Quantification of polyhydroxyalkanoates in the non-sulphur purple bacterium *Rhodospirillum rubrum* S1H

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Rhodospirillum rubrum is a non-sulphur purple bacterium well known for its huge metabolic versatility. Previous studies in the lab revealed its production of polyhydroxyalkanoates (PHAs) in different growth conditions (*fig.* 1). PHAs are bacterial biodegradable polymers which could be used to replace petroleum non bdegradable plastics.

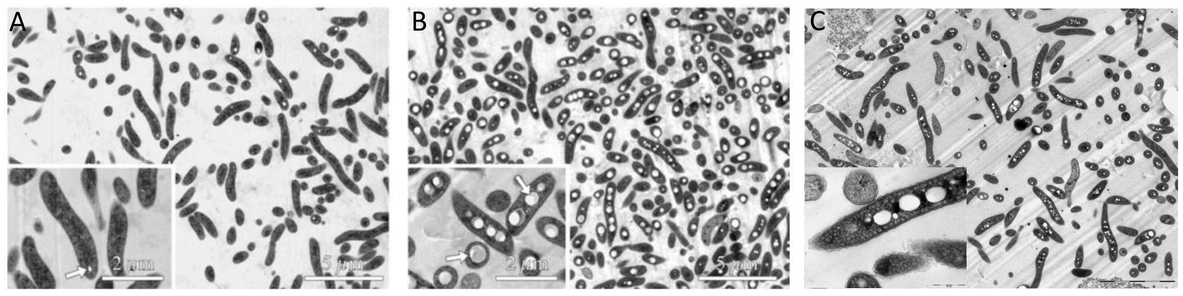

Fig. 1 : Transmission Electron Microscopy (TEM) pictures of *Rhodospirillum rubrum* in presence of different carbon sources showing PHA granules. A) succinate, B) acetate, C) butyrate

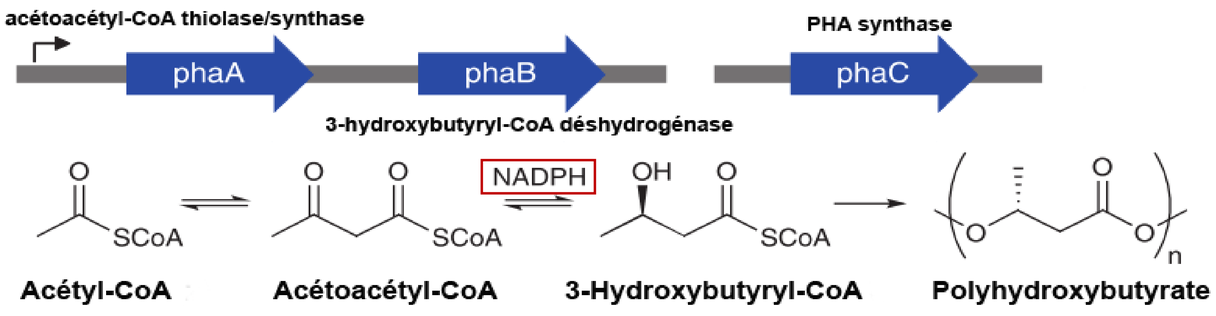

Fig. 2 : Metabolic pathway leading to the production of PHB and consuming a molecule of NADPH

Even if numerous bacteria are known to produce PHA with high yield, the industrial process is curbed by its production cost largely imputed to the carbon source. Henceforth, the use of waste coming from wastewater treatments like volatile fatty acids (VFAs, *e.g.* acetate, butyrate, propionate or valerate) could be a good solution to solve this issue. In our case, the hypothesis would be that a deregulation of the intracellular redox balance, induced by the use of a reduced carbon source, leads the production of PHA (*fig.* 2). Effectively, this mechanism is supposed to be driven by an excess of NAPDH in the cell.

Here, we have developed a reproducible method to quantify *Rhodospirillum rubrum* intracellular PHA content and determine the proportion of each monomer in the polymer.

In order to quantify PHA by GC-MS, these polymers have to be extracted. It implies their methanolysis *via* methanol and H_2SO_4 yielding the methyl ester species followed by their solubilisation in chloroform (*fig.* 3).

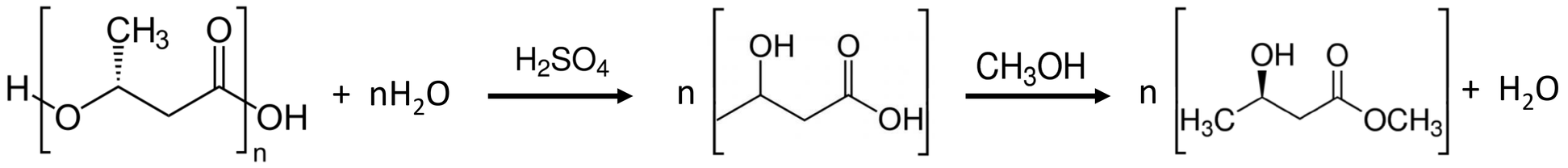

Fig. 3 : Methanolysis occurring during the extraction of the polymer yielding to its hydrolysis and the production of the methyl ester species

The determination of dynamic range has been done on the ion m/z = 103 ± 0.015 as this ion is present in both interest compounds. This dynamic range has been done for the two most common methyl ester species. The dynamic range indicates that injected quantity comprised between 2 and 10 ng is suitable for the quantitation of both compounds (*fig.* 5).

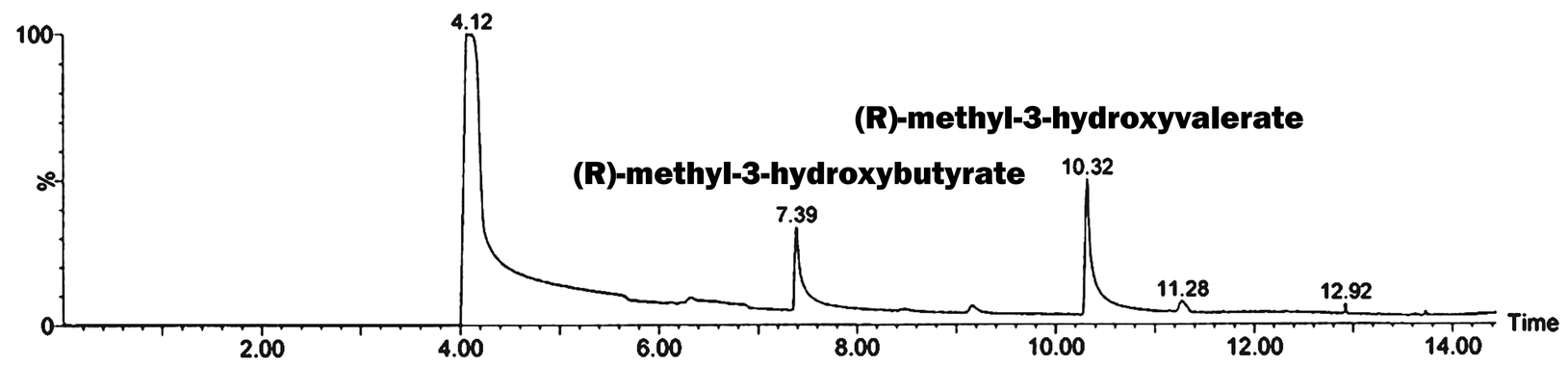

Fig. 4 : Retention time of the different standard (methyl-(R)-3 hydroxybutyrate and methyl-(R)-3-hydrovalerate) injected at 10 ng on column.

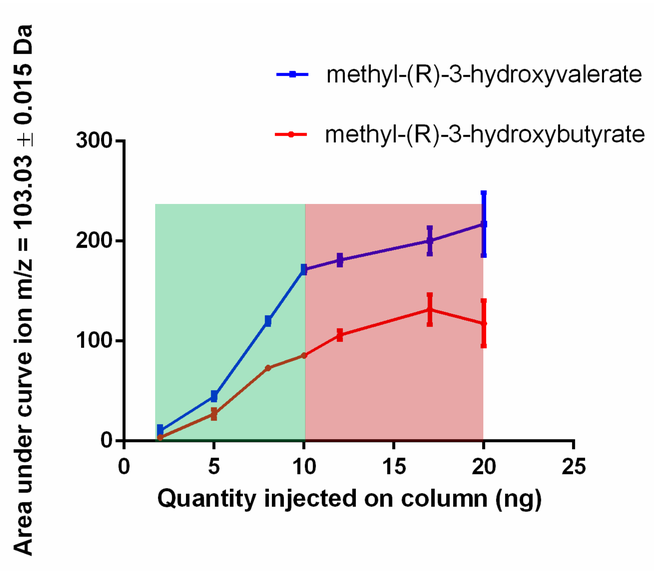

Fig. 5 : Dynamic range of both methyl ester species. The green area depicts the range of quantity on column where the quantitation is reliable.

First injections performed on GC-MS show the feasibility and the effectiveness of the protocol mentioned before. Moreover, the harvesting of small extract of of culture seems to be sufficient for the detection and the quantitation of PHAs (*fig.* 6).

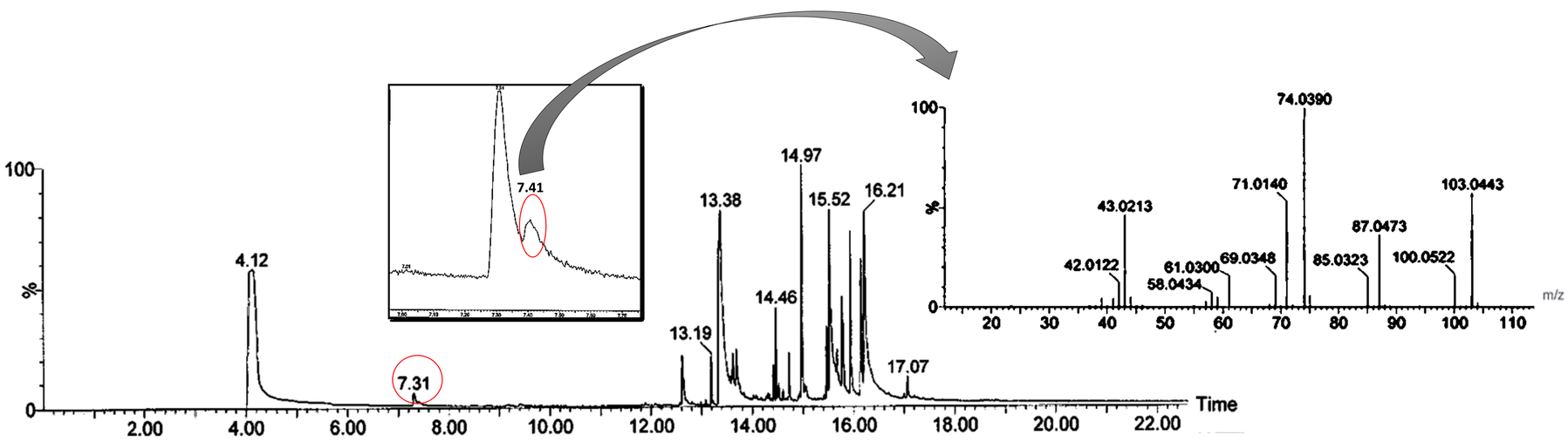

Fig. 6 : Chromatogram resulting from the extraction of PHAs in 0.5 mL of culture (O.D. ≈ 0.700) cultivated in 62.40 mM of acetate following the protocol described before (*fig.* 3).

Take-away message

The use of this method in complement with **cofactor quantification** will allow us to **link** the **PHA production** with the redox state of the **carbon source** and the **intracellular redox state**. The connection between them is of first interest in order to fully understand the PHA production leading to the production of **cheap microbial biodegradable plastics** using VFAs derived from **wastewater treatments**.

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