P-27 海洋性紅色光合成細菌によるバイオポリエステル生産システムの開発

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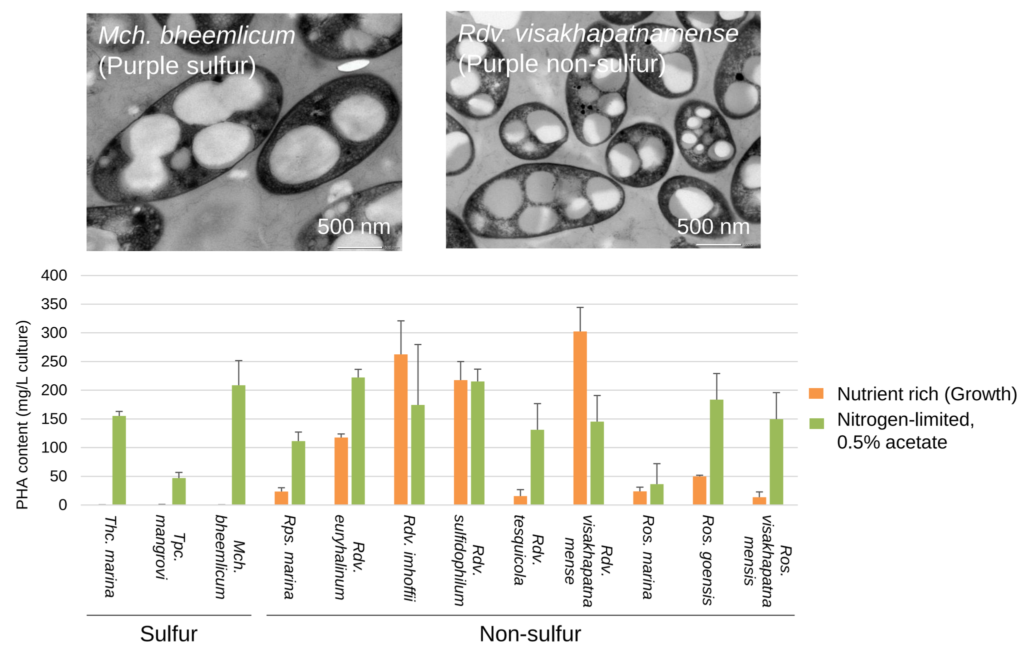
Advantages of photosynthetic bacteria

- Anoxygenic photosynthetic bacteria especially purple nonsulfur bacteria are known to produce high PHA content and shown to have nitrogen fixation ability as well as CO_2 fixation ability.
- The majority of purple nonsulfur bacteria can grow as photoautotrophs or photoheterotrophs in the light, and some strains can grow aerobically in the dark as chemoheterotrophs. This variety of growth style can expand the possibilities for wide range of application.
- We demonstrated that marine purple photosynthetic bacteria produced high molecular weight of polyhydroxyalkanoate (PHA) which is useful property for industrial polymer production and produced PHA using artificial seawater as a culture medium (Higuchi-Takeuchi M et al. 2016).

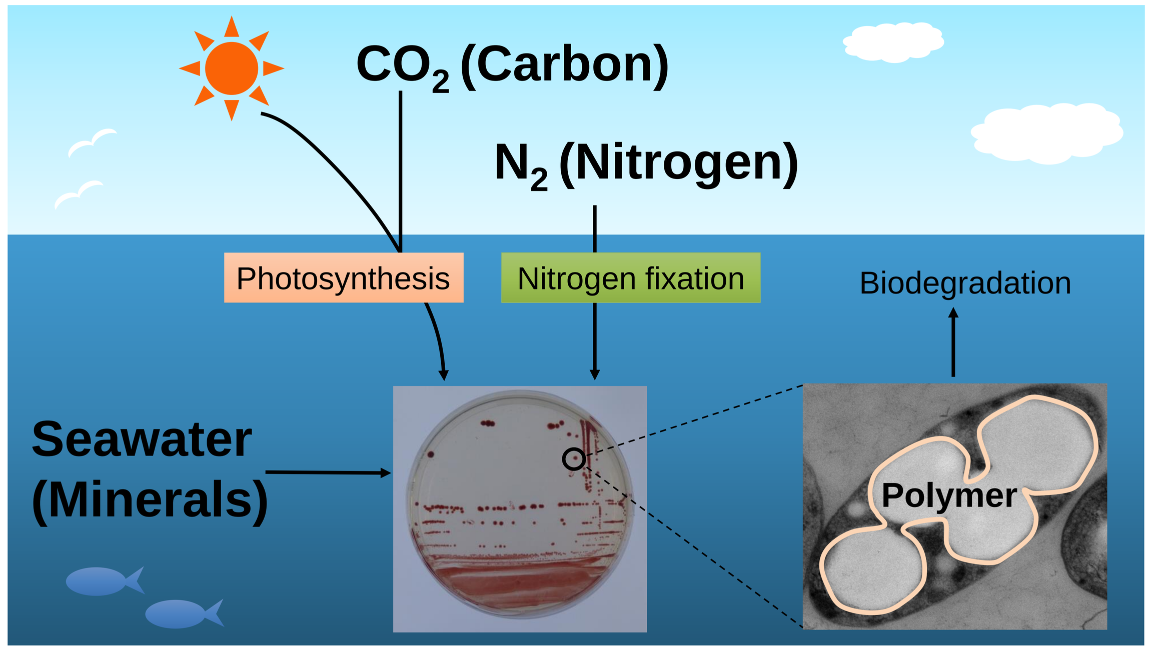
◆ Anoxygenic photosynthetic bacteria

Species	Electron donor	Growth condition
Purple sulfur	H_2S, H_2	Oxygen-limited or anaerobic conditions
Purple non-sulfur	Organic compounds, H_2	Facultative anaerobic (can grow with or without oxygen)
Green sulfur	H_2S, H_2	Obligatory anaerobic
Green non-sulfur (filamentous)	H_2S, Organic compounds	Facultative anaerobic, Weak CO_2 fixation activity
Aerobic photosynthetic	Organic compounds	Aerobic, No Rubisco activity

◆ PHA production by marine purple photosynthetic bacteria



◆ Marine purple photosynthetic bacteria factory



Effect of growth light conditions

- **PHA production and cell growth under different wavelengths of far-red light**
  - Bacterial cells grow well up to 4 days of cultivation under illumination with 800 nm and 850 nm LED compared to 730 nm LED. Whereas 730 nm LED lighting was the best for bacterial growth after 5 days of cultivation. These imply that shorter wavelength of LED light (730 nm) might be able to penetrate the cells even at high cell density.
  - PHA concentrations (mg/L) increased with increasing incubation time. PHA concentrations showed the highest after 4 days cultivation (1.2 g/L) at low light of 800 nm LED.

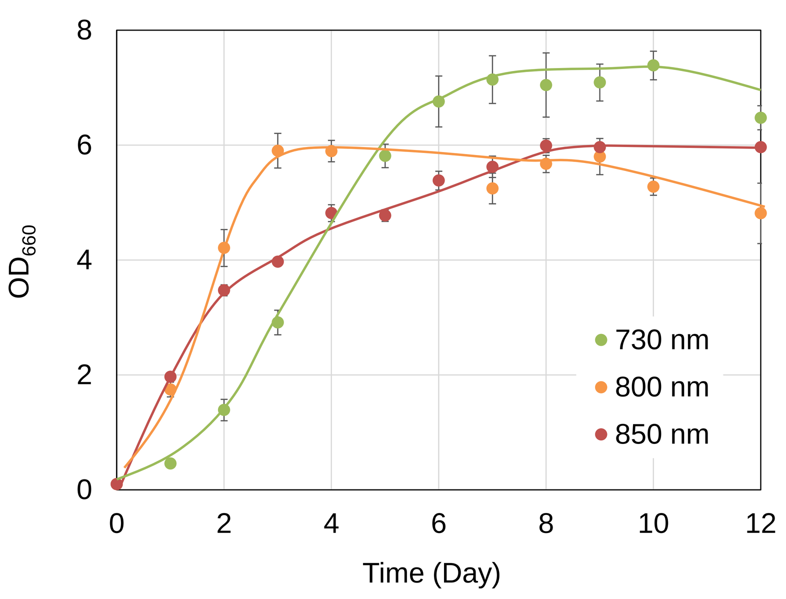


Fig. 1. Growth of marine purple nonsulfur bacteria under different light conditions. Cells were cultured for 1 to 12 days under 8 W/m² of 730 nm, 800 nm and 850 nm. Data are the mean ± SD of at least three cultures.

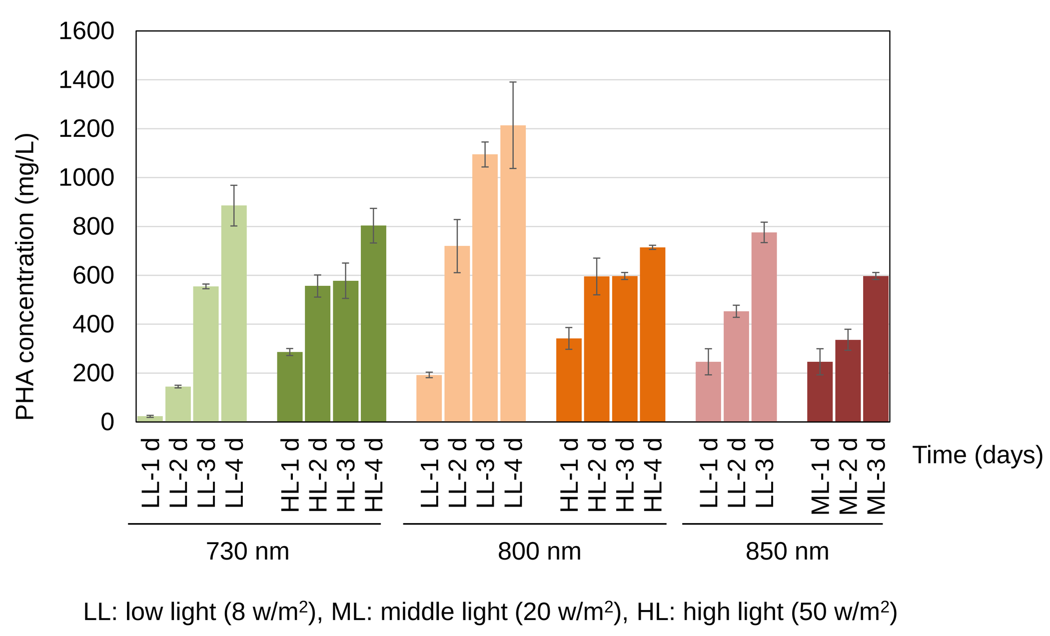


Fig. 2. PHA concentrations (mg/L) under different growth light conditions. Cells were cultured under low light (8 W/m²), middle light (20 W/m²: 850 nm) and high light (50 W/m²) conditions. Data are the mean ± SD of at least three cultures.

- **Relationship between PHA production and cell growth under different light intensities**
  - A linear correlation between PHA contents and dry cell weights (DCWs) were found under low light conditions (R^2 = 0.6289). This indicates that PHA production is dependent on cell growth under low light conditions. On the other hand, a correlation coefficient between PHA contents and DCWs under high light conditions were quite low (R^2 =0.0028), indicating there is no linear relationship.

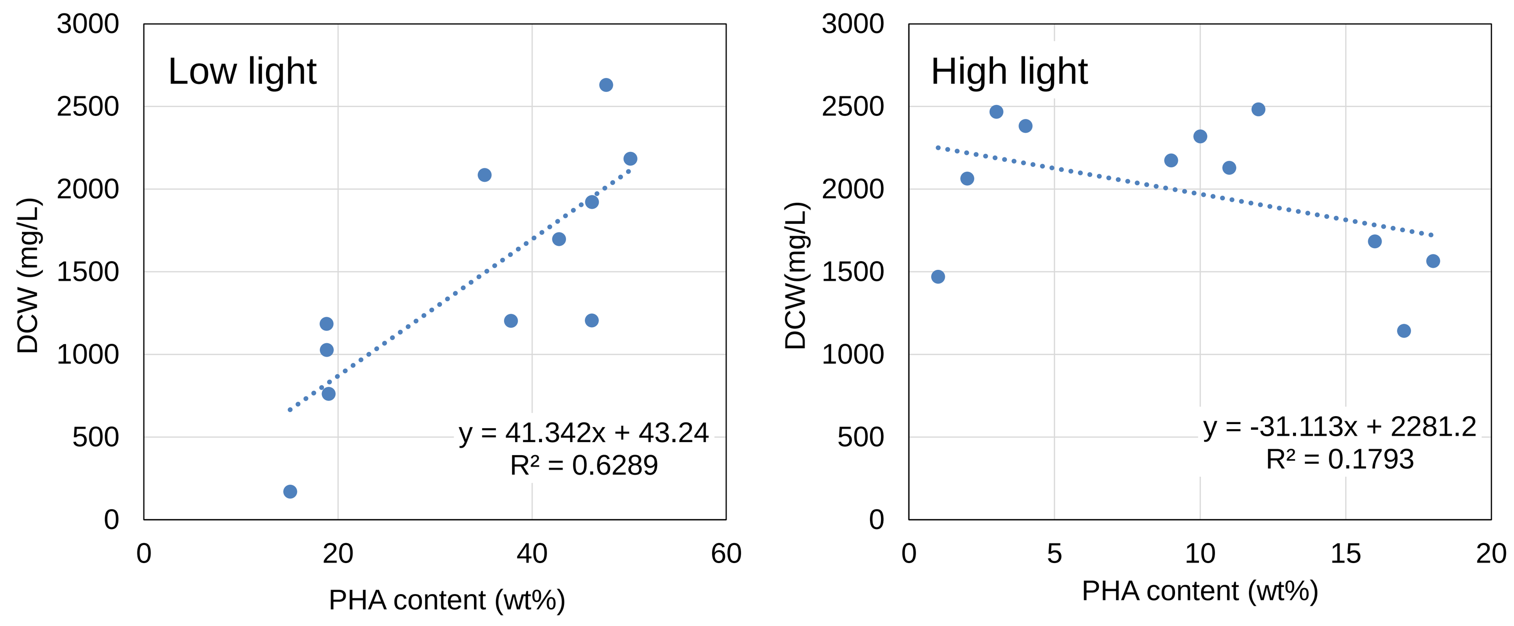


Fig. 3. Comparison between PHA contents (wt%) and DCW (mg/L) under low light (8 W/m²) grown cells (left) and high light (50 W/m²) grown cells (right). The regression lines and R^2 values were shown in each Figure.

◆ PHA production in purple non-sulfur bacteria is related to the cell growth.

PHA production under aerobic conditions

- **Enhancement of PHA production under aerobic condition by addition of acetate**
  - Purple nonsulfur bacteria are known to be able to grow in heterotrophically in the dark under aerobic condition. We found that *R. sulfidophilum, R. imhoffii* and *R. visakhapatnamense* cells hardly accumulated PHA under aerobic condition.
  - *R. sulfidophilum* accumulated 33% of PHA by addition of acetate under aerobic condition and PHA accumulation was not seen by the addition of reductants, suggesting that acetate-dependent pathway is involved in PHA accumulation.

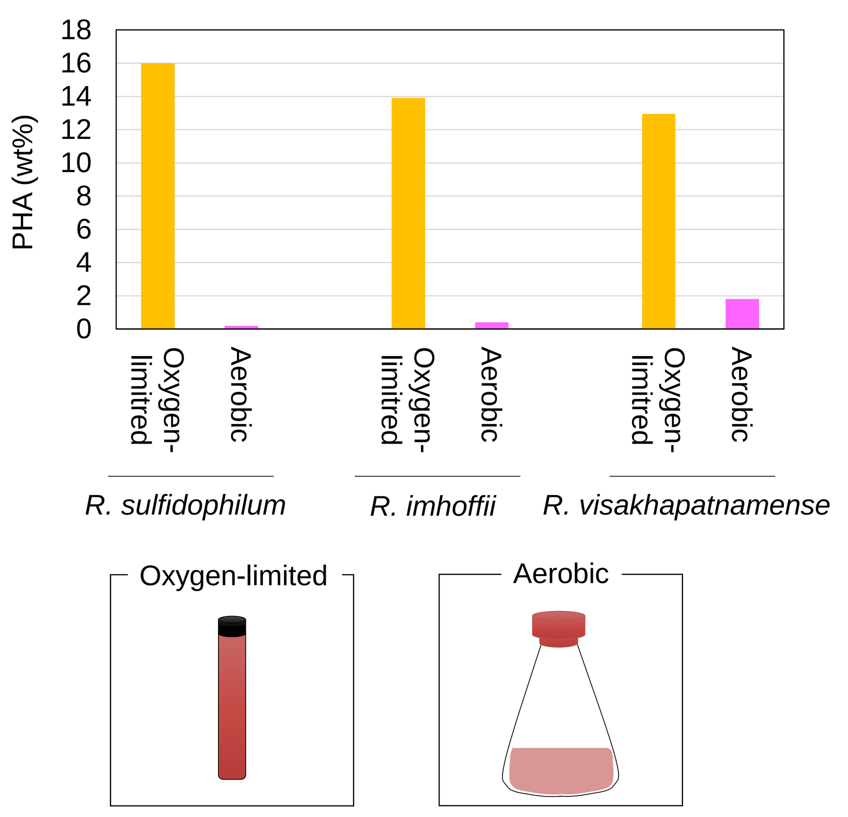
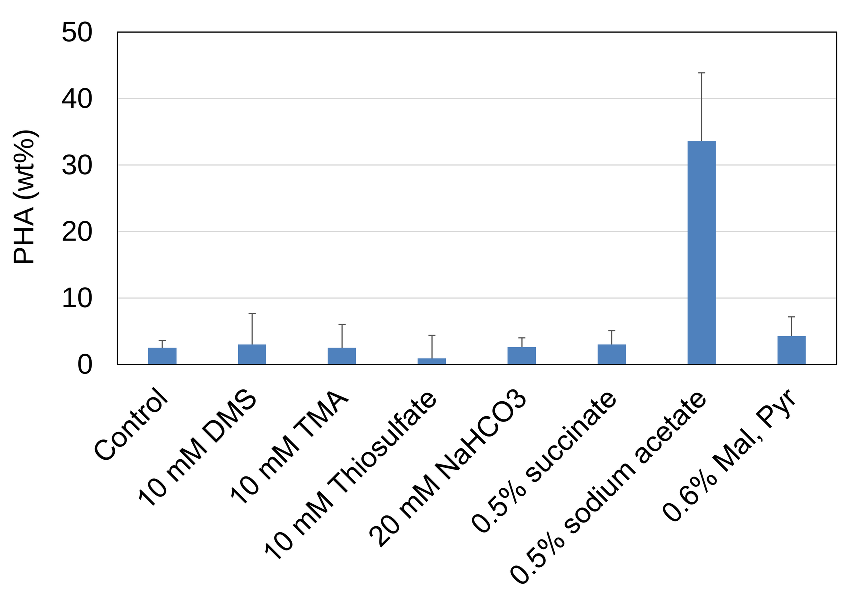


Fig.4. PHA contents under different oxygen growth conditions. Cells were cultured in 25 ml vial under aerobic and oxygen-limited conditions.

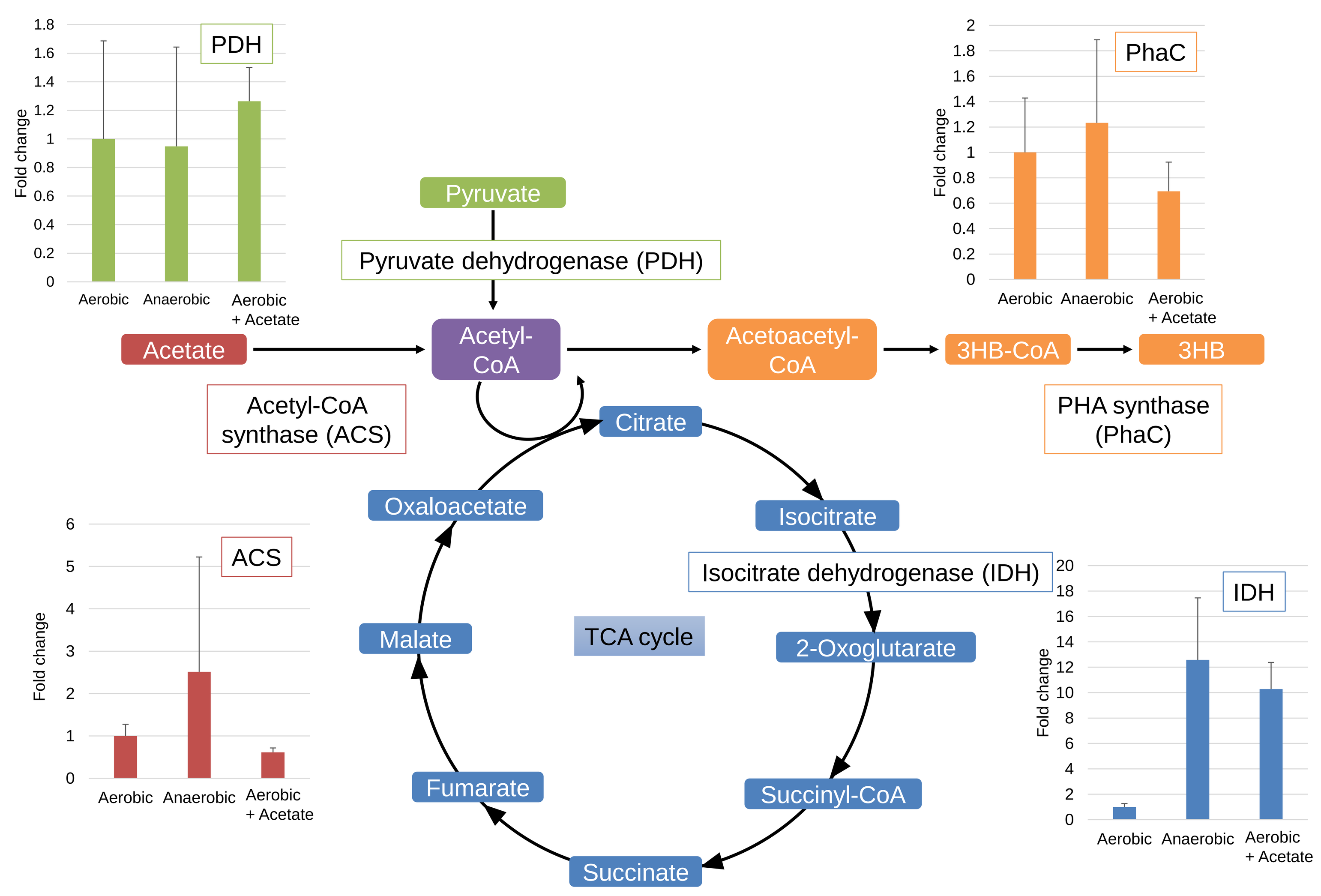


Growth medium: 0.3% sodium malate (Mal)
0.3% sodium pyruvate (Pyr)

DMS: Dimethylsulfoxide (C_2H_6S)
TMA: Trimethylamine (C_3H_9N)
Thiosulfate ($S_2O_3^{2-}$)

Fig.5. Effect of reductants and carbon sources on PHA production under aerobic condition. Cells were cultured under aerobic condition in the presence of malate and pyruvate supplemented with DMA, TMA and thiosulfate as reductants and sodium bicarbonate, succinate and acetate as carbon sources. .

- **Gene expression analysis**
  - Gene expression analysis revealed that the expression of isocitrate dehydrogenase in the tricarboxylic acid (TCA) cycle decreased under aerobic condition and increased by the addition of acetate, indicating that the TCA cycle activity is involved in PHA production under aerobic condition.



◆ Modification of metabolic pathway lead to PHA accumulation under aerobic condition in marine purple nonsulfur bacteria.

References
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2. Higuchi-Takeuchi M, Numata K. Acetate-inducing metabolic states enhance polyhydroxyalkanoate production in marine purple non-sulfur bacteria under aerobic conditions. Front Bioeng Biotechnol. (2019) 28;7:118.
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