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Microbial production of a precursor for biodegradable plastics

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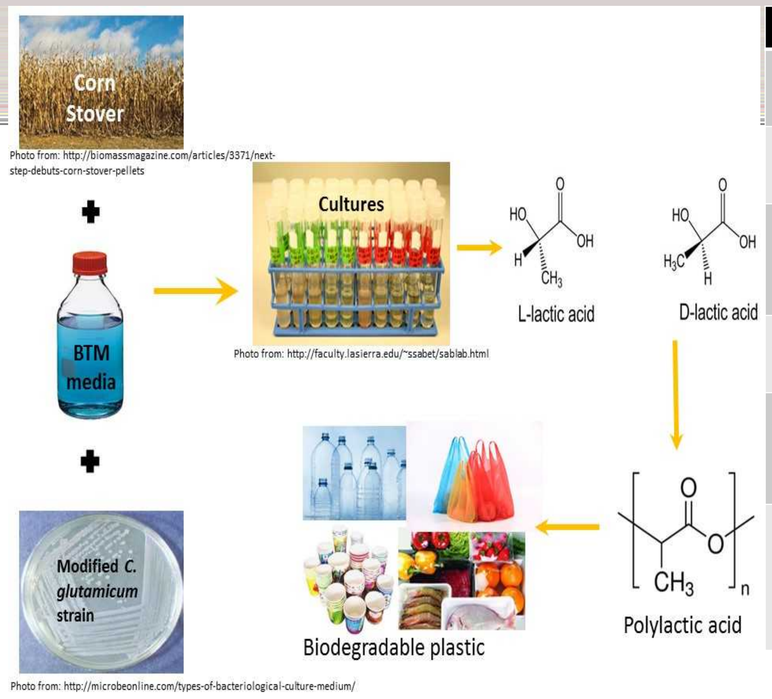
Abstract Plastics used today are made from raw materials that are extracted from oil, coal, and natural gas. Approximately 140 million tons of petroleum-based plastics are produced per year, and most of these plastics are introduced into the ecosystem as waste, resulting in increasing water, air, and land pollution. Polylactic acid (PLA) is a fully biodegradable, cost-competitive substitute for many synthetic polymers with versatile uses in industry and biomedicine. *Corynebacterium glutamicum* has been genetically modified into the following strains: AV41, AV42, AV43, AV44, and AV46. These strains have been engineered to produce D-lactate, a precursor to PLA. The ability of these strains to convert renewable biomass to D-lactate was tested under different conditions to obtain high yield and productivity.

Analysis Culture samples were analyzed using r-Biopharm D-Lactic acid/L-Lactic acid analysis kit.

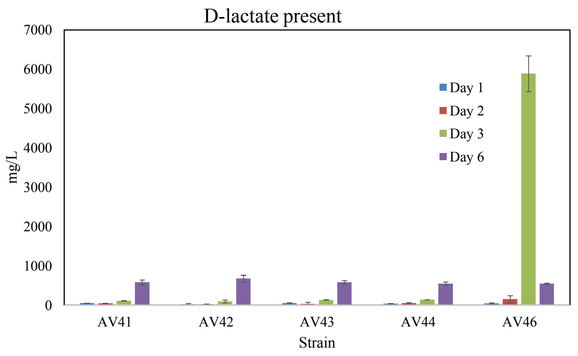


Outline of methods and the expected outcome.

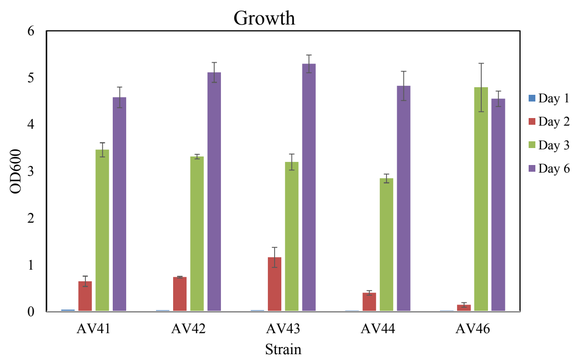
Plasmid	Description
AV40	ATCC13032 markerless *ldhA* null mutant
AV41	AV40 Ptac::*ldhA* – *(Lactobacillus delbrueckii*
AV42	AV40 Ptac::*ldhA* – *(Lactobacillus delbrueckii* - Codon optimized)
AV43	AV40 Ptac::*gldA101* – *Bacillus coagulans*
AV44	AV40 Ptac::*gldA101* – *Bacillus coagulans* & Codon optimized
ATCC13032/pCRB204 (AV46)	PldhA::*ldhA* – *Lactobacillus delbrueckii*

Description of modified *C. glutamicum* strains.

Production of D-lactate in BTM + 4% glucose cultures inoculated with 1mM IPTG

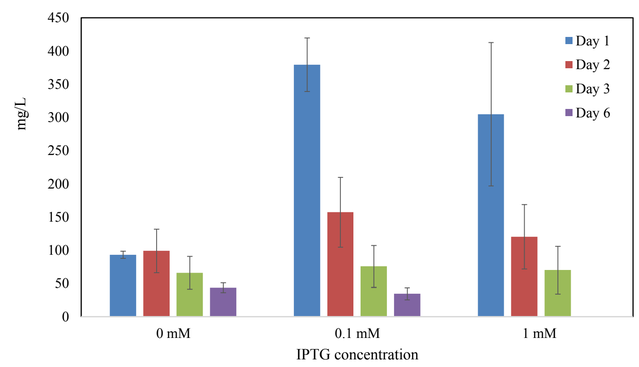


Displaying the volume of D-lactate present in BTM + 4% glucose cultures of all strains.



Displaying the growth of all strains in BTM + 4% glucose cultures.

Production of D-lactate in BTM (no glucose) + 20% DMR AV43 cultures inoculated with various concentrations of IPTG

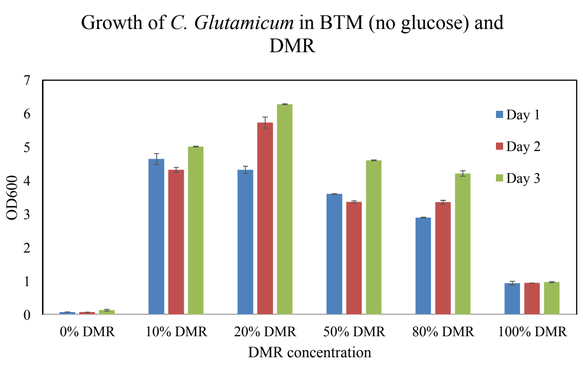


Displaying the volume of D-lactate present in BTM + 20% DMR AV43 cultures with various IPTG concentrations.

Introduction of DMR (corn stover)

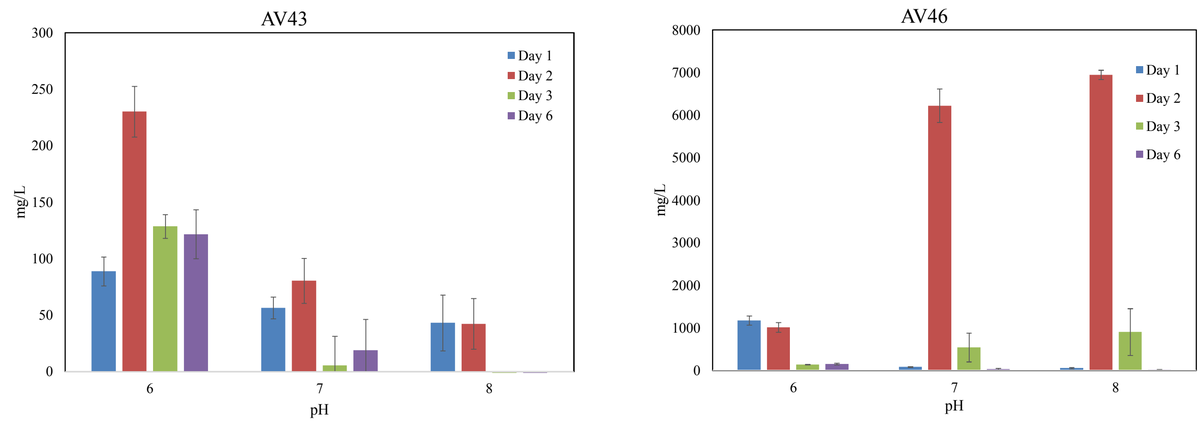
Cellulose (g/L)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Lactate (g/L)	Acetate (g/L)
1.872644	111.4133	49.41555	52.26962	1.924259	0.2218476
1.904026	112.4036	49.92332	54.08814	1.915772	0.2193445

Composition of DMR (corn stover).



Displaying the growth of *C. glutamicum* in various DMR concentrations.

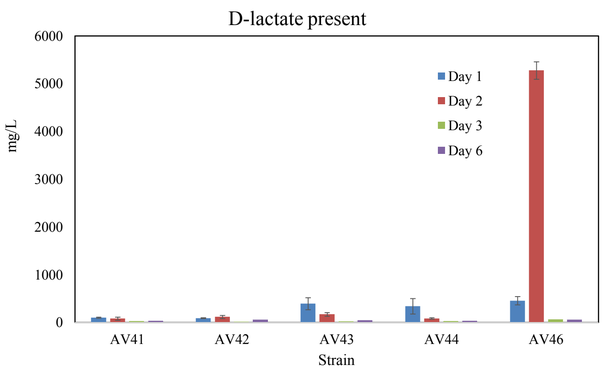
Production of D-lactate in BTM (no glucose) + 20% DMR AV43 & AV46 cultures of various pH levels



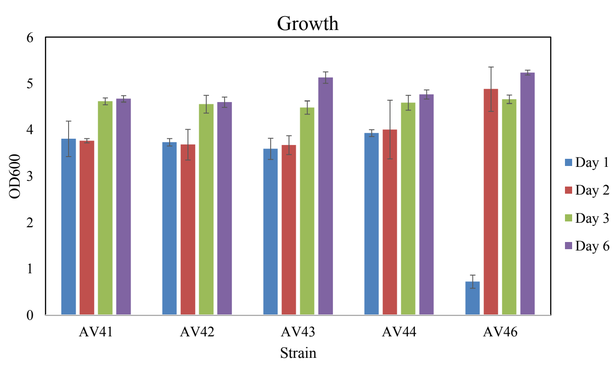
Displaying the volume of D-lactate present in BTM + 20% DMR AV43 cultures of different pH levels.

Displaying the volume of D-lactate present in BTM + 20% DMR AV46 cultures of different pH levels.

Production of D-lactate in BTM (no glucose) + 20% DMR cultures inoculated with 1 mM IPTG



Displaying the volume of D-lactate present in BTM + 20% DMR cultures of all strains.



Displaying the growth of all strains in BTM + 20% DMR cultures.

Conclusion We have demonstrated the conversion of biomass to the bioplastic precursor of PLA. We found that AV46, with the natural promotor, performs better than the other strains. Further pathway engineering and well controlled bioreactor studies can be performed to improve the product concentration rate.

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