

Towards a Metabolic Engineering Strain “Commons”: An *Escherichia coli* Platform Strain for Ethanol Production

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Review

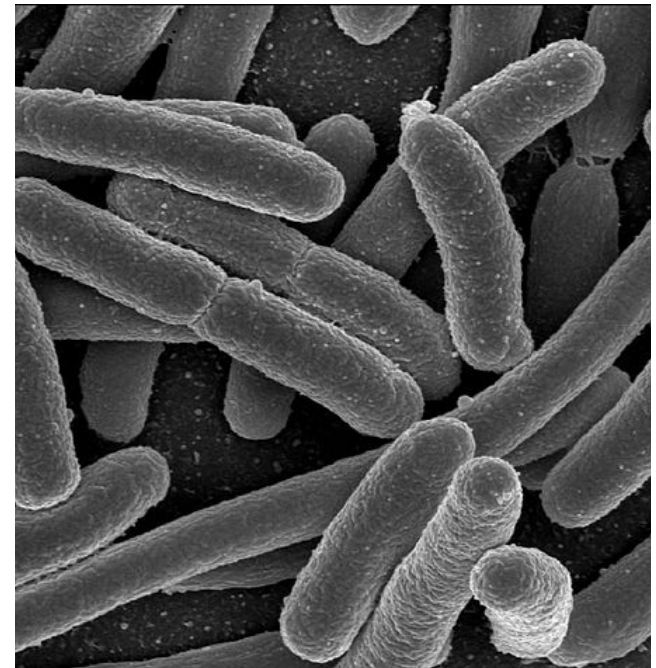
Scaling up synthetic biology: Do not forget the chassis

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“ Synthetic biology puts together two separate entities: a **program** (conceptual extension of the genetic program) and a **chassis** (conceptual extension of the living cell).”



Open source commons for production strains

- Motivation

- Genome engineering tools benefit from a set of platform strains
- “decrease the cost of engineering metabolic pathways, make metabolic engineering more predictable with less guesswork” Keasling(2008)
- Direct comparisons between studies and labs

Challenges for commons for production strains

- Synthetic biology



BioBricks
FOUNDATION

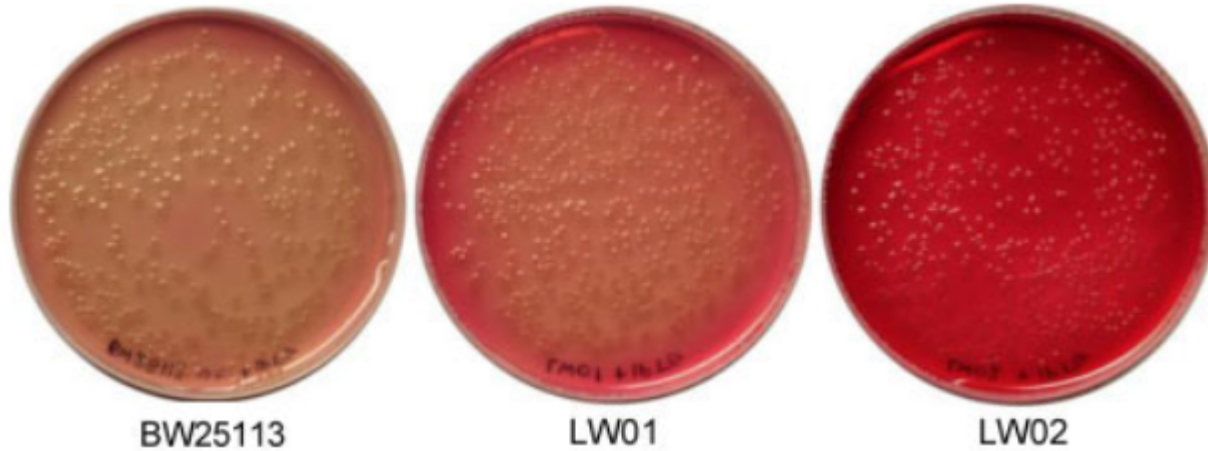
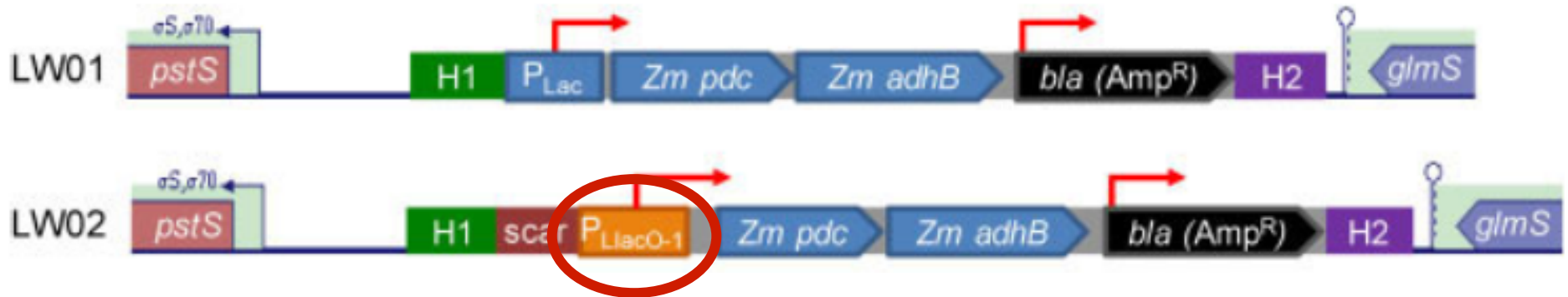
Biotechnology in the public interest

**Registry of Standard
Biological Parts**

- Metabolic engineering

- A single “commons” collection
- Third-party to maintain and distribute (ATCC)
- Universal commons agreement (BioBricks Public Agreement)

Constructing platform ethanol production strain



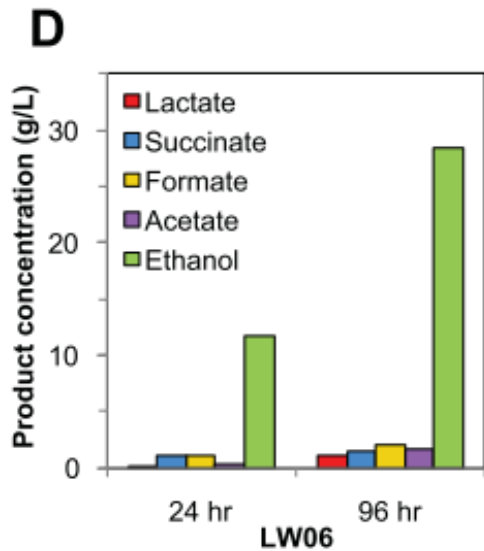
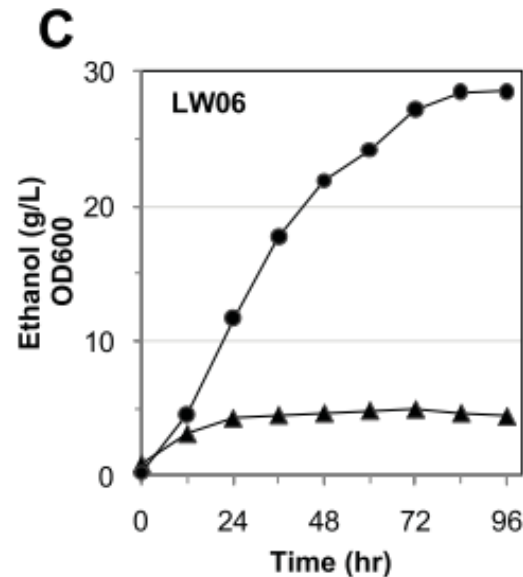
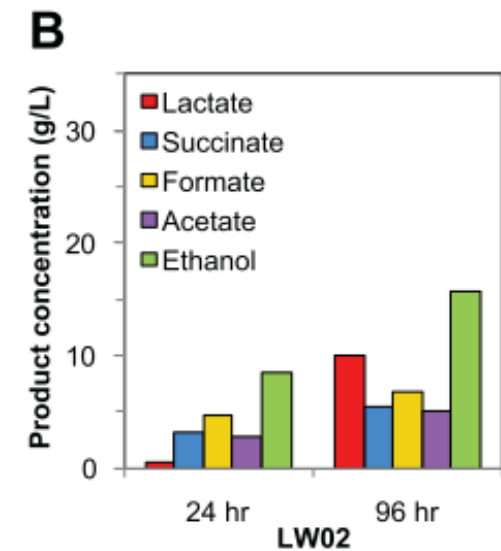
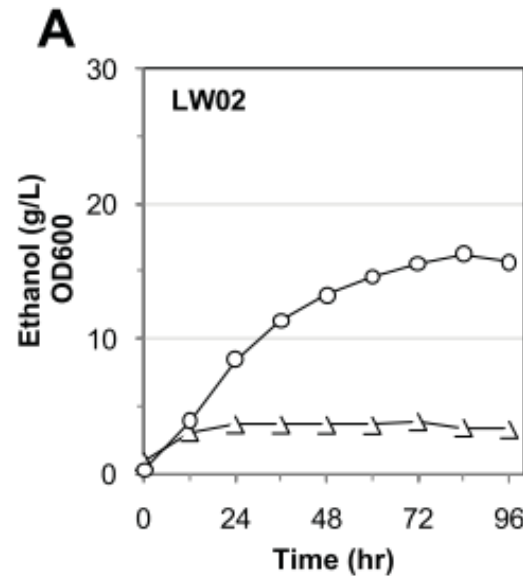
Accumulation of additional fermentative byproducts

- LW06

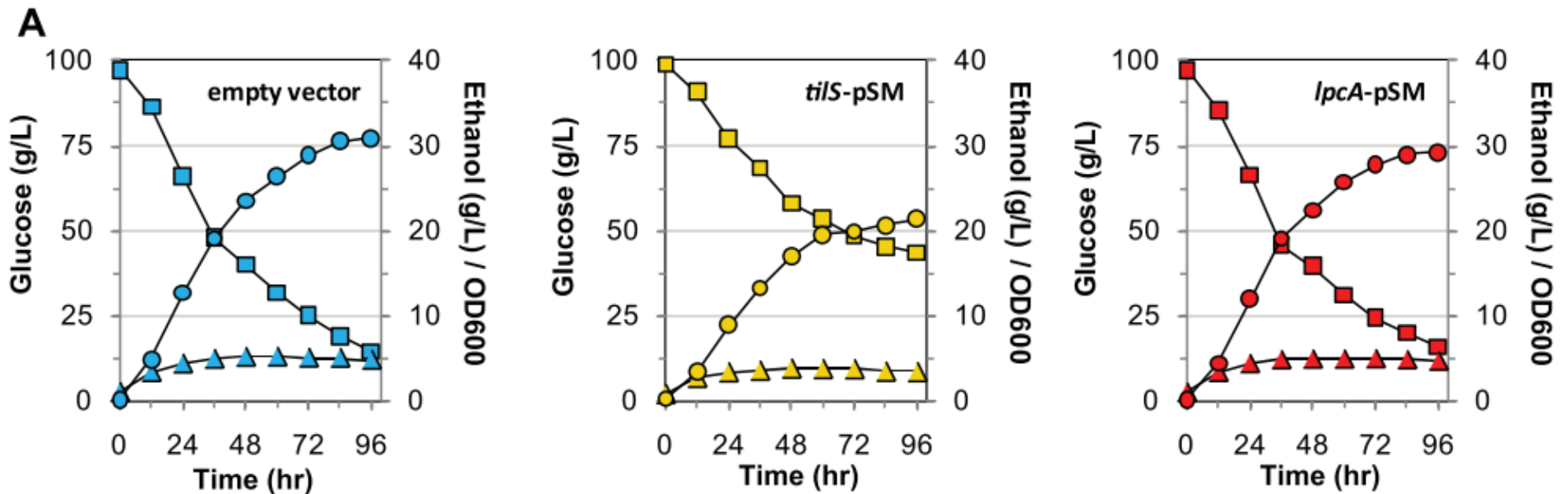
Knocked out enzymes for:

acetate (*ackA*) succinate (*frdABCD*) lactate (*ldhA*)

native ethanol (*adhE*)

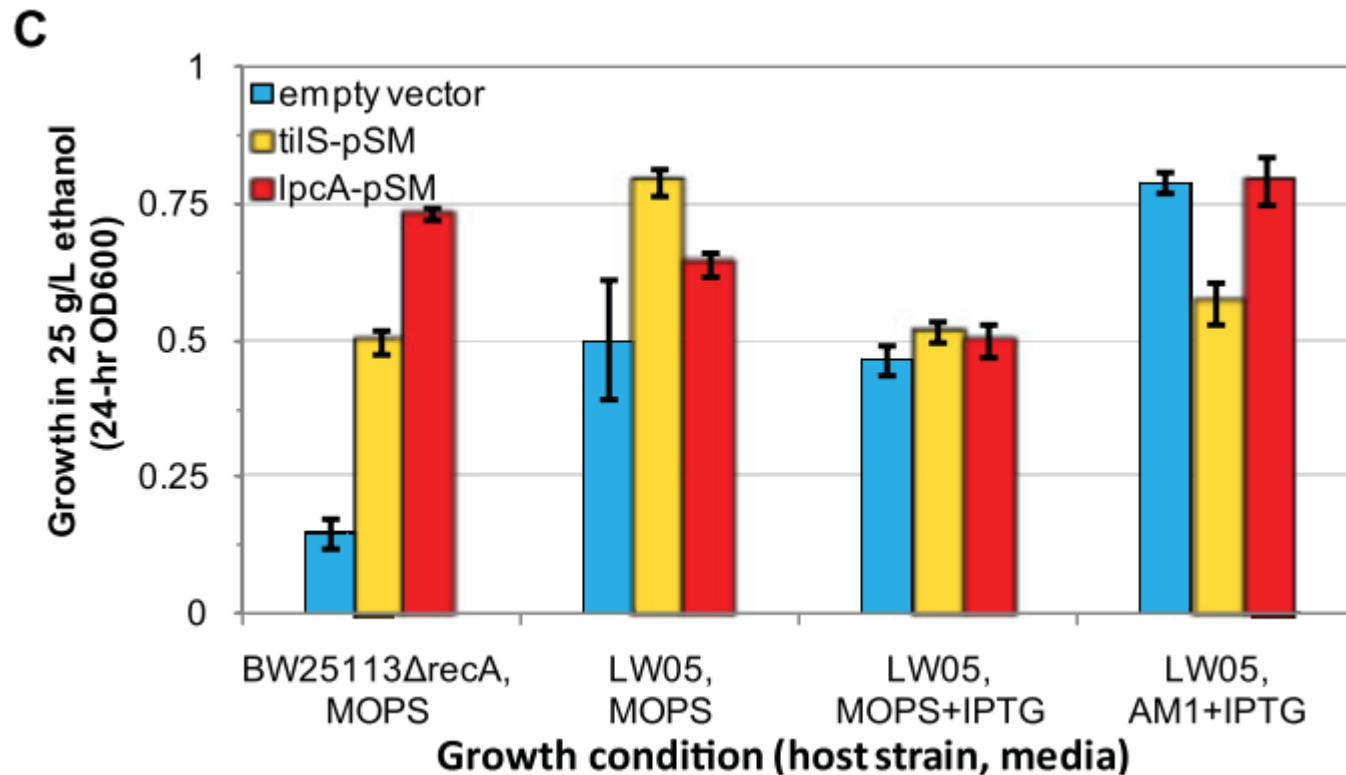


Ethanol tolerance genes did not improve ethanol production



- *lpcA*: enzyme for biosynthesis of lipopolysaccharide
- *tilS*: discrimination of isoleucine and methionine

Ethanol tolerance genes did not improve ethanol production or tolerance under production conditions



MOPS media used for tolerance testing

AMI1 minimal media used for ethanol production

Assumptions & Limitations

- Leakiness of the IPTG-inducible promoter
- LW05 vs LW06
- Knockout of byproduct formation pathways can affect regulatory network
- Enhanced production of ethanol not super impressive

Significance of the work

- Open-source ethanol production strain
 - Inducible promoter
 - Minimal polar effects
 - Transposable elements not used
 - Competing fermentative pathways knocked out
 - No other genetic modifications to enhance production
- Importance of growth environment and host strains