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# Metagenomics and *in situ* analyses reveal *Propionivibrio* spp. to be abundant GAO in biological wastewater treatment systems

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## BACKGROUND

Enhanced biological phosphorus removal (EBPR) is widely applied to remove phosphorus (P) from wastewater. The process relies on polyP accumulating organisms (PAOs) that are able to take up P in excess of what is needed for growth, whereby P can be removed from the wastewater by wasting the biomass. However, glycogen accumulating organisms (GAOs) may reduce the EBPR efficiency as they compete for substrates with PAOs, but do not store excessive amounts of polyP. The model PAOs are the betaproteobacterial "*Candidatus* Accumulibacter spp." (Accumulibacter). Here, we report the discovery of a GAO from the genus *Propionivibrio*, which is closely related to Accumulibacter.

## STUDY PERSPECTIVES

The *Propionivibrio* spp. are abundant GAO in EBPR systems. Results from studies applying the PAOmix probes should be interpreted with care given they also target a GAO. Comparative expression studies of the Accumulibacter PAO, and the closely related *Propionivibrio* GAO, may be key to determining the genetic and regulatory mechanisms that give rise to the PAO phenotype.

## IDENTIFICATION OF A NOVEL GAO

A 6L lab scale sequencing batch reactor configured for EBPR was operated for 3 months using volatile fatty acids (VFAs) as the carbon source. Biochemical transformations indicated a mixed PAO-GAO community.

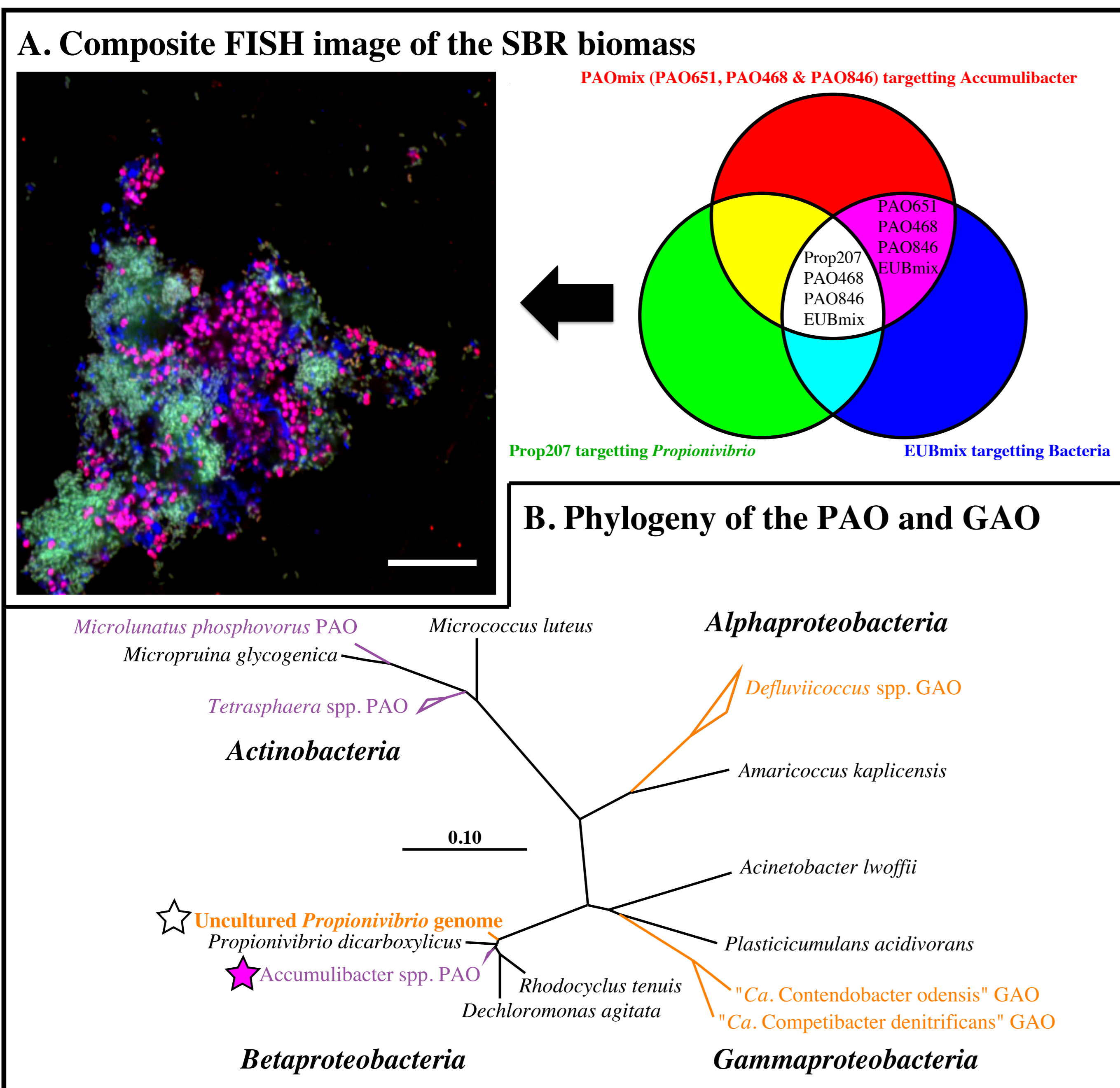


Fig. 1. Phylogeny (16S rRNA gene) and FISH analysis of the reactor biomass community

Phylogenetic (Fig. 1B) and FISH (Fig. 1A) analyses revealed that the reactor was dominated by two populations representing related genera - Accumulibacter and *Propionivibrio*. The PAOmix probes designed to target the Accumulibacter target both populations Fig. 1. A novel probe was designed to target the *Propionivibrio* (Prop207). The PAO651 probe alone is recommended to target the Accumulibacter.

Surveys of full scale EBPR systems reveal a high abundance of the *Propionivibrio* – sometimes in higher abundance than the Accumulibacter PAO.

## GENOMIC ANALYSES

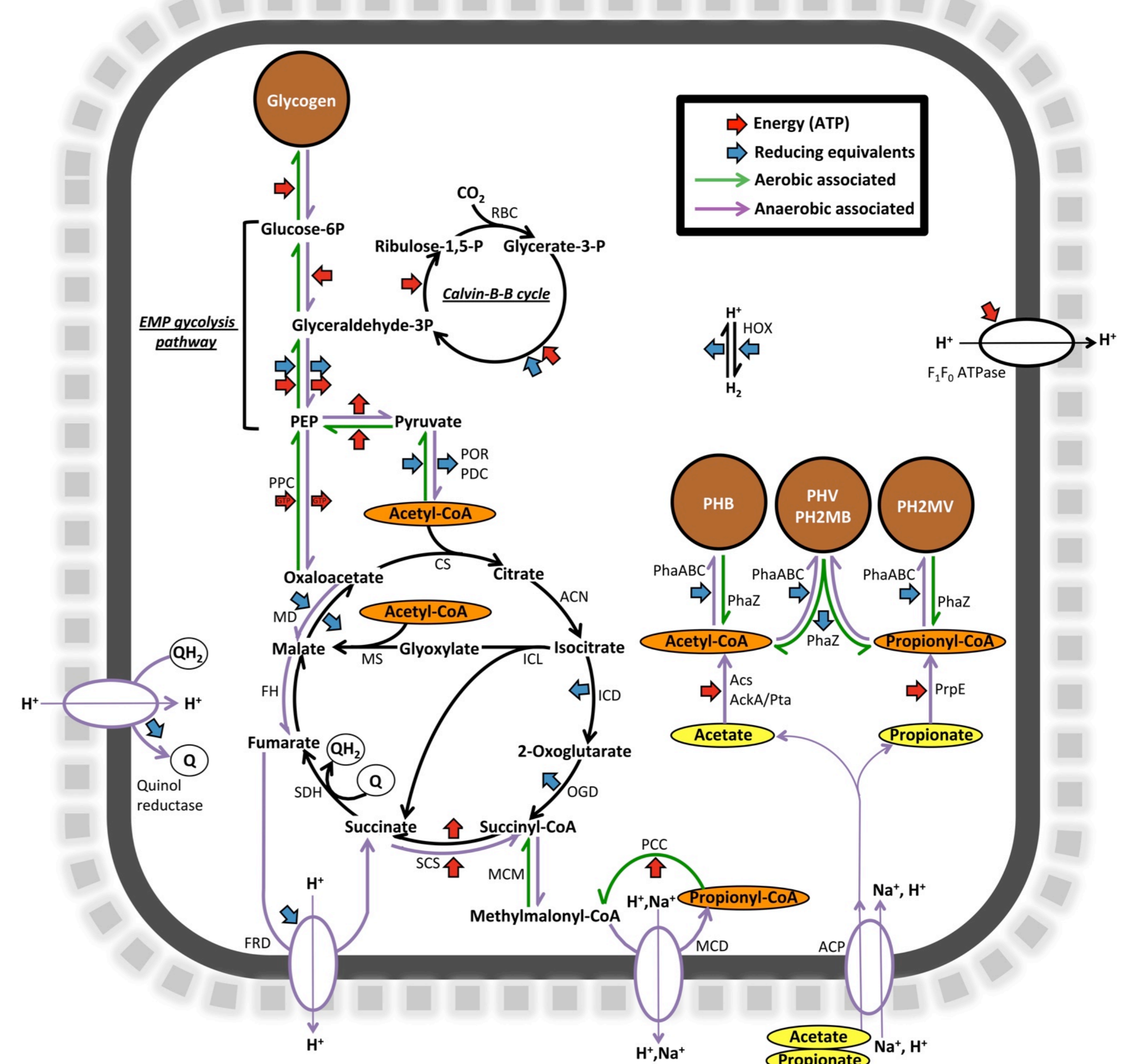


Fig. 2. Metabolic model for the *Propionivibrio* GAO in EBPR systems

Metagenomics was applied to obtain representative genomes from the abundant Accumulibacter and *Propionivibrio* species. Annotation supported a typical GAO physiology for the *Propionivibrio* spp. where aerobically stored glycogen is predicted to provide the energy and reducing equivalents to support anaerobic uptake and storage of VFAs as PHAs (Fig. 2).

## IN SITU ANALYSES

*In situ* staining revealed that both Accumulibacter spp. and *Propionivibrio* spp. stored VFAs as polyhydroxyalkanoates (PHAs) under anaerobic conditions while only the former cycled polyP (Fig. 3). This is consistent with the Accumulibacter and *Propionivibrio* behaving as PAO and GAO, respectively.

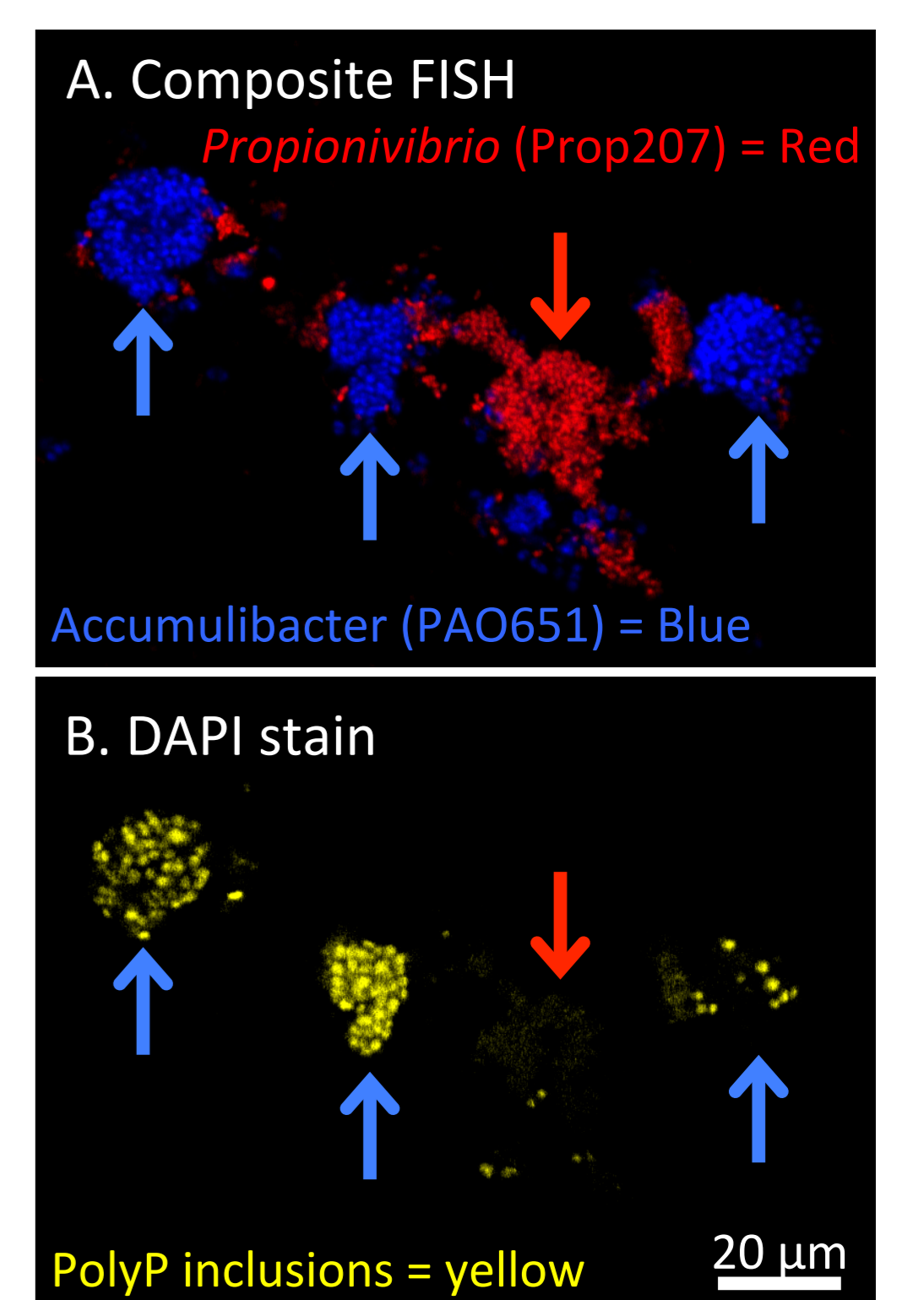


Fig. 3. *In situ* staining of the biomass