Engineering Pseudomonas putida biocatalysts for the production of antimicrobial biopolyesters

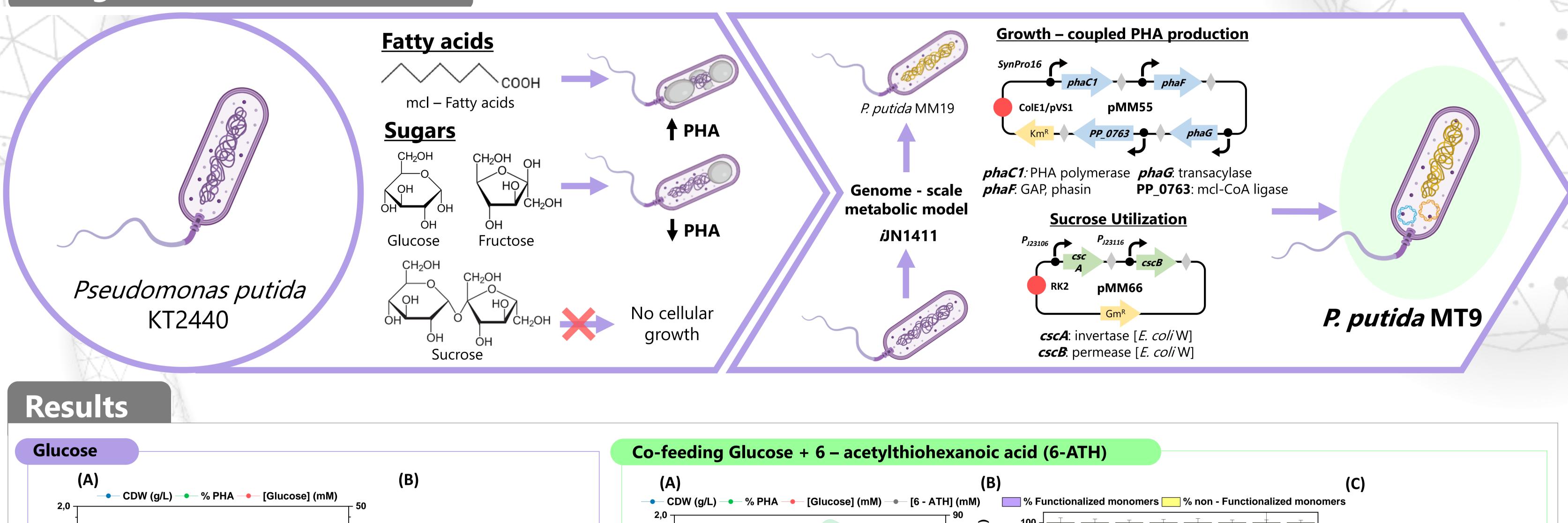
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Introduction

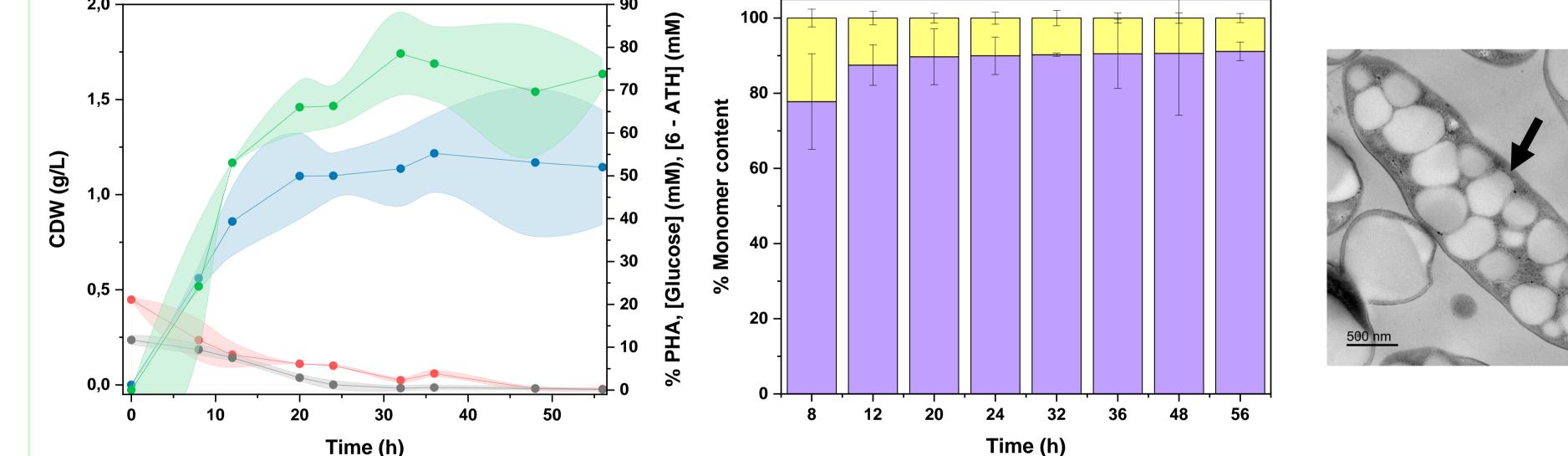
Biomaterial-associated infections (BAIs) are one of the main issues to overcome in regenerative medicine. A promising alternative is offered by PHACOS, a naturally functionalized bacterial polyhydroxyalkanoate (PHA) with thioester groups in the lateral chains that confer antimicrobial activity to this biopolymer. In this work, we studied the production of PHACOS by using an engineered Pseudomonas putida strain that utilizes sugars, e.g., monosaccharides and disaccharides, as an inexpensive and highly abundant feedstock.

Background and Procedure





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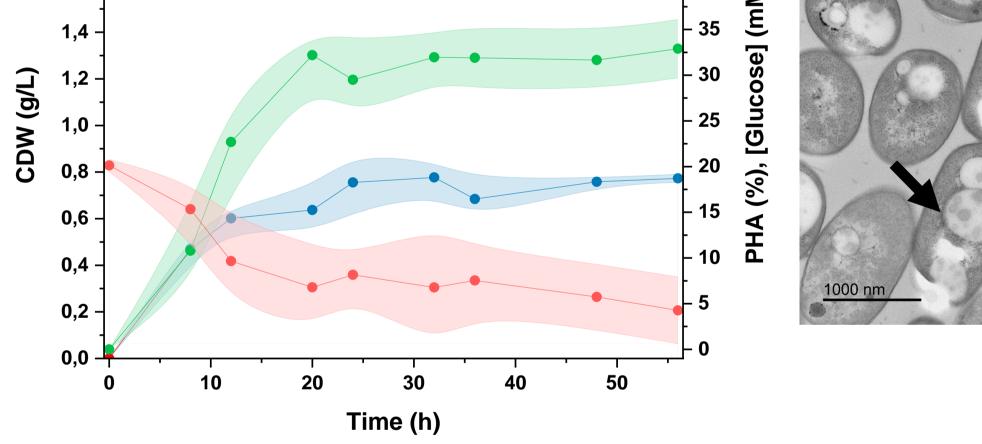
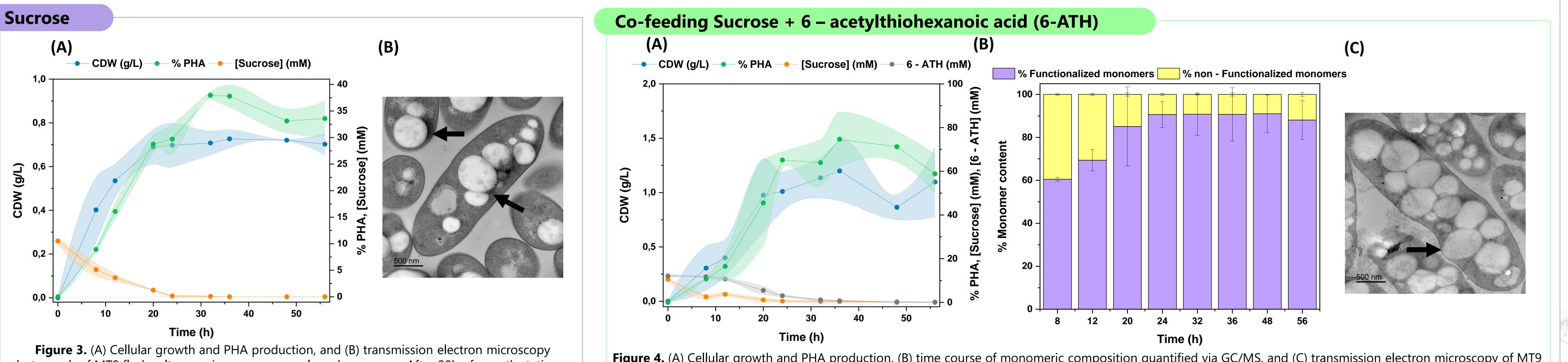


Figure 1. (A) Cellular growth and PHA production, and (B) transmission electron microscopy photograph of MT9 flask cultures using glucose as sole carbon source. After 24h of growth stationary phase is reached achieving 0.7 g/L of final biomass and 32.5% CDW of PHA. Intracellular PHA granules are indicated with arrows.

Figure 2. (A) Cellular growth and PHA production, (B) time course of monomeric composition quantified via GC/MS, and (C) transmission electron microscopy of MT9 flask cultures co-feeding glucose as carbon source and 6-ATH. Stationary phase is reached after 24h where both biomass concentration and biopolymer accumulation increased up to 1.1 g/L and 75% respectively compared to cultures without 6 – ATH in the cellular broth. Intracellular PHA granules are indicated with arrows.



photograph of MT9 flask cultures using sucrose as sole carbon source. After 20h of growth stationary phase is reached achieving 0.7 g/L of final biomass and 37% CDW of PHA. Intracellular PHA granules are indicated with arrows.

Figure 4. (A) Cellular growth and PHA production, (B) time course of monomeric composition quantified via GC/MS, and (C) transmission electron microscopy of MT9 flask cultures co-feeding sucrose as carbon source and 6-ATH. Stationary phase is reached after 24h where both biomass concentration and biopolymer accumulation increased up to 1.1 g/L and 70% respectively compared to cultures without 6 – ATH in the cellular broth. Intracellular PHA granules are indicated with arrows.

Conclusions

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- A model-driven recombinant *P. putida* KT2440 strain able to metabolize sucrose (pMM66) and to produce PHA coupled to cellular growth (pMM55) (*P. putida* MT9) accumulates around 35% CDW PHA when cultivated in glucose and sucrose as sole carbon sources.
- Co-feeding of glucose/sucrose and 6-ATH increases up to 70% CDW PHA accumulation using recombinant strain MT9.
- Functionalized monomers are predominant in the PHA produced when 6-ATH is present, revealing for the first time the biological production of PHACOS from sugars.





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