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Bioprocessing of biomass-derived diverse carbon substrates into D-lactate by a substrate promiscuous bacterium

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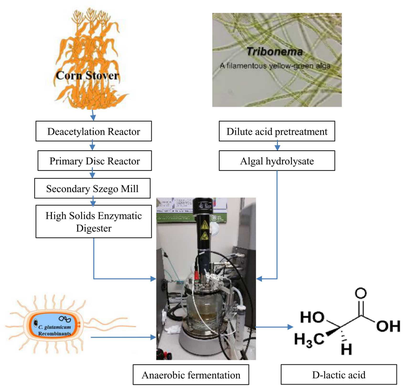
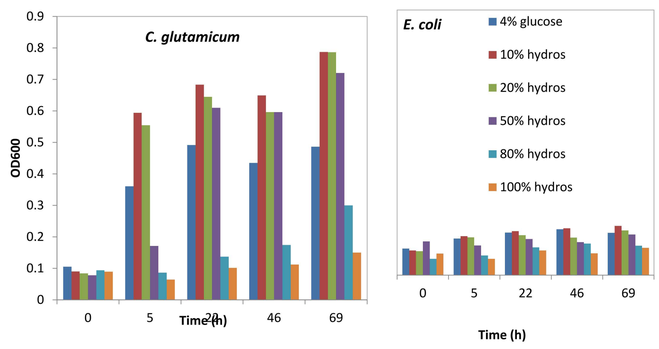
Motivation and Strategy

- The aim of this study is to increase the ability of *C. glutamicum* to utilize all major carbon sources present in different biomass sources such as lignocellulosics and algal biomass.
- D-lactate is a precursor for poly-D-lactic acid (PDLA), a bio-based polymer, is a renewable packaging material for fresh fruit containers, drinking cups, lamination films, and other items.
- Recombinant strains of *Corynebacterium glutamicum* were generated after knocking out the L-lactate production pathway and overexpression of ***ldhA*** *-Lactobacillus delbrueckii*, ***gldA101*** *– Bacillus coagulans*, and codon optimized ***gldA101**** and designated AV46, SSL01, and SSL02, respectively, for the production of D-lactate.
- The D-lactate producing strains were grown with glucose and different aromatics such as benzoic acid, cinnamic acid, vanillic acid, and coumaric acid directly.
- Xylose and arabinose uptake pathways were introduced to increase the ability of these strains to utilize xylose and arabinose.
- U-13C fingerprinting study carried out by supplementing different aromatics and U-13C glucose to the cells exhibited significant assimilation of aromatics especially in case of coumaric acid and cinnamic acid.
- Fed-batch fermentation gave high D-lactate titer and productivity of 14.41 g/L and 0.075 g/L/h, respectively.

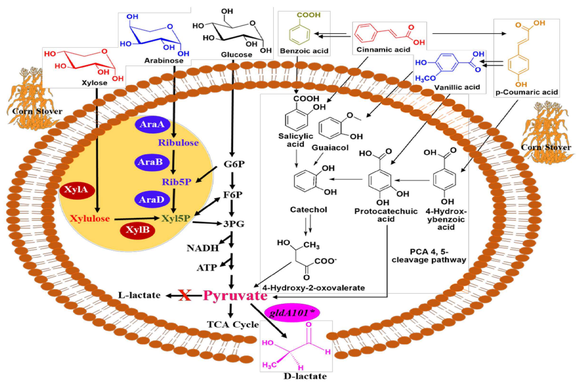
Overall Strategy



Resistivity of Microbes to Lignocellulosic Hydrolysates



Metabolic Engineering strategy

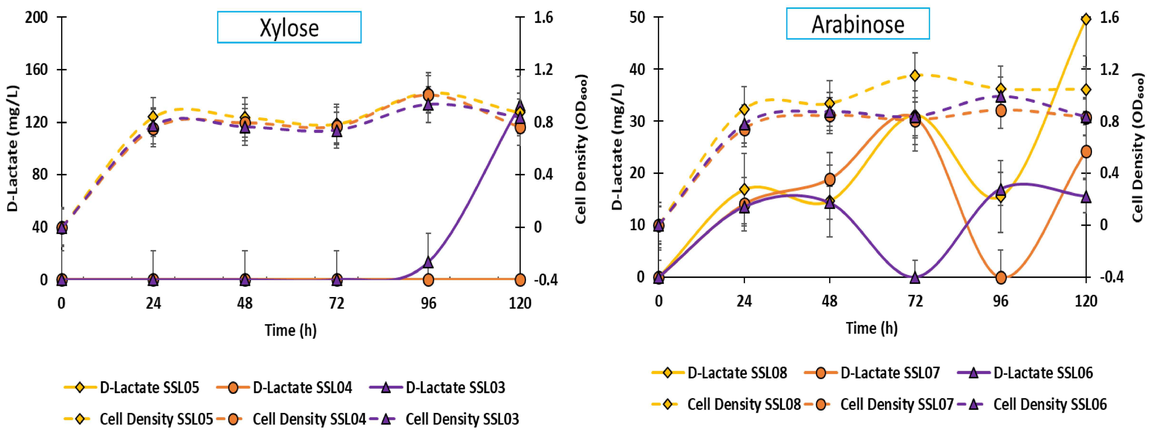


SSL02 strain: P_{ldhA} — *gldA101**

SSL04: P_{ldhA} — *gldA101* — P_{tac} — *xylA* — *xylB*

SSL08: P_{ldhA} — *ldhA* — P_{tac} — *araA* — *araB* — *araC*

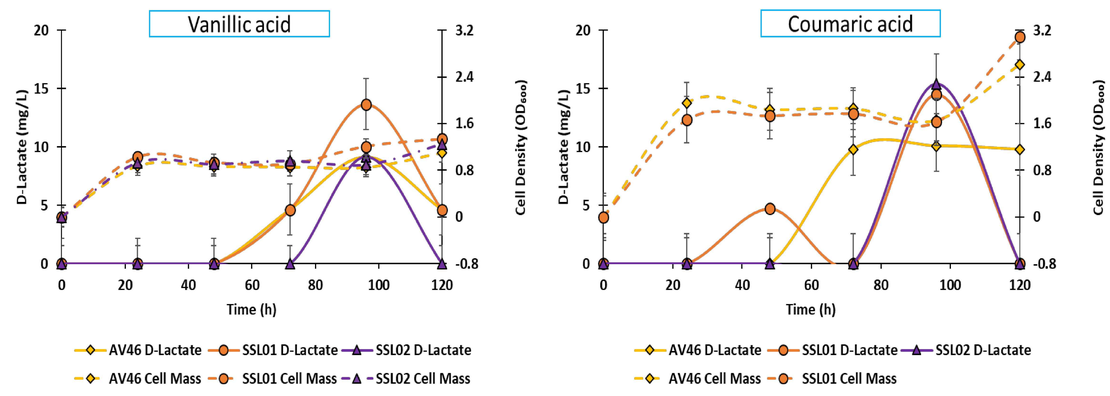
Effect of xylose and arabinose on D-lactate production



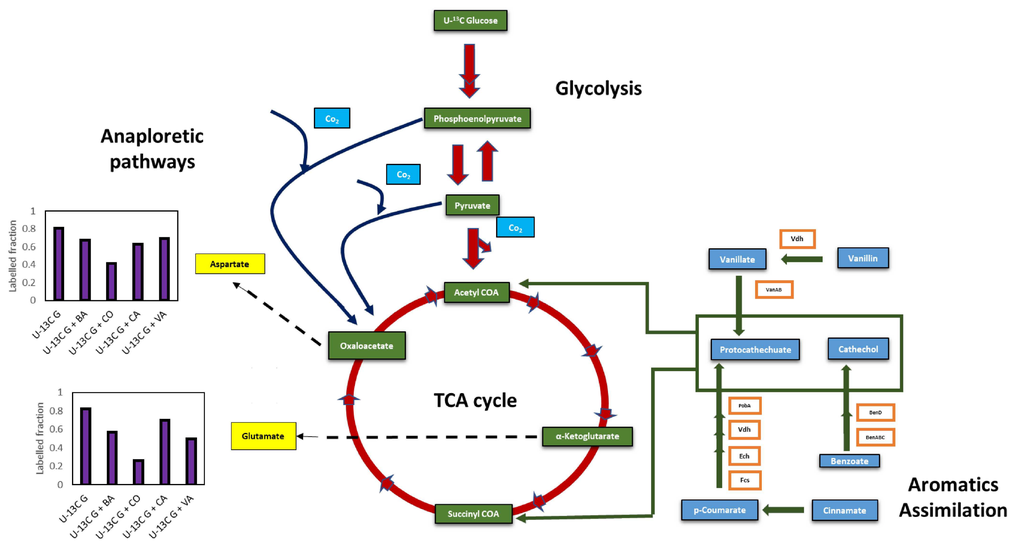
References

1. Klotz S, Kaufmann N, Kuenz A, Prüße U; Biotechnological production of enantiomerically pure d-lactic acid. *Appl Microbiol Biotechnol*, 2016 Nov; 100 (22):9423-9437.
2. Sievert C, Nieves LM, Panyon LA, Loeffler T, Morris C, Cartwright RA, Wang X; Experimental evolution reveals an effective avenue to release catabolite repression via mutationsin XylR. *PANS*, 2017 Jul 11;114(28):7349-7354.

Effect of Aromatics on D-lactate production

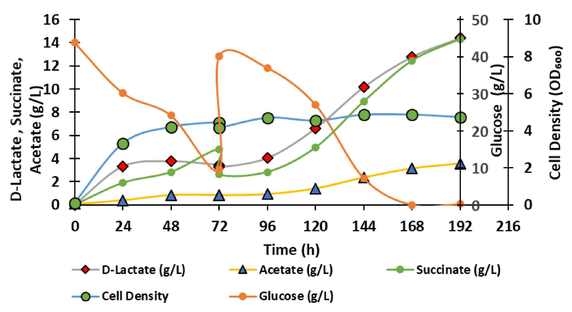


U- ^{13}C fingerprinting study on aromatics assimilation



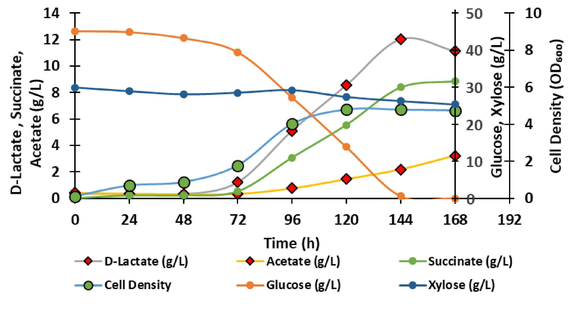
Batch and Fed batch studies on D- lactate production

D-lactic acid production from Glucose



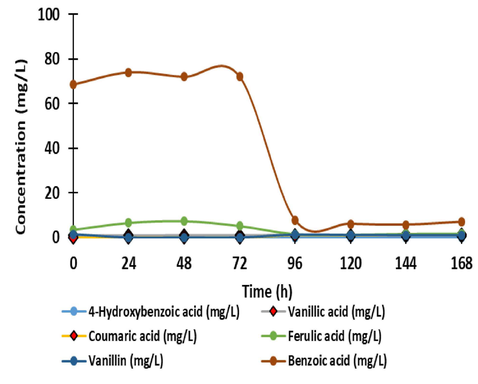
Conditions: Anaerobic, fed-batch: 40 g/L (0 h) + 30 g/L (72 h), pH 7, SSL02, BTM2

D-lactic acid production from DMR hydrolysate



Conditions: Anaerobic, batch, pH 7, SSL09, DMR hydrolysate

Aromatics utilization



Conclusions & Significance

- Demonstrated the conversion of diverse biomass substrate to D-lactate, a bioplastic precursor of PDLA, in an industrial bacteria *C. glutamicum*.
- Effective utilization of coumaric acid demonstrated by U-13C fingerprinting demonstrates promising perspectives for lignin utilization
- Production of D-lactate from hydrolysate in *C. glutamicum* opens the possibility of production of other chemicals.

Ongoing R&D

- Development of recombinant *C. glutamicum* for proteneaous algae biomass
- Co-culture of carbohydrate and protein utilizing strains for algae hydrolysate valorization

Acknowledgements

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Co-Optimization of Fuels & Engines

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