DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Degradable Biocomposite Thermoplastic Polyurethanes

4/5/2023

Plastics Deconstruction and Redesign

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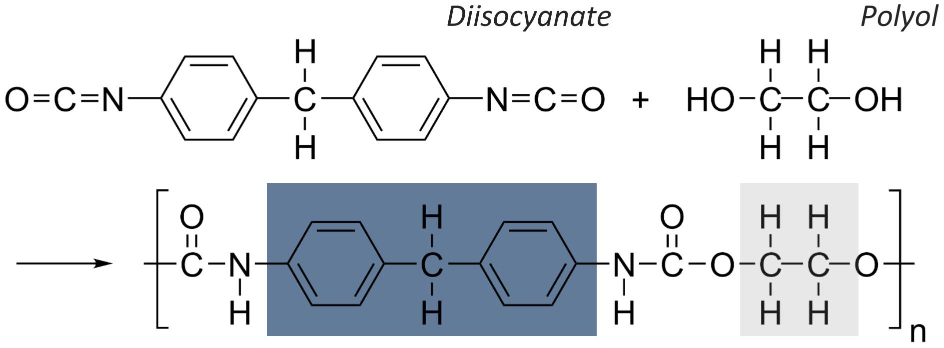
University of California, San Diego

Project Overview

Motivation and Background: Thermoplastic Polyurethanes (TPUs)

- **Specialty elastomer:** highly elastic / resistant to stress
- **Various applications:**
  - Automotive parts
  - Flexible tubes / hoses
  - Medical devices
  - Cases for electronic devices
  - Sports goods and footwear



RSC Adv., 6, 114453 (2016)

- **No commercially-accepted recycling stream for TPU.**
- **In the US alone, 1.3 M tons/year of TPU waste is generated*.**

1)

Approach

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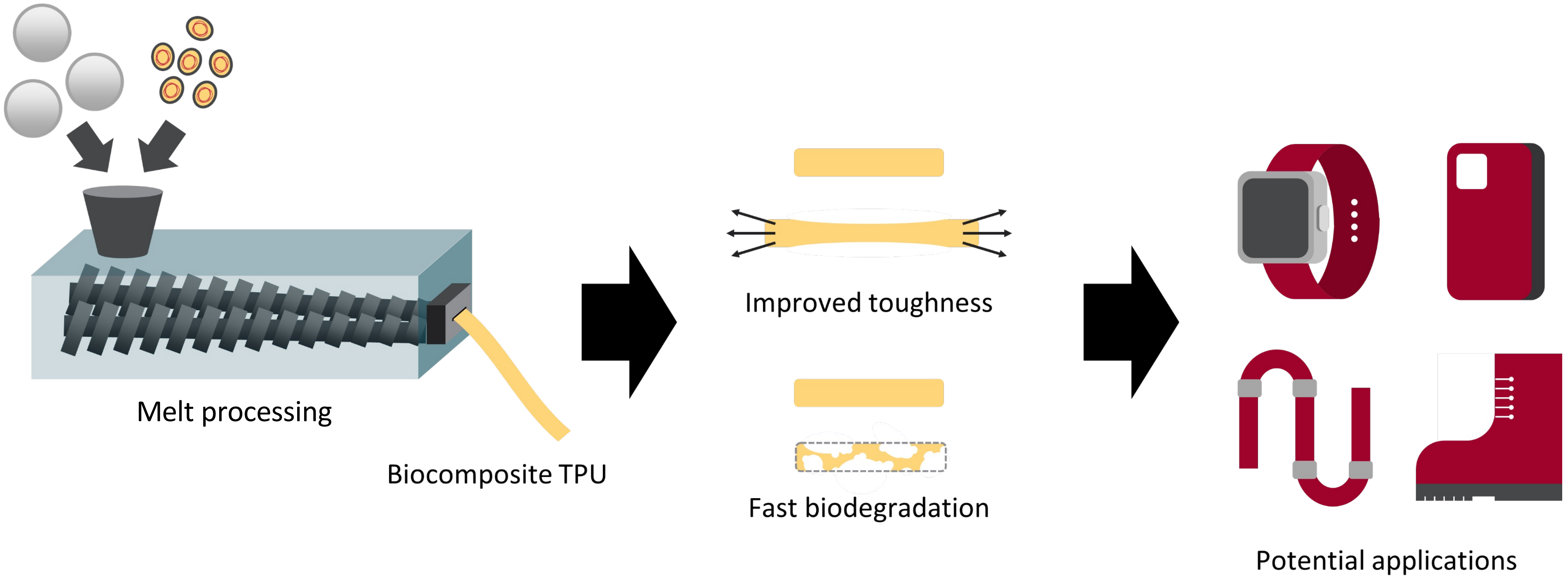
Generating Biodegradable TPUs while Improving Mechanical Properties

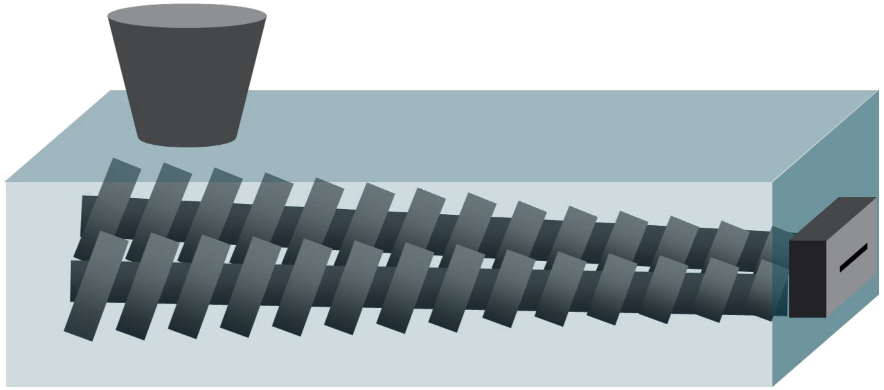
Incorporating **spore-forming, TPU-degrading bacteria** into TPU



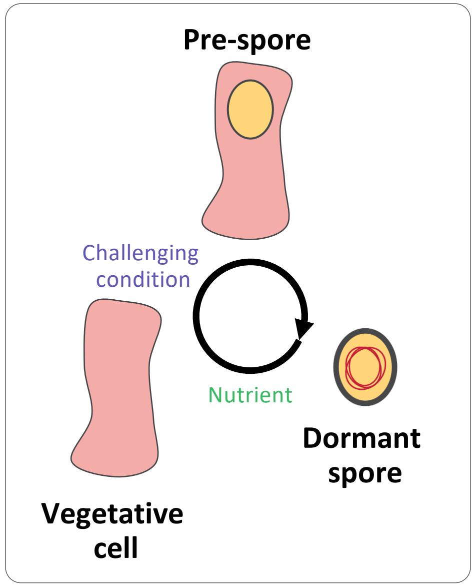
- Spores serves as a sub-micron filler to improve the mechanical properties of biocomposites
- Bacterial strains have demonstrated potential to enhance biodegradation

How do spores achieve our goals?

Why Choose Melt Processing?

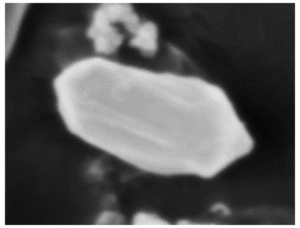


- ✓ **Scalable** process
- ✓ **Continuous** and solvent-free process
- ✓ Uniform **dispersion** of additive



Outer coat = 69 nm (Proteins)
Inner coat = 50 nm (Proteins)
Cortex = 132 nm (Peptidoglycan)
Core = 380 nm
Total = 631 nm

ISBN 978-1-4398-3415-2 (2010)



Bacterial spores

- ✓ **Submicron** / **Soft** / **Living / Green** filler materials for polymer
- ✓ Enhance **toughness** if dispersed
- ✓ Improve **biodegradation**

Bacillus subtilis for as a Polymer Filler

Polyester Degradation

Bacterial strain	Average weight loss (mg)	Visible degradation
Control – abiotic	0.0 ± 0.1	No
Control – autoclaved biomass	0.4 ± 0.1	No
Acinetobacter sp. ATCC 31012	1.2 ± 0.1	No
Aeromonas sp. ATCC 55641	1.4 ± 0.1	No
Bacillus sp. ATCC 19385	0.8 ± 0.3	No
Bacillus subtilis ATCC 6051	1.4 ± 0.3	Yes
Bacillus subtilis ATCC 21332	2.0 ± 0.1	Yes
Corynebacteria sp. ATCC 21744	0.9 ± 0.1	No
Corynbacteria hydrocarbooxydans ATCC 21767	0.8 ± 0.1	No
Delftia acidovorans soil isolate	1.2 ± 0.2	No
Escheria coli	0.5 ± 0.1	No
Pseudomonas sp. 273	0.6 ± 0.3	No
Pseudomonas aeruginosa PA01	1.5 ± 0.2	No
Pseudomonas fluorescens ATCC 13525	1.3 ± 0.1	No
Pseudomonas oleovorans ATCC 29347	0.5 ± 0.1	No
Pseudomonas putida ATCC 12633	1.0 ± 0.2	No
Rhodococcus sp. ATCC 29671	0.6 ± 0.2	No
Rhodococcus erythropolis ATCC 4277	0.6 ± 0.1	No
Rhodococcus rhodochrous ATCC 13808	0.6 ± 0.2	No
Streptomyces clavulegirus ATCC 27064	0.9 ± 0.1	No
Sphingobium herbicidovorans ATCC 70029	0.9 ± 0.1	No

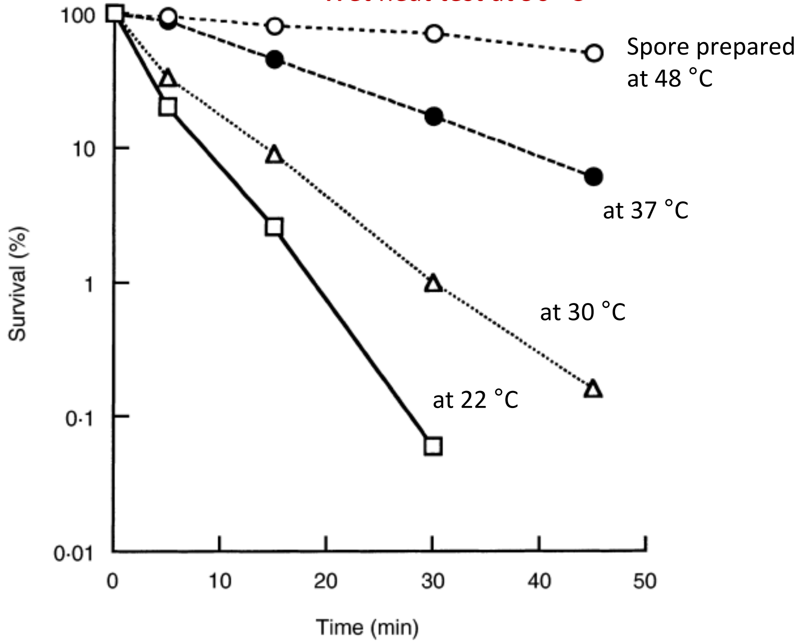
Polym. Degrad. Stab., 93, 1479-1485 (2008)

Non-Pathogenic

GRAS:
This bacterium is benign to human.

Appl. Microbiol. Biotechnol., 105, 6607-6626 (2021)
Nutrients, 13, 733 (2021)

Thermal Stability



J. Appl. Microbiol., 92, 1105-1115 (2002)

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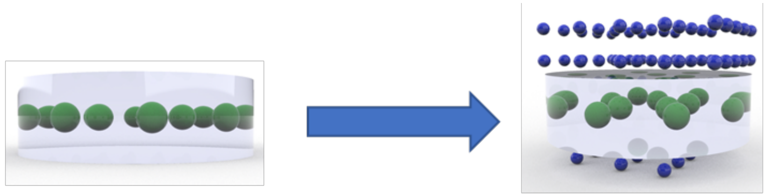
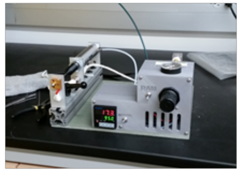
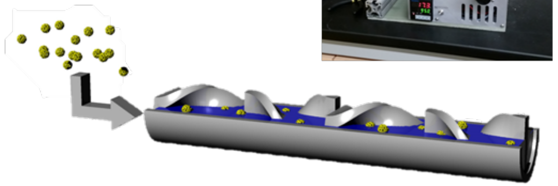
Iterative Feedback and Laboratory Evolution



Spore Germination/Proliferation
(UCSD, Pokorski & Feist)





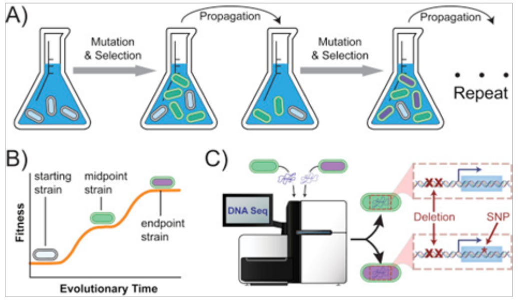
Pilot Processing of TPU Composites
(UCSD, Pokorski)

Spore Production

TPU Expertise

Scale-up & Commercialization
(BASF, Rahman)

Biodegradation
(UGA, Locklin)



Optimization of Bacterial Strains
(UCSD, Feist)

Challenges to the Technical Approach

- ☹ Spores can not survive temperatures relevant for extrusion
- ☹ Homogeneous dispersion of a uniform filler is critical to improve mechanical properties
- ☹ Spore must use TPUs as a sole carbon source at an appreciable metabolic rate

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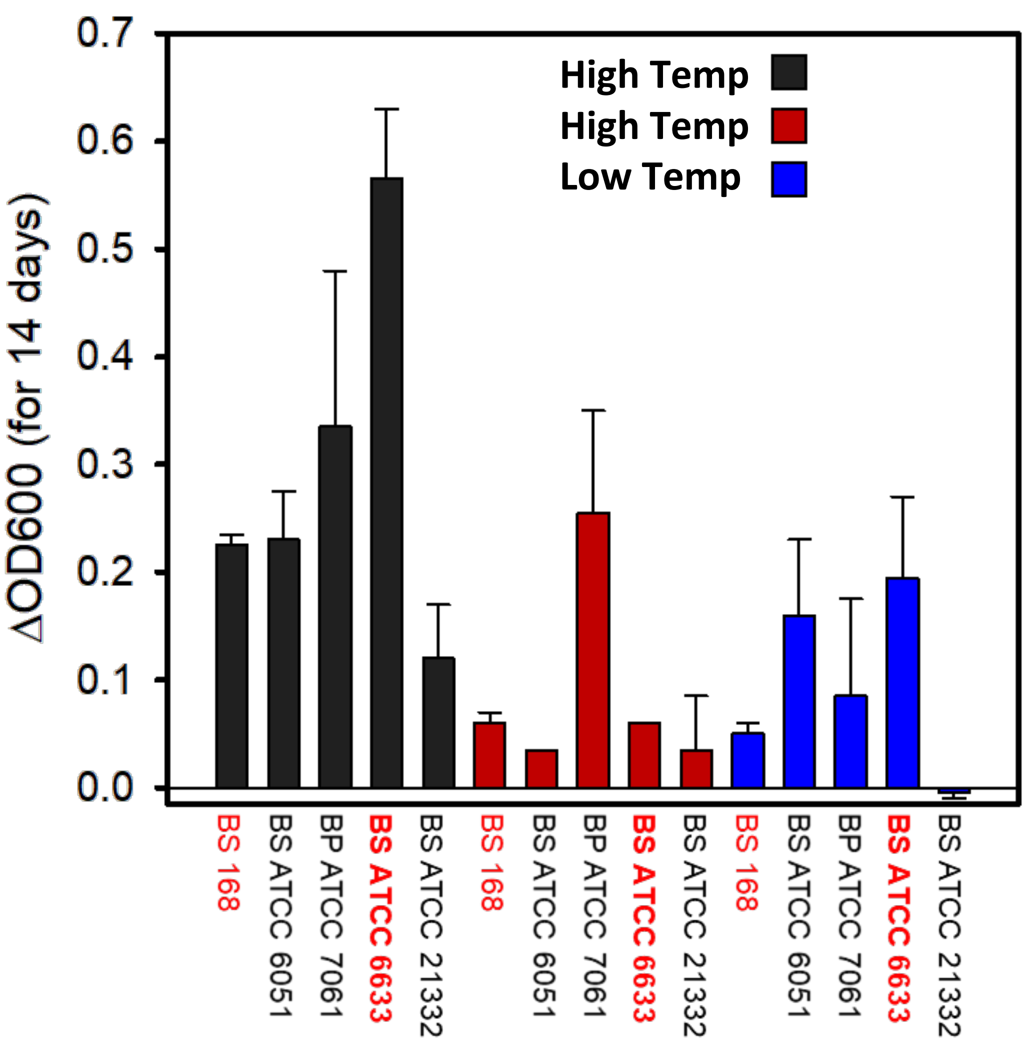
- ✓ Use evolution to generate heat shock tolerant strains
- ✓ Optimize mixing by varying temperature, screw speed, etc. during extrusion
- ✓ Screen and evolve strains to use TPU as a sole carbon source

Progress and Outcomes

Choosing Strains that Utilize TPU as a Sole Carbon Source



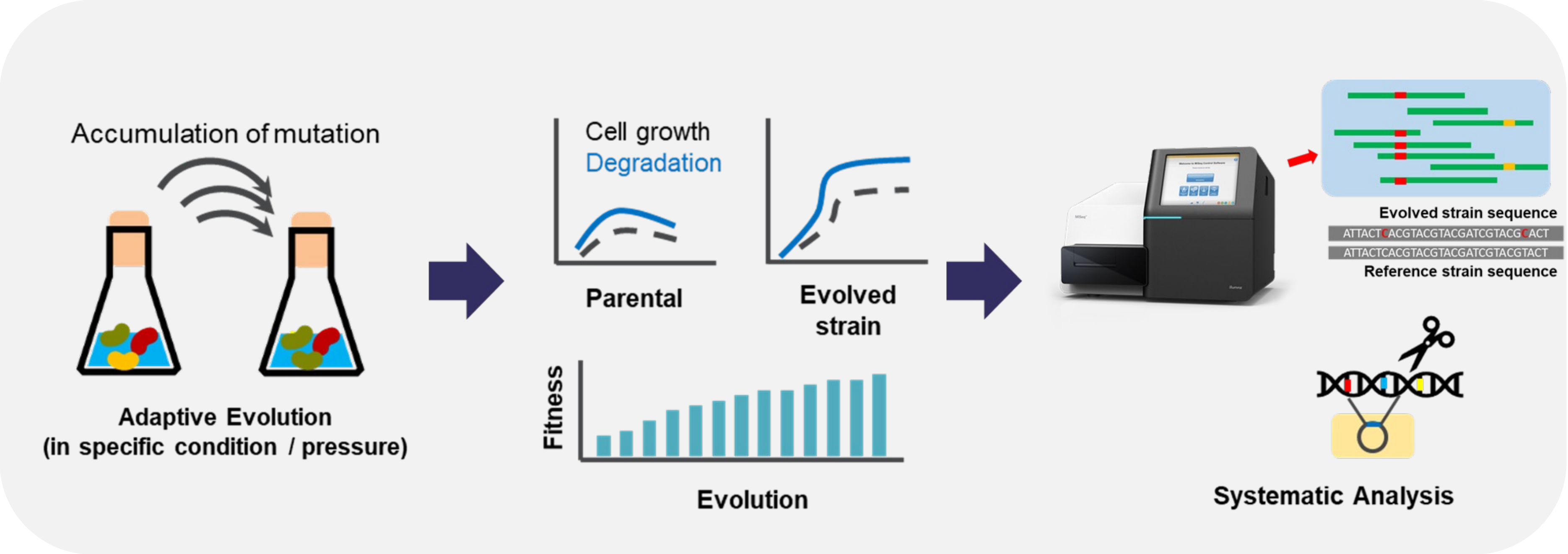
	TPU assimilation
ATCC 6633	High Temp (+) High Temp (+++) Low Temp (++) Low Temp (++)
BS168	High Temp (+) High Temp (++) Low Temp (-) Low Temp (-)
ATCC 6051	High Temp (-) High Temp (+++) Low Temp (++) Low Temp (N/A)
BP ATCC 7061	High Temp (++) High Temp (+++) Low Temp (+) Low Temp (N/A)
ATCC 21332	High Temp (-) High Temp (+) Low Temp (-) Low Temp (N/A)

- Low temperature polymers are soft and processed at ~135 °C
- High temperature polymers are more mechanically robust and processed at ~170 °C

Overall Implementation of ALE

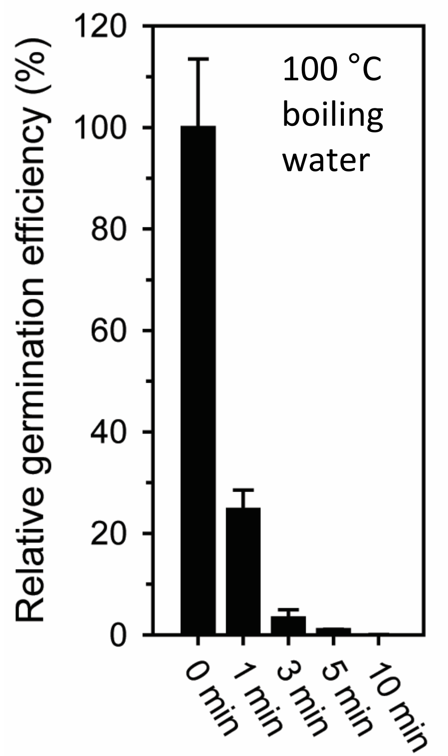
Adaptive Laboratory Evolution (ALE)

- Engineering strategy relies on naturally occurring mutations
- Mutations that meet specific conditions can be accumulated to have the desired phenotype
- Evolved strains can be analyzed through DNA/RNA sequencing

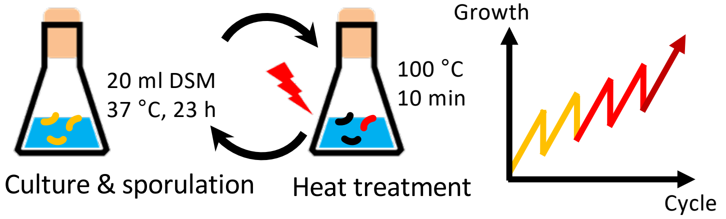


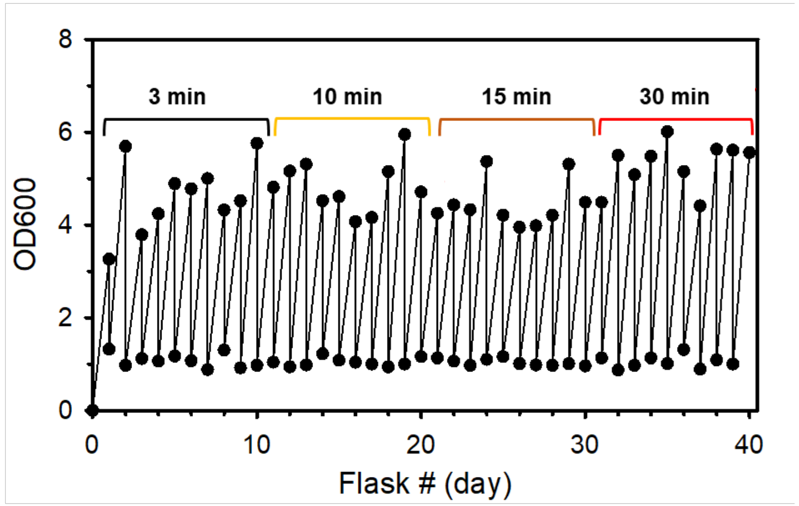
Adaptive Laboratory Evolution of *B. subtilis: Thermal Tolerance*

Heat Stability of WT *B subtilis*

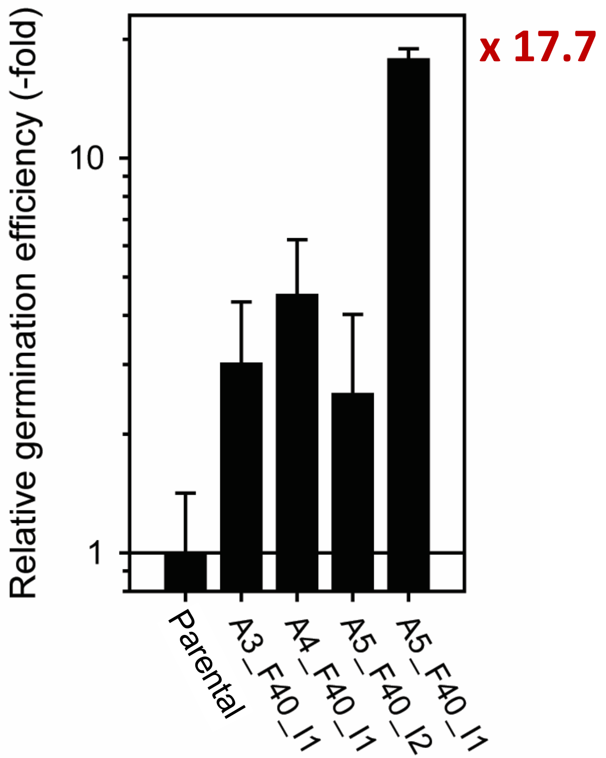


Adaptive Laboratory Evolution (ALE)



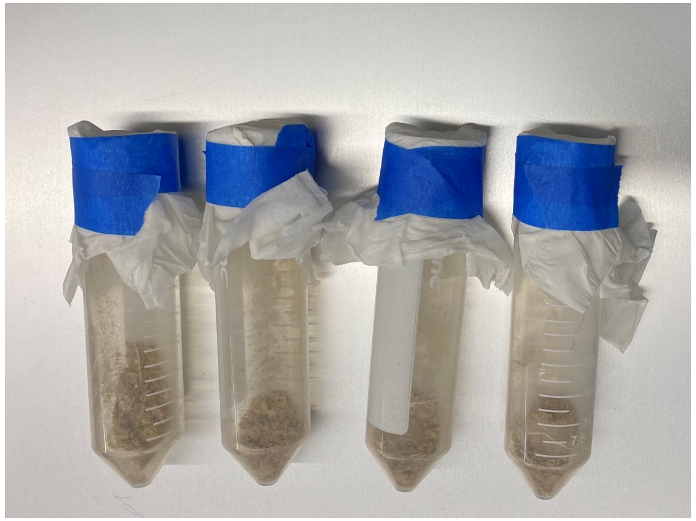


Improved Heat Stability after ALE



Production of *B. subtilis* Spore

Cultivation
Sporulation
Purification
Lyophilization

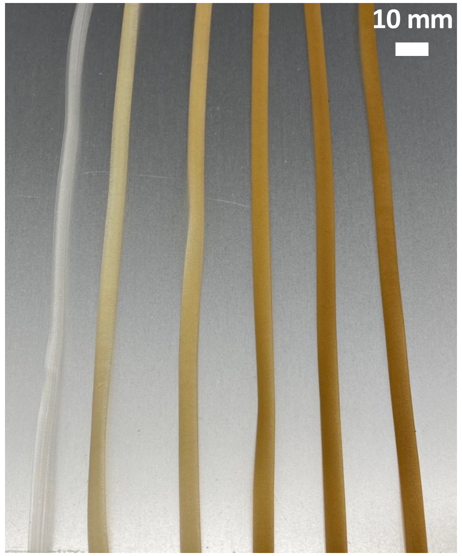


Spore powder

Fabrication of Biocomposite TPU

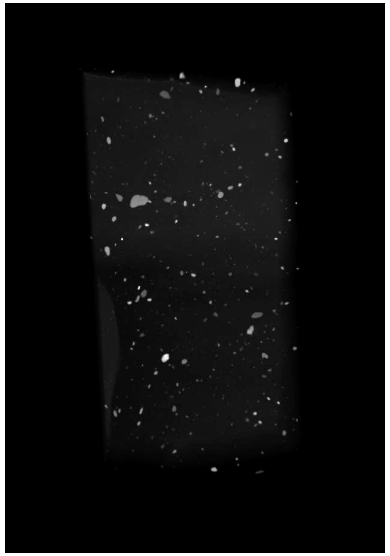
135 °C / 36 rpm / 5 min

135 °C / 36 rpm / 15 min



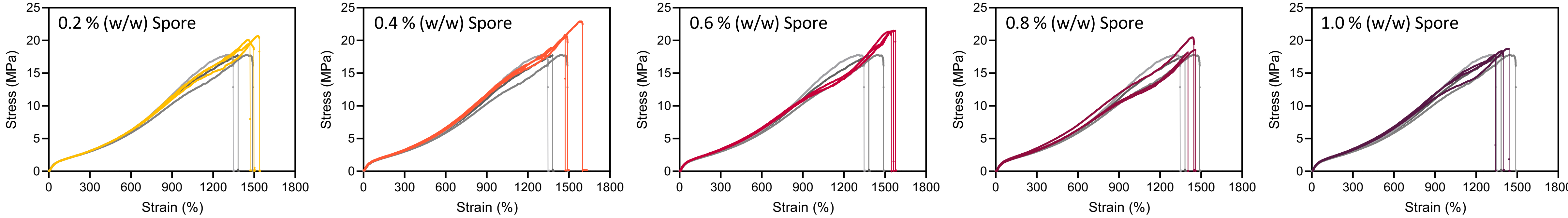
0% 0.2% 0.4% 0.6% 0.8% 1.0%

Spore loading (w/w)

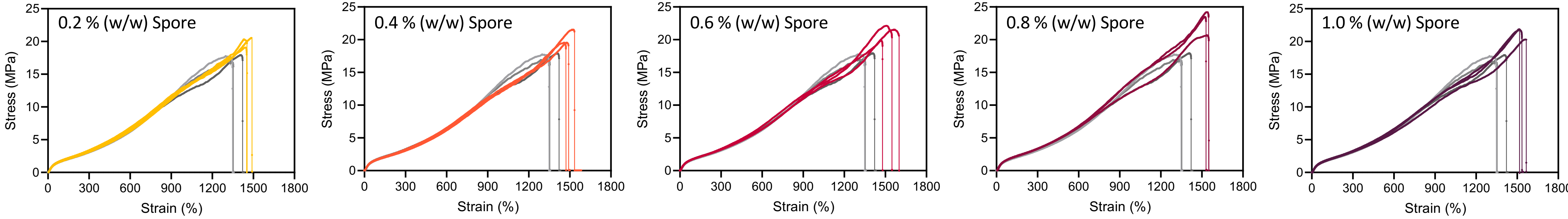


Tensile Testing

Biocomposite TPUs with ATCC6633 wild type (WT)

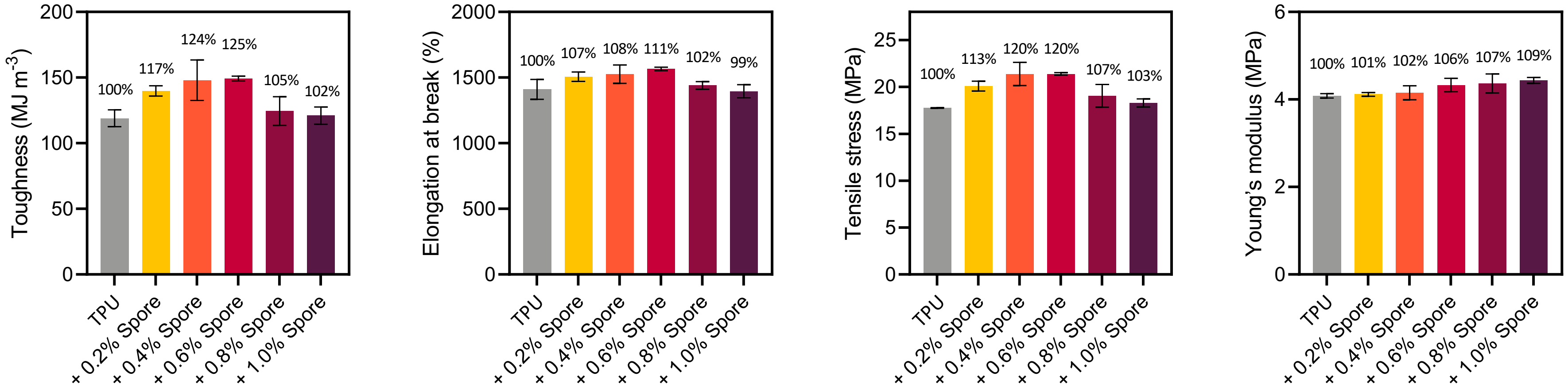


Biocomposite TPUs with ATCC6633 Heat Shock TALE (HST)

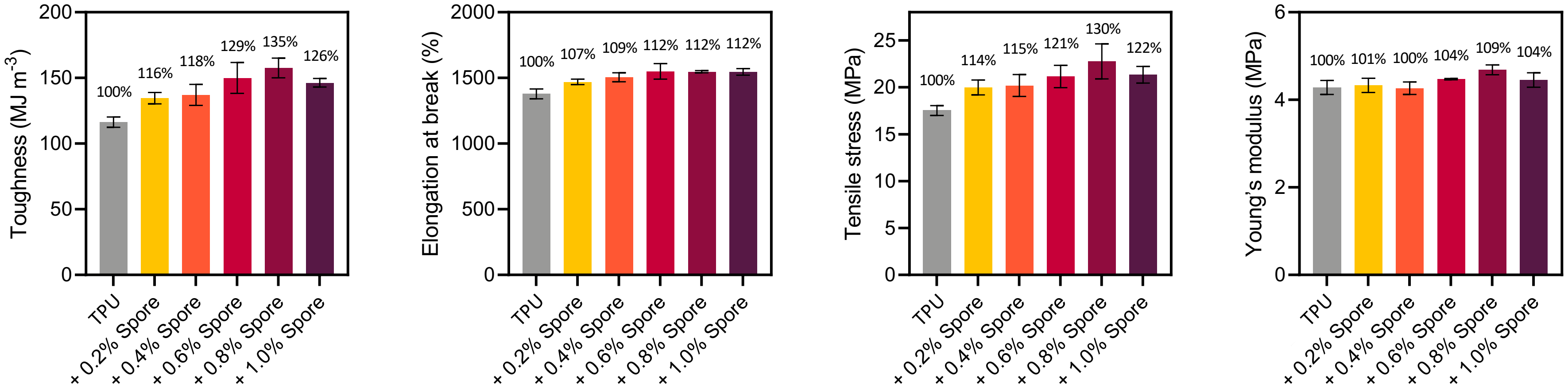


Heat Tolerant Spores Improved Tensile Toughness

ATCC6633 WT

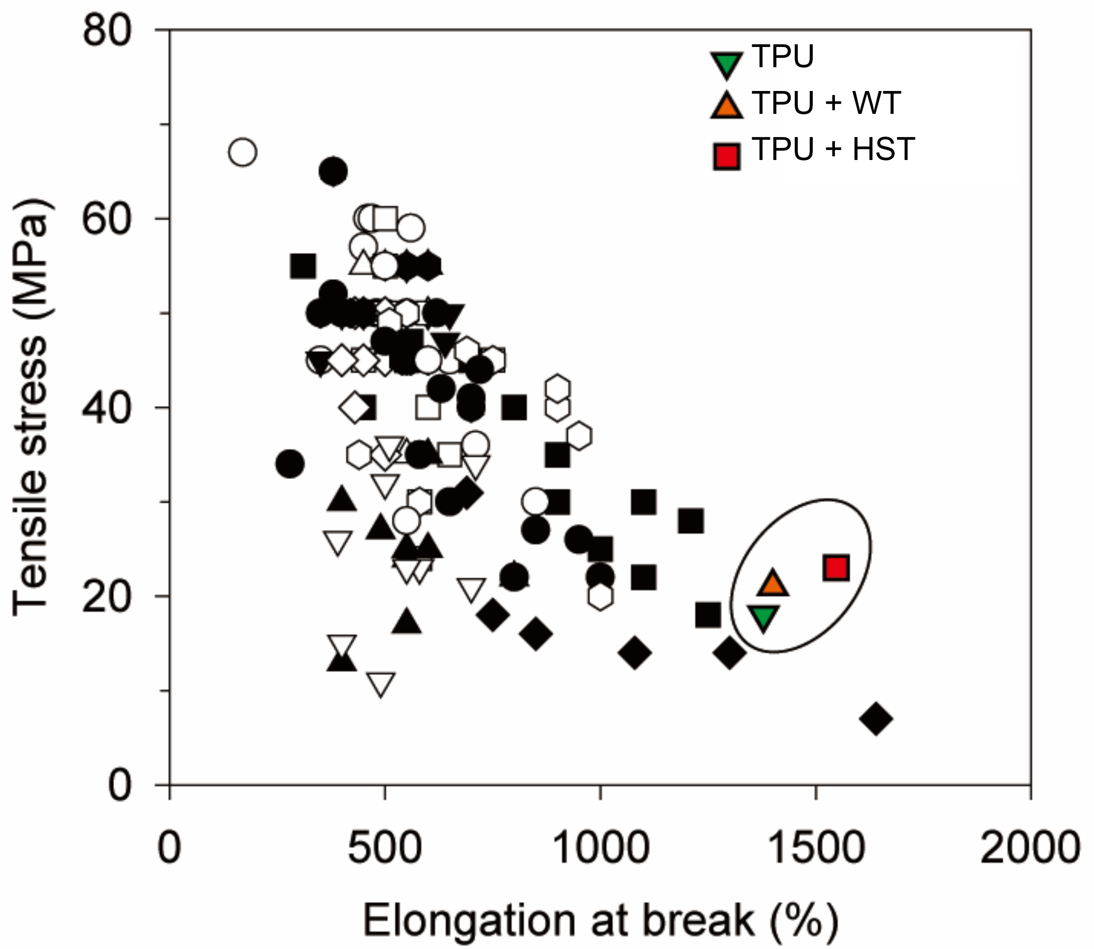


ATCC6633 HST



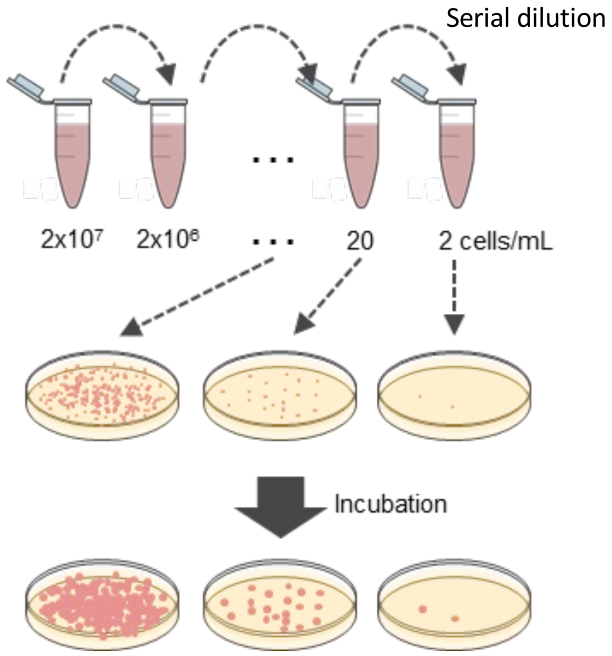
- **35% Improvement in Toughness, 12% EAB, 30% Tensile Stress**

Spores Significantly Improved the Tensile Properties of TPUs

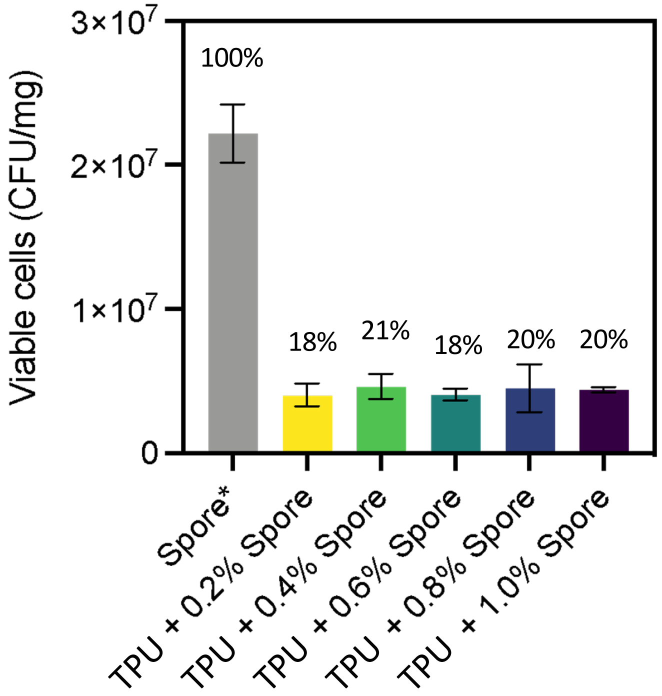


Spores Retained Viability after Melt Processing (135 °C / 36 rpm)

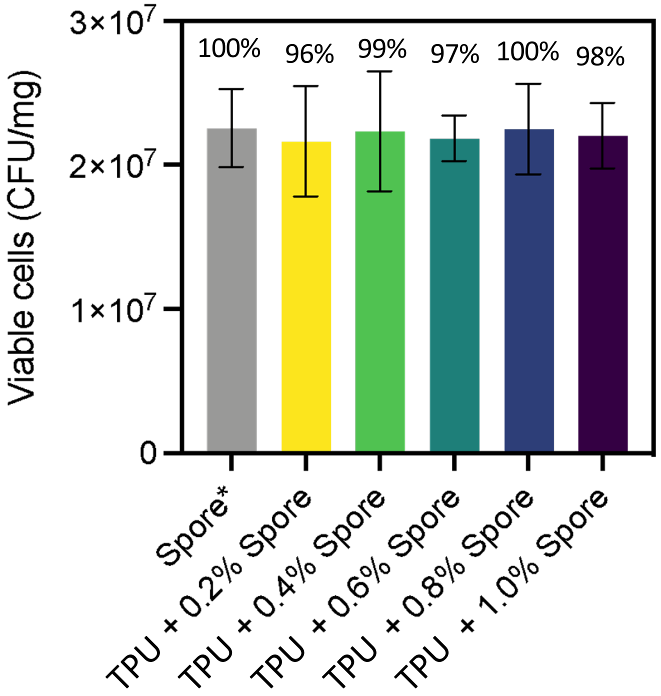
Colony forming unit (CFU) assay



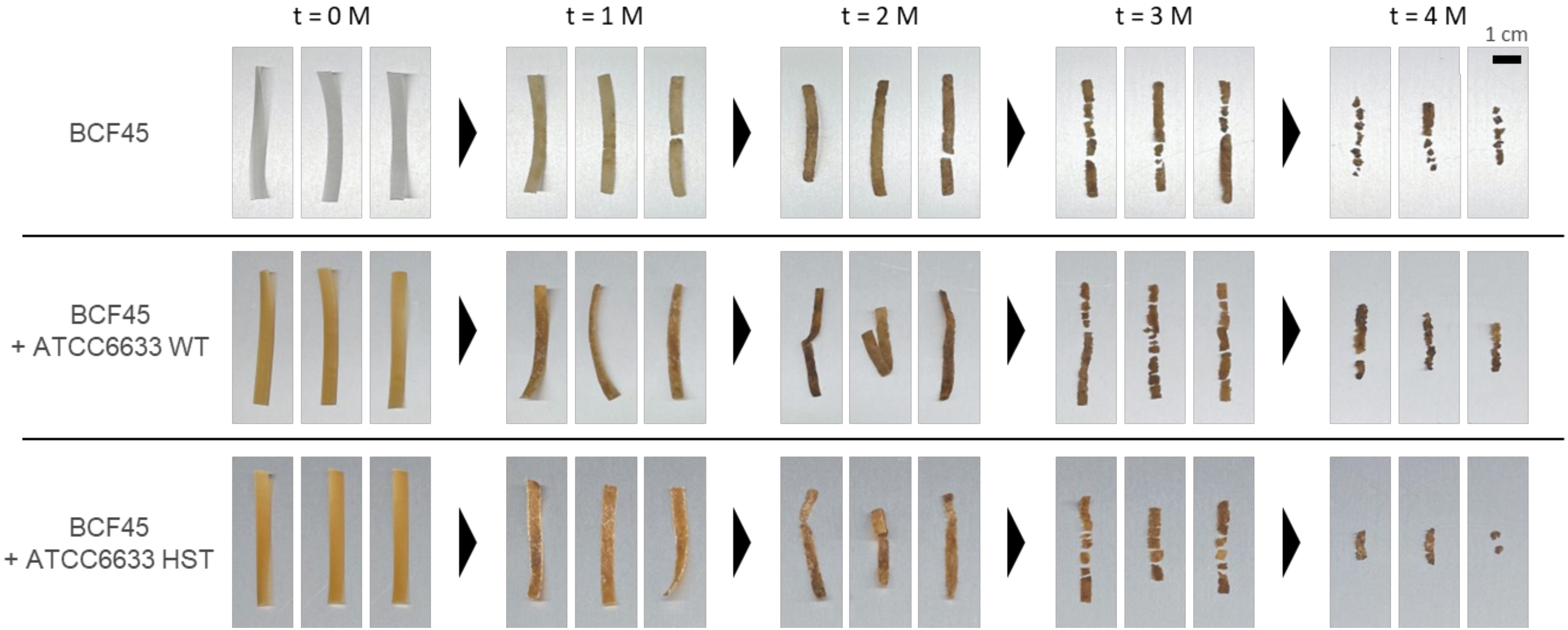
ATCC6633 WT



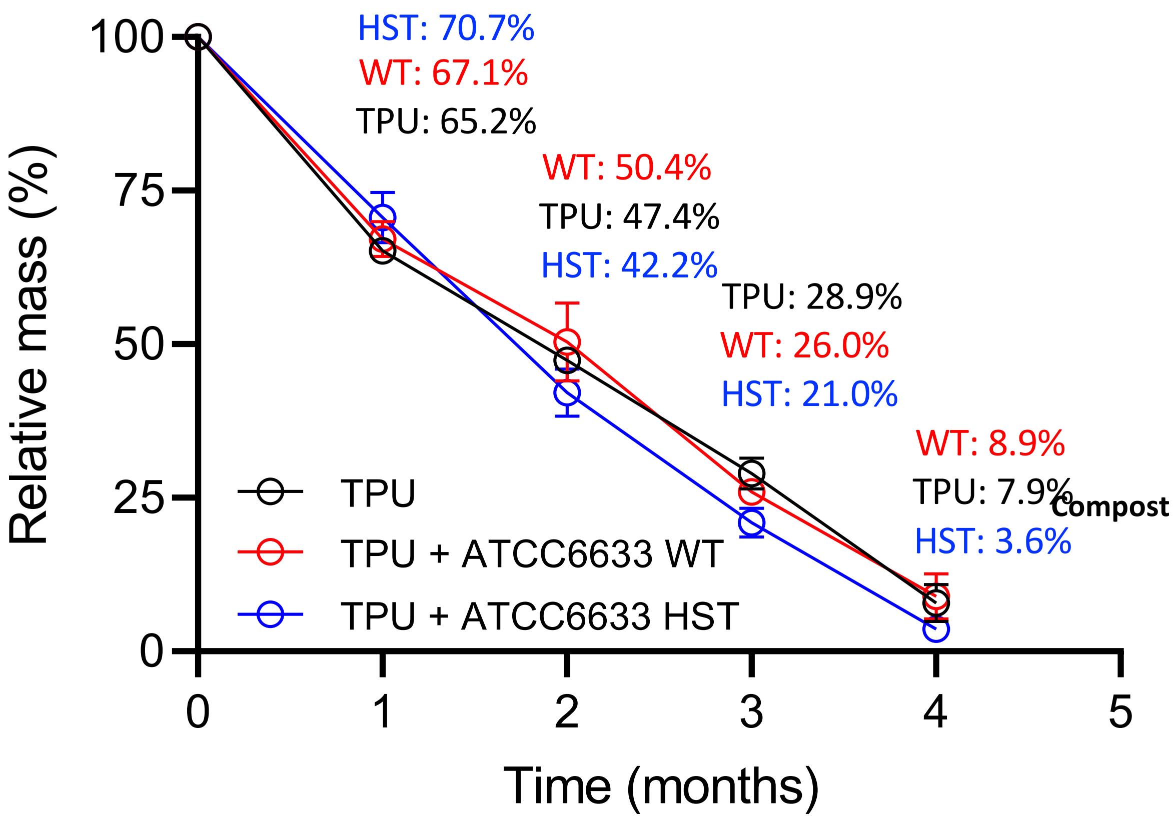
ATCC6633 HST

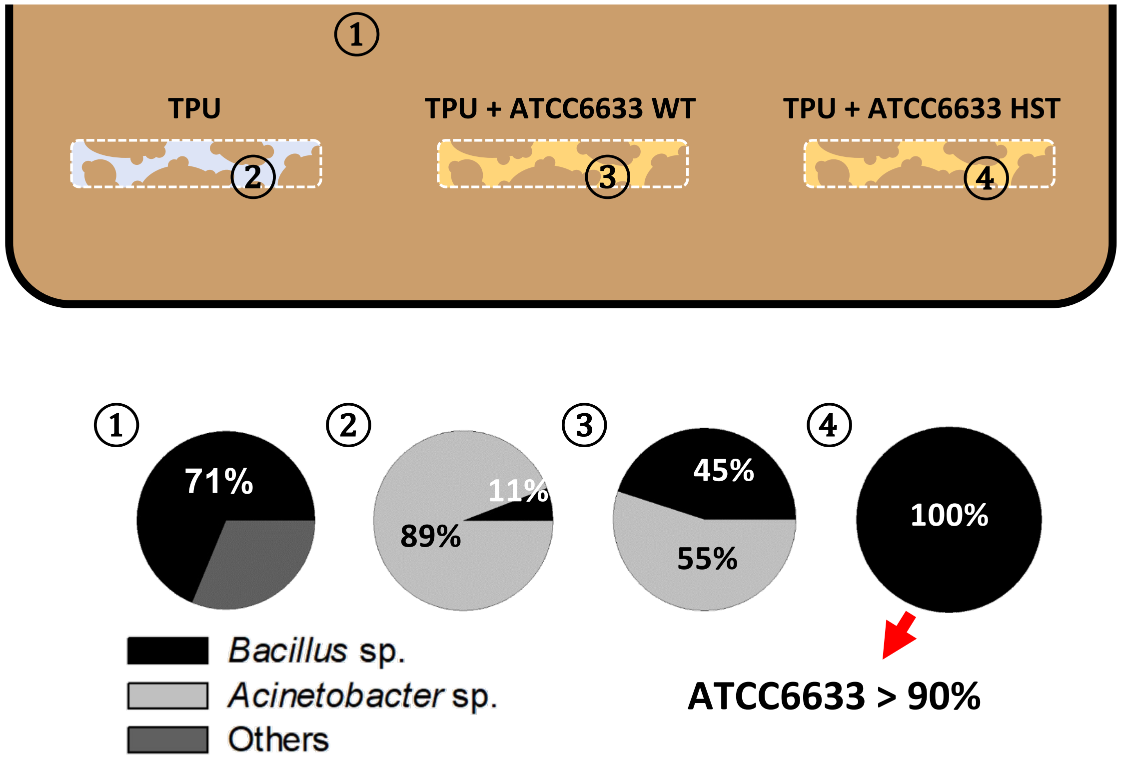


Biodegradation



Biodegradation and Microbial Consortium





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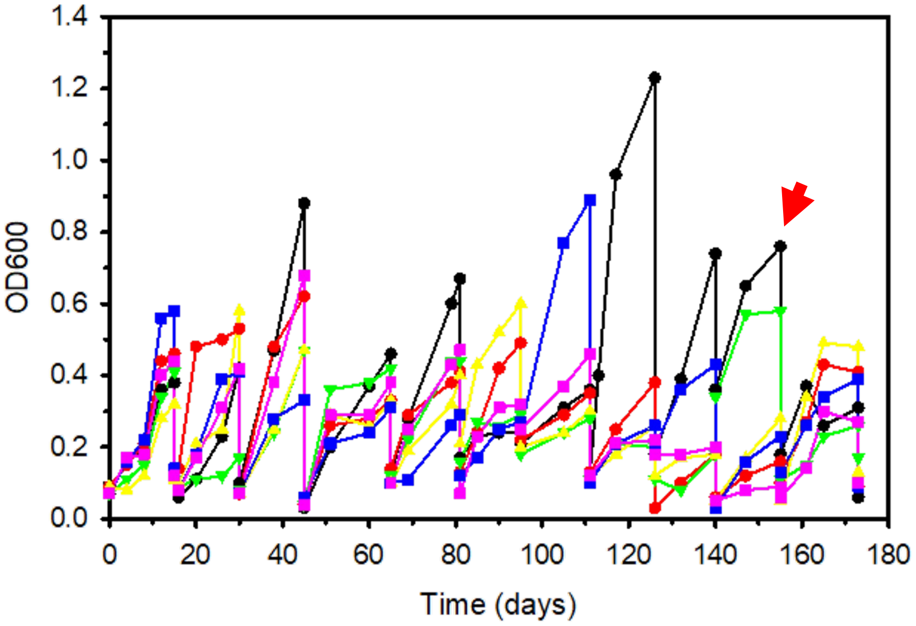
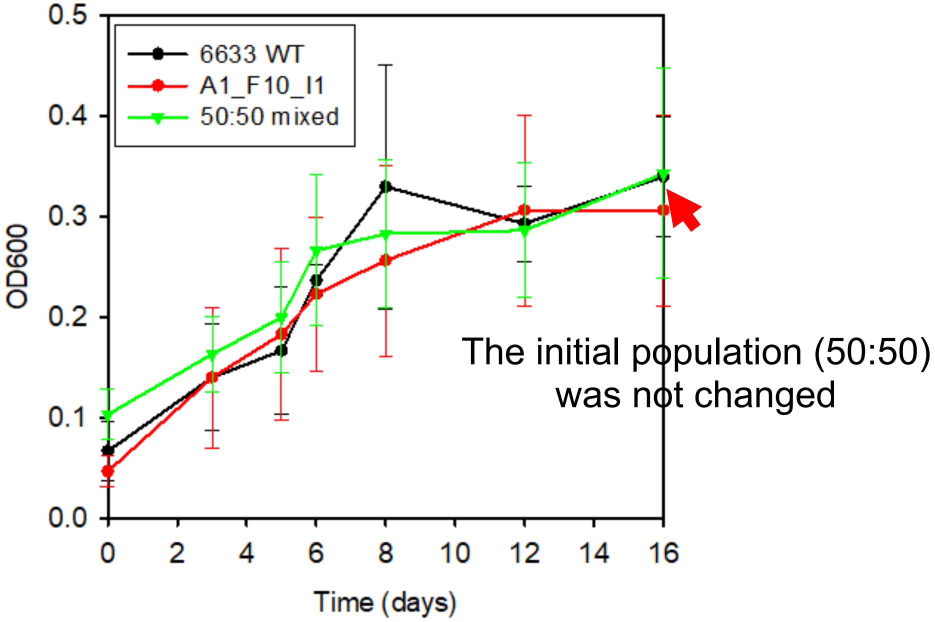
On Going Work: TPU degradation ALE of ATCC 6633

- 10 g/L TPU 785A (powder) was supplied as a sole carbon source
- ALE experiments have been conducted for 6 months

  - Growth too slow to accumulate mutations, so far

785A 10, 12th flask, ~ 6 months





Impact

Summary/Conclusions

- Evolutionary engineering of *B. subtilis* improved the heat tolerance of spore by up to **17.7-fold** in boiling water (100 °C for 10 min).
- Toughness of TPUs was increased by up to **35%** with spore addition.
- Spores retained **~100%** viability after melt processing of TPU at 135 °C at 36 rpm.
- Evolved spores were able to colonize TPU material and serve as the primary strain for degradation.
- ALE for degradation is the major priority for the remainder of the project

Quad Chart Overview

Timeline
- *4/1/21*
- *3/31/24*

	FY22 Costed	Total Award
DOE Funding	*4/01/2021 – 3/31/2024*	*$2,088,114*
Project Cost Share *	$522,129	

TRL at Project Start:
TRL at Project End:

Project Goal
Improve degradability of thermoplastic polyurethanes while improving their mechanical properties by incorporating evolved bacterial spores.

End of Project Milestone
Spores show viability >20% versus control, mechanical properties of the composite are improved by 10%, and degradation kinetics in excess of 50% versus control TPUs.

Funding Mechanism
BOTTLE

Project Partners*
- Partner 1
- Partner 2

*Only fill out if applicable.