

# Biotechnological Valorization of PLA Hydrolysates via Microbial PHA Synthesis by *Pseudomonas* spp.

Katerina Foka<sup>1</sup>, Kleoniki Mitili<sup>1</sup>, Efstratios Nikolaivits<sup>1</sup>, Christina Ferousi<sup>1</sup>, Christina Gkountela<sup>2</sup>, Stamatina Vouyiouka<sup>2</sup>, Evangelos Topakas<sup>1\*</sup>

<sup>1</sup>Industrial Biotechnology & Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

<sup>2</sup>Laboratory of Polymer Technology, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

\*vtopakas@chemeng.ntua.gr

## Introduction

Poly(lactic acid) (**PLA**) is a widely used bioplastic, but its presence in recycling streams creates challenges for plastic recovery. Hydrolysis of PLA generates **lactic acid**, which can serve as a valuable substrate for microbial bioconversion. While *Ralstonia eutropha* is the only bacterium known to produce polyhydroxyalkanoates (**PHAs**) directly from lactic acid<sup>1</sup>, *Pseudomonas* spp. represent a promising alternative due to their metabolic flexibility and capacity for synthesizing diverse PHA types. This study investigates the ability of selected *Pseudomonas putida* strains to utilize lactic acid and PLA hydrolysates for growth and PHA production, advancing strategies for sustainable bioplastic upcycling.

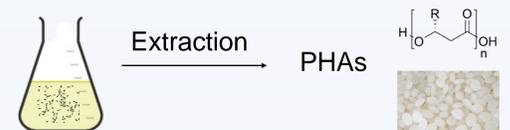
1. Huschner F, Grousseau E, Brigham CJ, Plassmeier J, Popovic M, Rha C, Sinskey AJ (2015)

*Process Biochemistry* 50:165–172

2. Jiang Y, Mikova G, Kleerebezem R, van der Wielen LAM, Cuellar MC (2015) *AMB Express* 5:1–13

## Materials & Methods

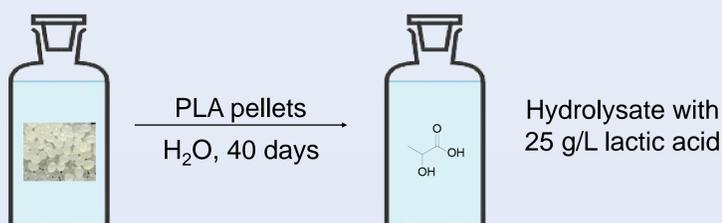
- ✓ *Pseudomonas putida* strains Mt2, Ca3 & KT2440 were cultivated in mineral salts medium using **lactic acid** and **PLA hydrolysate** as the sole carbon source.
- ✓ For lactic acid cultures two PHA extraction protocols<sup>2</sup> were compared:
  1. **0.2 M NaOH, 30 min** → aimed at maximum recovery.
  2. **0.2 M NaOH + 0.2 % w/v SDS, 1 h** → aimed at maximum purity.
- ✓ **Nitrogen limiting conditions** were also tested only with pure lactic acid to assess their effect on PHA accumulation.



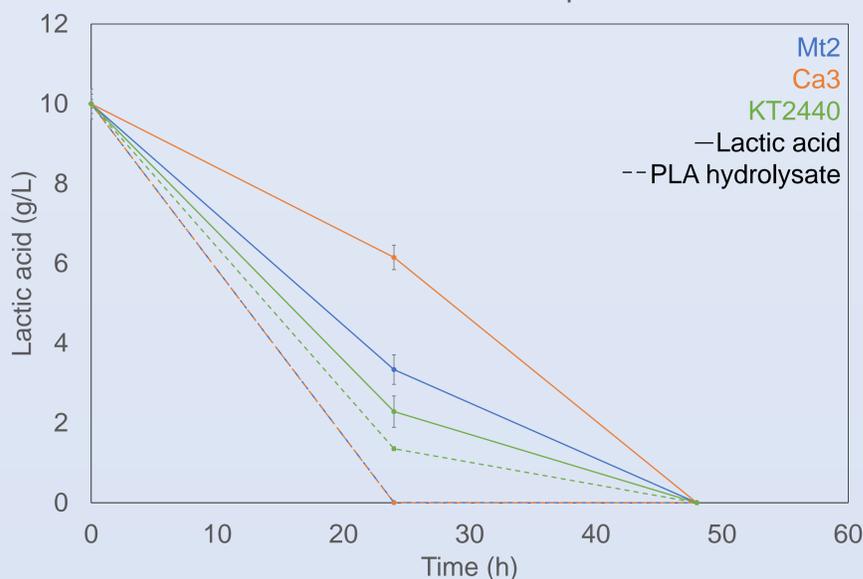
## Results & Discussion

Strain/ N-condition	0.2 M NaOH, 30 min		0.2 M NaOH + 0.2 % w/v SDS, 1 h	
	PHAs (%DCW)	PHAs (g/L)	PHAs (%DCW)	PHAs (g/L)
Mt2	45.85 ± 3.36	1.28 ± 0.12	38.96 ± 5.22	1.09 ± 0.18
Mt2 N-depletion	63.70 ± 2.08	0.77 ± 0.05	<b>62.94 ± 2.92</b>	0.76 ± 0.06
Ca3	27.80 ± 2.31	0.65 ± 0.05	23.73 ± 2.46	0.65 ± 0.06
Ca3 N-depletion	59.04 ± 5.01	0.96 ± 0.11	55.53 ± 4.92	0.90 ± 0.5
KT2440	48.65 ± 4.07	<b>1.41 ± 0.12</b>	43.26 ± 3.36	<b>1.26 ± 0.10</b>
KT2440 N-depletion	<b>67.01 ± 0.34</b>	1.11 ± 0.01	54.53 ± 2.61	0.90 ± 0.04

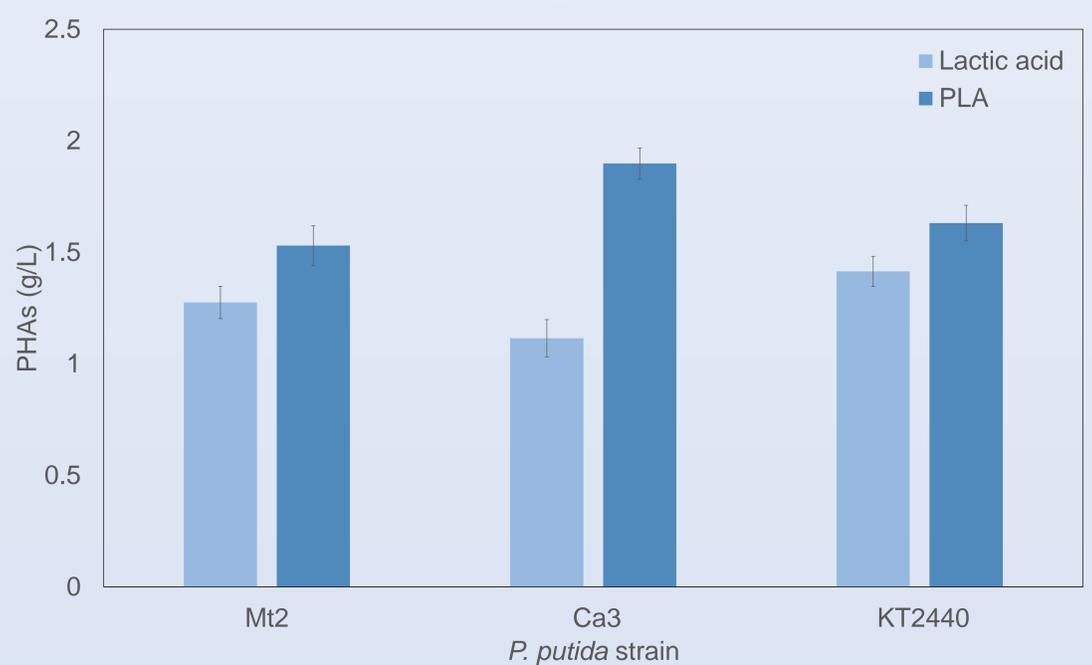
- ✓ Both extraction protocols produced **comparable amounts of PHAs**, with extraction using only NaOH resulting in slightly higher yields.
- ✓ Under nitrogen limitation, **PHA concentration increased** while **absolute yields decreased**.
- ✓ Among the tested strains, **KT2440 performed best**.



Substrate consumption



PHAs production



- ✓ All strains consumed lactic acid from **PLA hydrolysate** more rapidly than pure lactic acid.
- ✓ PHA titers (g/L) were consistently higher with the hydrolysate.
- ✓ **Ca3** emerged as the best-performing strain.

## Acknowledgements

The research project "EnZyReMix – Chemoenzymatic Recycling of Mixed Plastic Waste" is implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union – NextGenerationEU (H.F.R.I. Project Number: 15024).