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ABSTRACT

- Biosynthesis 3-HP has been developed using recombinant microorganisms, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas denitrificans*...
- Using *Pseudomonas denitrificans* for 3-HP production has several advantages, including: (i) aerobically organism; (ii) naturally producing B₁₂ and (iii) 3-HP inducible systems is well-characterized.
- In attempt to screen various hosts which has potential to produce 3-HP and also can utilize several different carbon sources (such as gluconate, glucose...), *Pseudomonas putida* could be a potential candidate.
- A strategy to produce 3-HP from glycerol using recombinant plasmid was applied and the recombinant strain could produce 3-HP.

METHODS

- Confirmation 3-HP degradation pathway in wild-type strain.
- Deletion of 3-HP degradation pathway using homologous recombinant method (pop-in pop-out).
- 3-HP degradation examination by growing cells and resting cells with wild-type and mutant strains with various carbon sources.
- 3-HP production pathway at plasmid level was introduced and 3-HP production experiment was carried out.

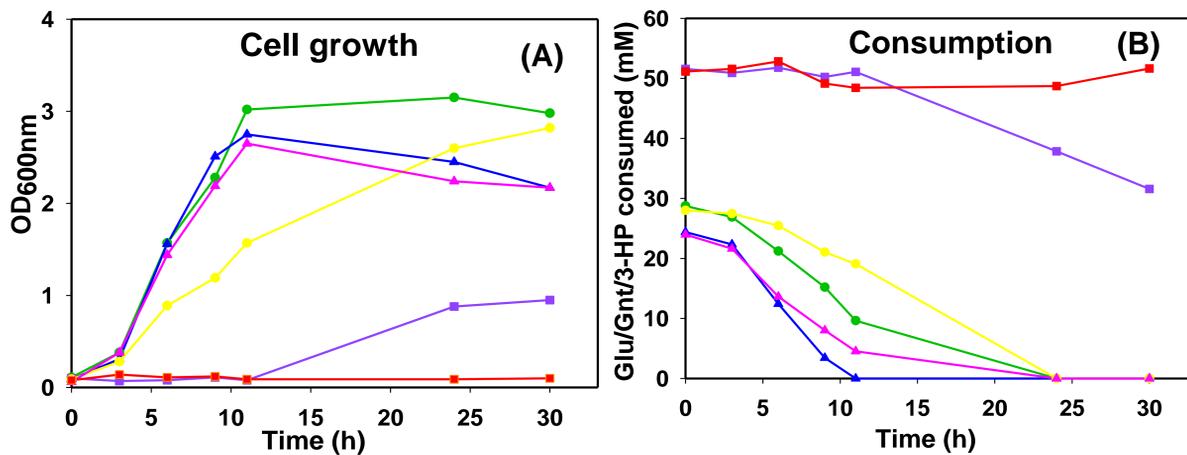


Fig. 2. 3-HP degradation examination with growing cells of wild-type and deletion mutant strains: (A) cell growth, (B) consumption

- 3-HP degradation pathway in wild-type strain was confirmed by culturing on medium containing 3-HP (Fig. 1).
- Major genes responsible for 3-HP degradation was deleted and 3-HP degradation experiments were done to confirm deletion of those genes with growing cells (Fig. 2A and 2B) and resting cells (Fig. 3) and compared to wild-type.
- Introduction of recombinant plasmid for 3-HP production pathway with mutant strain and recombinant strain was successful (Fig. 4).

RESULTS

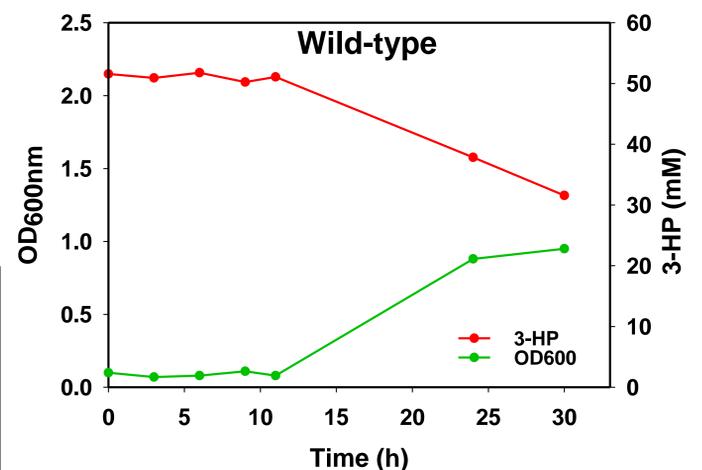


Fig. 1. Confirmation of 3-HP degradation pathway

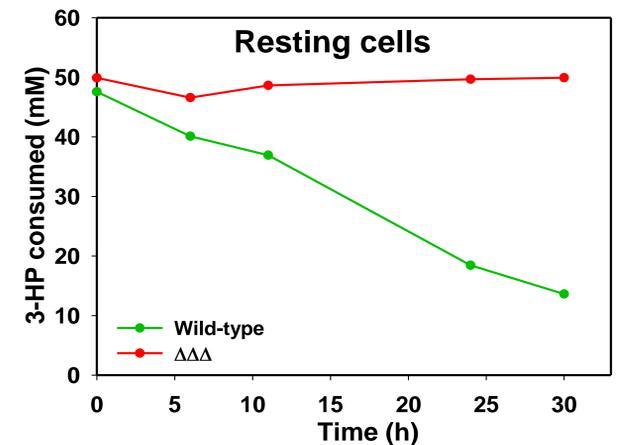


Fig. 3. 3-HP degradation examination with resting cells

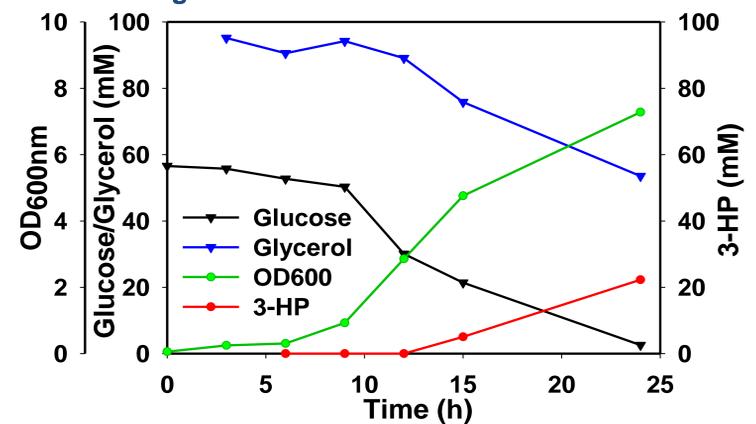


Fig. 4. Production of 3-HP with recombinant strain

CONCLUSIONS

- The potential of using several different carbon sources (such as gluconate, glucose...) of *Pseudomonas putida* was exploited.
- Comparison of 3-HP degradation genes between *P. denitrificans* and *P. putida* was carried to confirm potential of this strain.
- Generation host for 3-HP production was carried by removal of 3-HP degradation genes in chromosome.
- Production of 3-HP from glycerol by introducing recombinant plasmid into mutant host was successful.

REFERENCES

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