*TKL1*, *TAL1*, *RPE1*, *RKI1*, *XKS1*, and Gxf1) was constructed in the plasmid pYPK0 under the control of different terminators/promoters (TP). Each EBY.VW4000 strain clone was mated with the CEN.PK111-61A strain. Candidate XI enzymes were functionally screened on solid D-xylose medium.

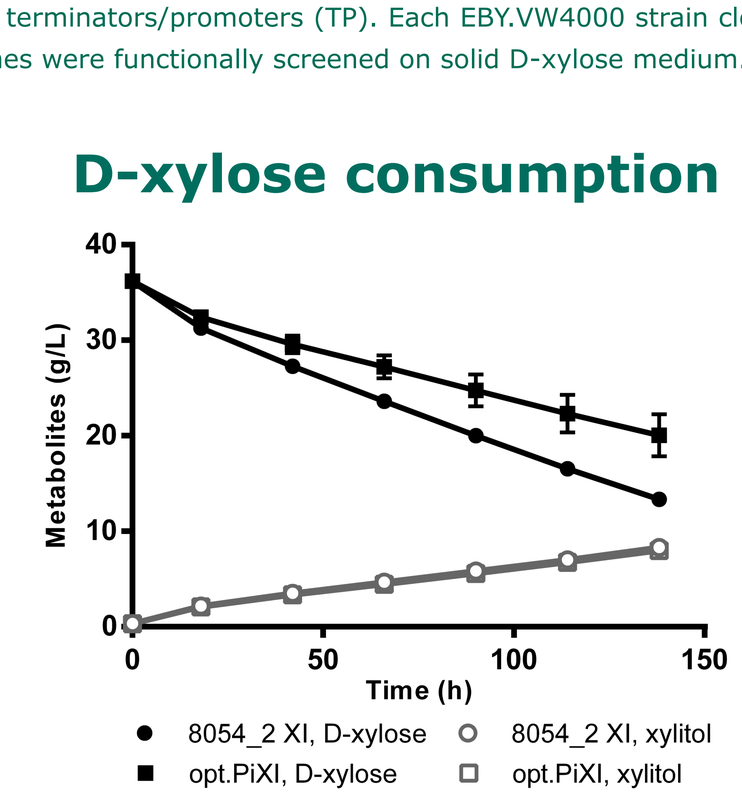
Growth on D-xylose



XR/XDH: 0.10 h^{-1} 8054_2: 0.06 h^{-1} opt.PiXI: 0.04 h^{-1}

Expression of the new XI, 8054_2, resulted in **50% faster aerobic growth** than the XI from *Piromyces* sp. (opt.PiXI) in synthetic medium with D-xylose as the sole carbon source.

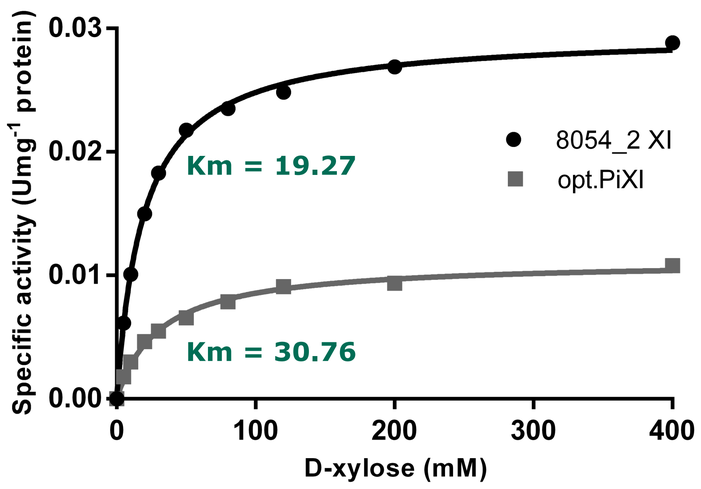
D-xylose consumption



The D-xylose isomerization rate conferred by 8054_2 was **72% higher** than the XI from *Piromyces* sp. (opt.PiXI).

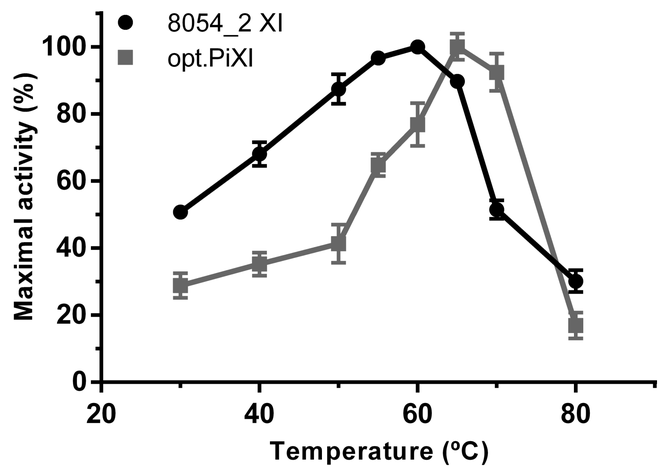
Increasing concentrations of xylitol (up to 8 g/L) appeared **not to inhibit** D-xylose consumption.

Kinetic parameters



8054_2 operates at a **2.6 times higher V_{max}** and with **37% higher affinity** for D-xylose than the XI from *Piromyces* sp. (opt.PiXI).

Optimal temperature



8054 exhibited higher activity over a broader temperature range, retaining **51% of maximal activity at 30 ºC** compared with only 29% activity for the *Piromyces* XI (opt.PiXI).

Conclusions

⇒ The new enzyme, **8054_2**, showed higher specific activity and affinity for D-xylose than the current gold-standard from *Piromyces* sp., as well as substantially higher relative activity at 30 ºC.

⇒ The novel XI represents a highly valuable addition to the *S. cerevisiae* molecular toolbox and shows promise for improved industrial conversion of carbohydrate substrates.