

## Master of Science HES-SO in Life Sciences

# Specificity of an isolated thermophilic isolate regarding poly(3-hydroxyalkanoate) production

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## DESCRIPTION

Poly(3-hydroxyalkanoates) (PHAs) are a class of **polyesters** that can be produced from renewable and biobased feedstocks. PHAs are **biosynthesized** and stored as intracellular granules in microorganisms such as bacteria, yeasts, or archaea. They present thermoplastic to elastomeric properties depending on their monomeric units and are therefore defined in two main categories: **short chain length** (scl, **less** than **6 carbons**, thermoplastic), and **medium chain length** (mcl, **more** and equal than **6 carbons**, elastomeric). One current focus is to study **thermophilic strains** and processes to reduce PHA production cost by mitigating the cooling power needs by operating at higher temperatures. Moreover, thermophilic microorganisms also present the advantage of **limiting the risk for contamination** by mesophiles during cultivation.

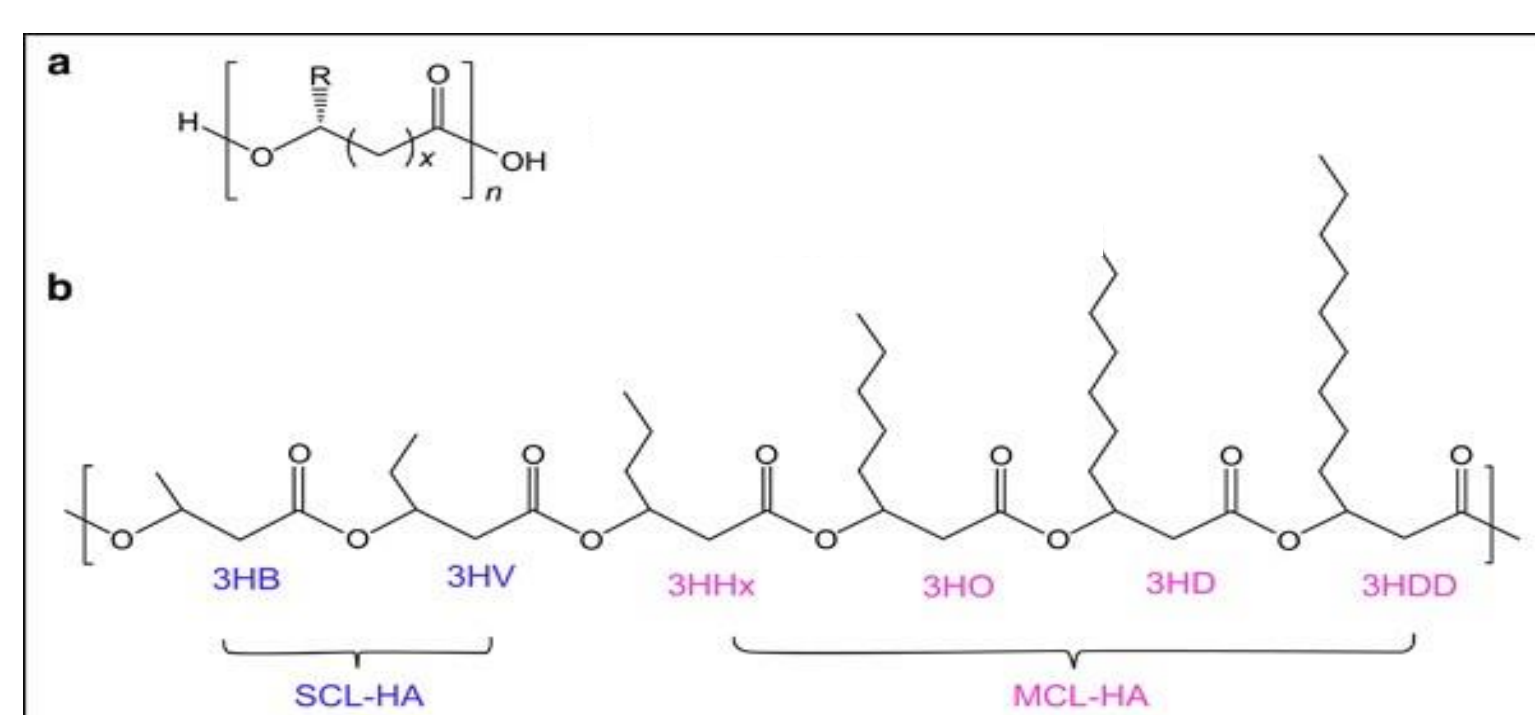


Figure 1 : Picture of the general molecular formula of PHAs. (a) General chemical composition of a PHA monomer. (b) Some commonly synthesized short-chain-length PHA monomers (scl-PHA)

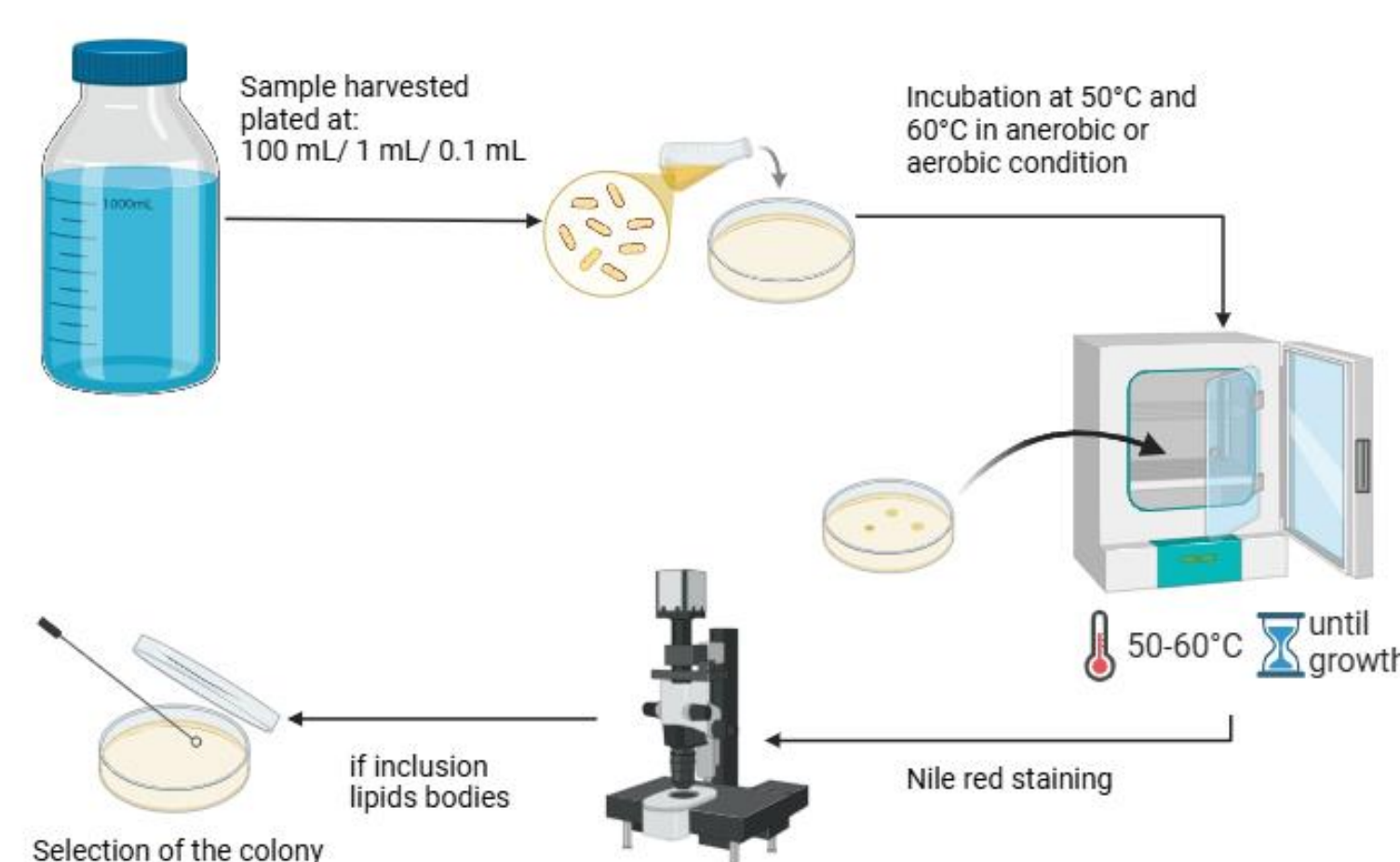
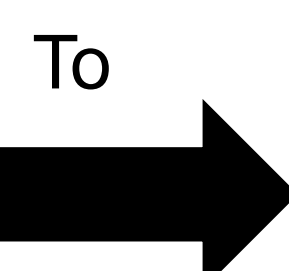


Figure 2: description on BioRender of the isolation process used to obtain the strain of interest of the X03 project

## OBJECTIVES

During the previous work: X02, many strains were isolated from hot spring in Switzerland. One of them was selected, as it shown initial hint of being able to produce PHA.

From hot springs



Bioplastics



The **objectives** of this project are:

- **identify** this new isolated strain
- confirm the **PHA production** capacity
- obtain a general understanding of the **PHA pathways** of this strain
- understand the **condition of accumulation** needed to enhance its production capacity

## RESULTS

### Results overview:

- Biomass analyses showed scl-PHA by gas chromatography upon methanolysis with a cell content of **25 wt%**.
- Microscopic observations revealed a **pleomorphic strain**
- **3HV** accumulated on valeric acid shows a yield up to **70 mol%**
- **P(3HB-3HPte)** with a monomeric composition of 3HPte up to **90 mol%**

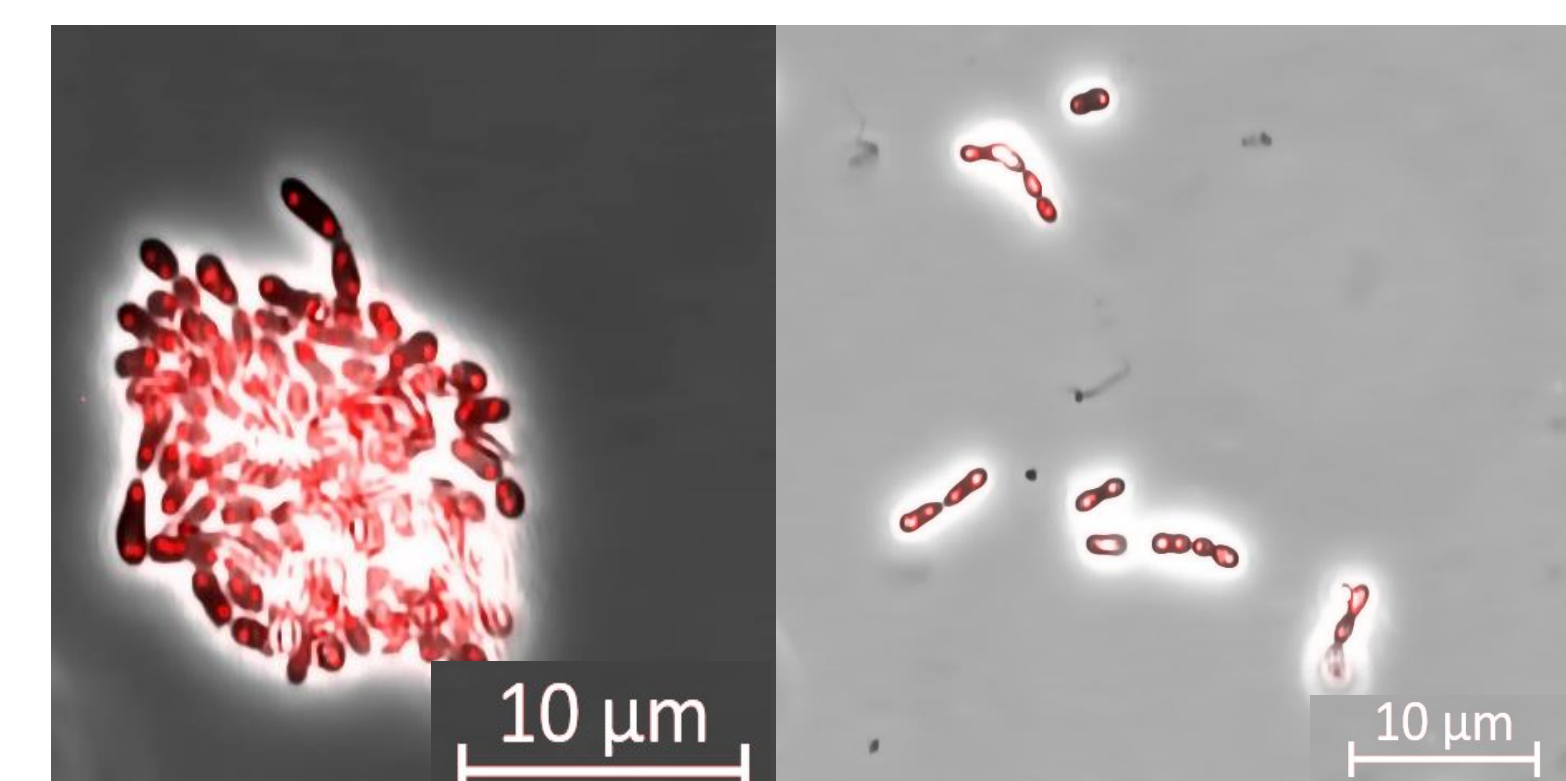


Figure 3: Nile red staining of PHB in liquid *Thermus* media : start (left) and after accumulation for 12h (right).

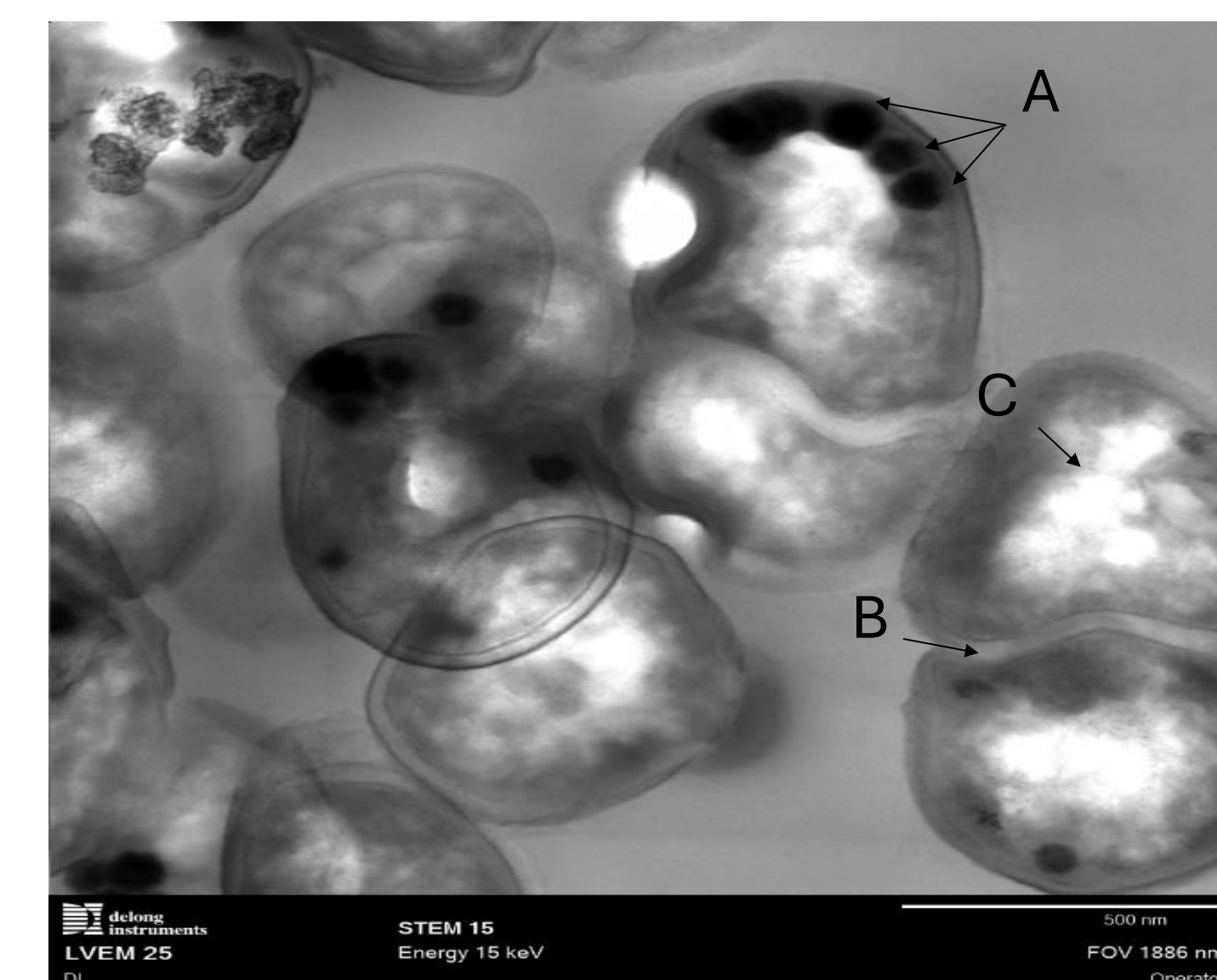
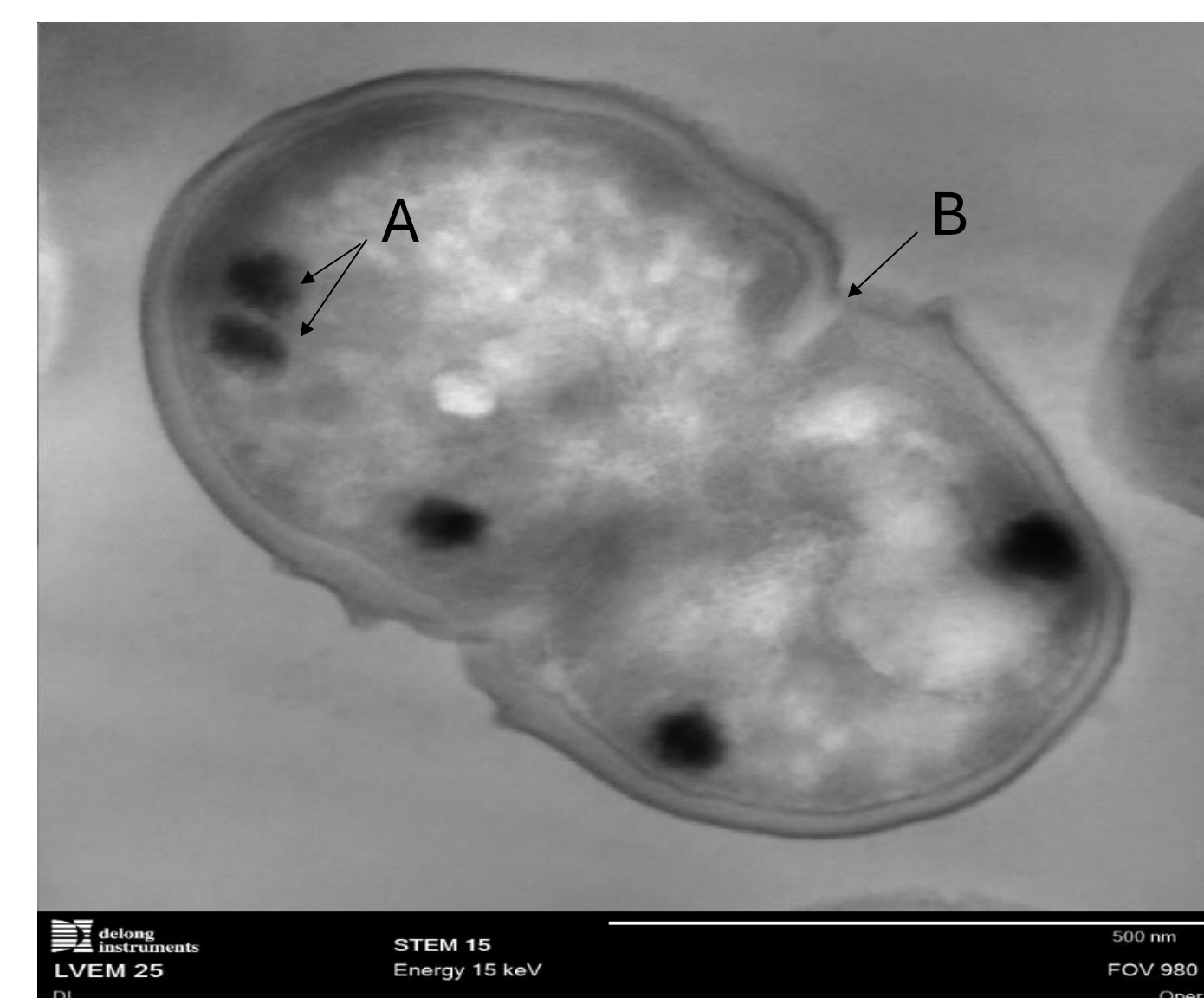


Figure 4: Picture of *Rubrobacter* sp. using TEM, a single duo of diplococcus cells (left), multiple cells simultaneously (right) with A: intracellular dense structures or microcompartments with a hexagonal shape, B: cell wall between 2 cells, and C: low density electron structure in granules shape identified as PHA's granules.

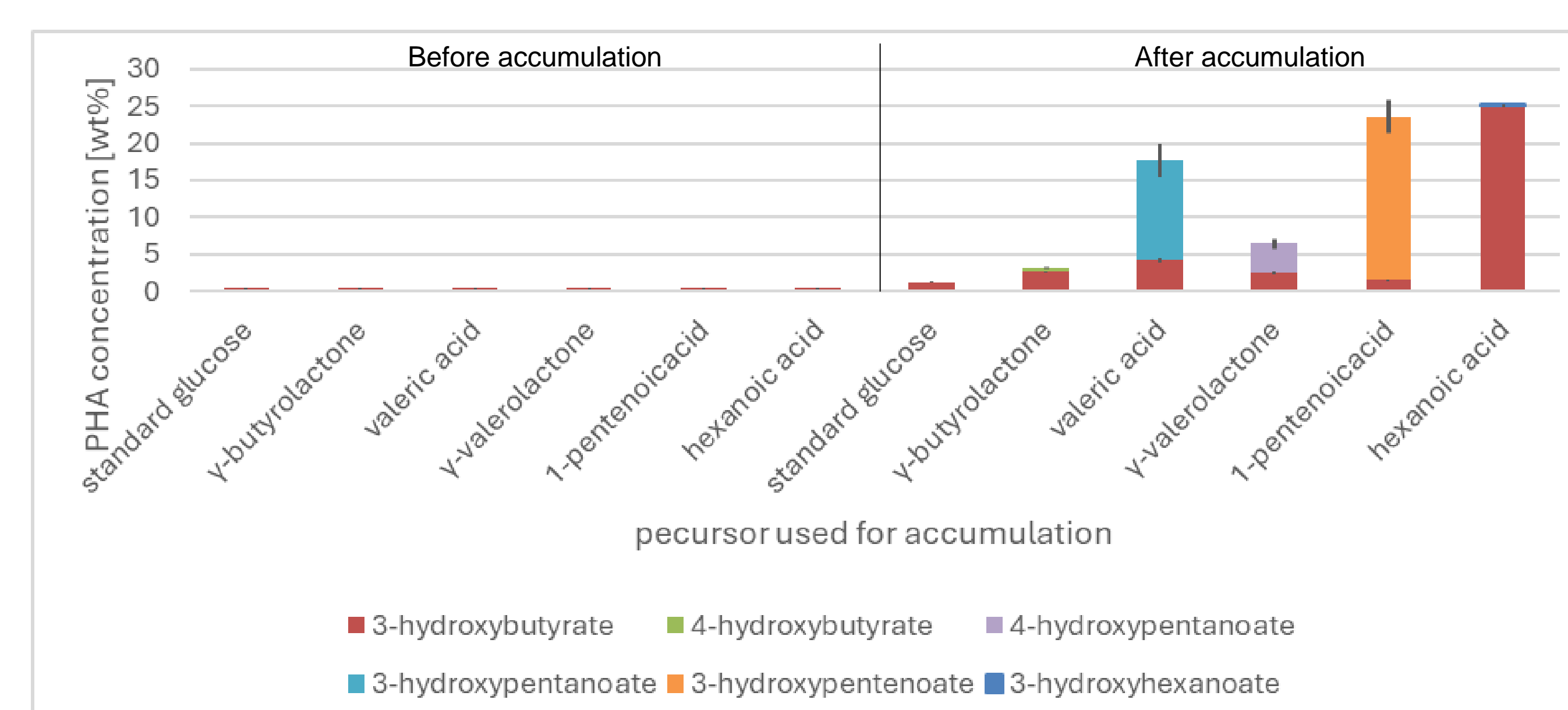


Figure 5: Precursor test with glucose,  $\gamma$ -butyrolactone, valeric acid,  $\gamma$ -valerolactone, 1-pentenoic acid, and hexanoic acid, the culture grew for 24h and 100 ml were taken for GC measurement (left). Then the precursor was added in the culture at 0.5 g L<sup>-1</sup> for 24h and the biomass was harvest (right).

## CONCLUSION

We identified a thermophilic strain call: ***Rubrobacter* sp.** We also found 2 type of polymerases: phaC (**class III**) is able to produce PHA or only the phaC (**class I**). Furthermore, it would be interesting to invest more time in the accumulation properties of the strain, to **confirm the accumulation of 3HPte**, and to study its mechanical properties.

The strain, in the PHA field, as not any production interest, with a **low  $\mu_{max}$  (< 0.07 h<sup>-1</sup>)** and an **accumulation up to 25 wt%**, and these values are **not compatible with industrial applications**. However, the high content of the polymerization of **3HV** and **3HPte** under **valeric acid** and **1-pentenoic acid** seems promising for academic research. The goal would be in future research to **extract the polymerase** and add it in a **plasmid**, to **study the accumulation** of those compounds in other organisms such as ***E. coli***, in the direction to exploit the production specificities of the strain.