

Development of improved gene expression cassette for glycerolar dehydratase enzyme in *Pseudomonas denitrificans*

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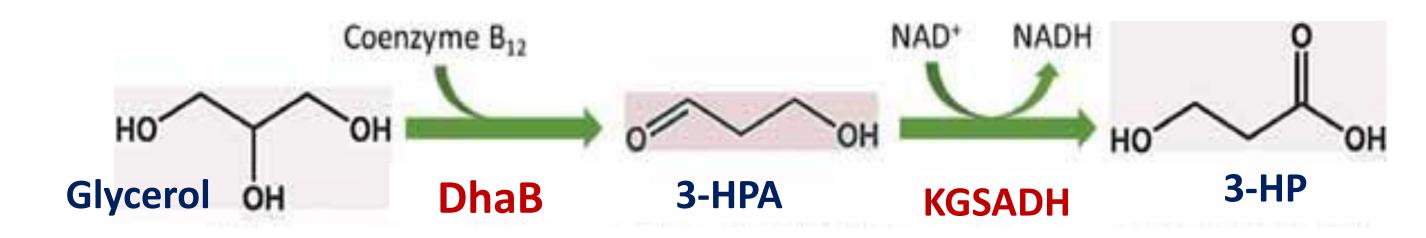
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Abstract

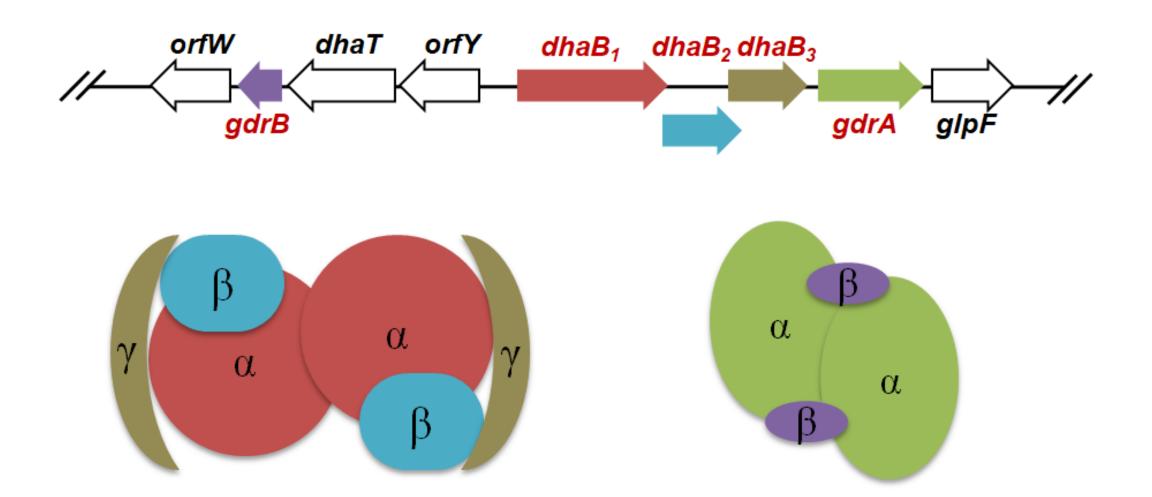
- □ 3-hydroxypropioinc acid (3-HP) can be produced from glycerol by glycerol dehydratase (GDHt) and 3-hydroxypropionaldehyde dehydrogenase (ALDH).
- □ When these enzymes are highly expressed from multi-copy plasmid(s), recombinant microorganisms can produce 3-HP efficiently. However, for industrial purposes, the enzymes should be expressed from the chromosome.
- ☐ In this study, using the recently-discovered novel promoter which is induced by the target product 3-HP, expression cassette for a GDHt was developed.
- □ The GDHt expression/activity, under both non-induced and induced conditions, could be varied in a wide range by proper combination of the gene expression control methods.

Introduction

3-HP production pathway



The dha regulon of Klebsiella pneumonia and Functional form

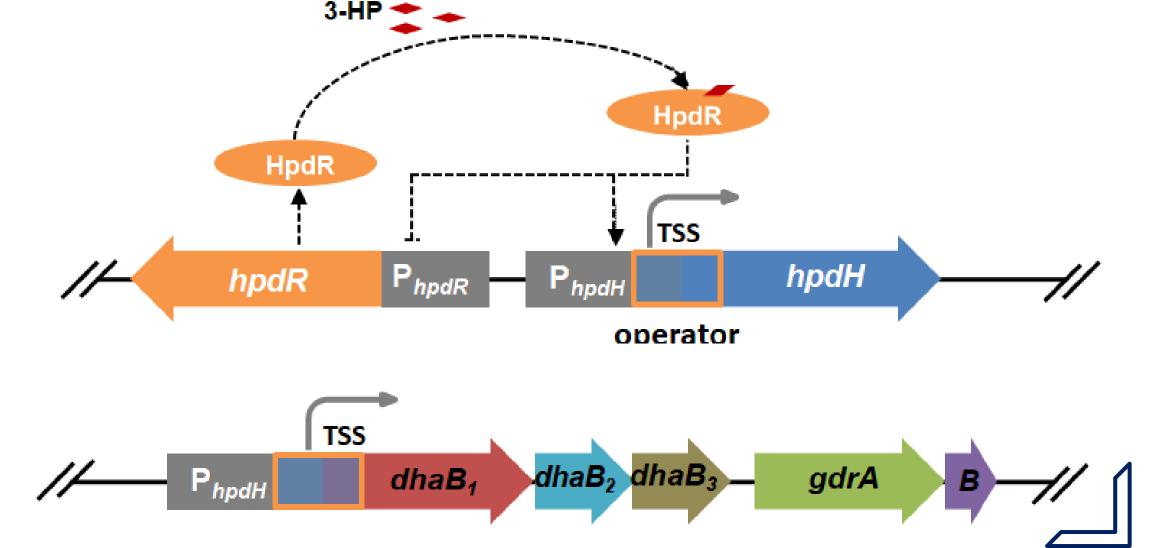


Glycerol dehydratase (GDHt)

GDHt reactivase

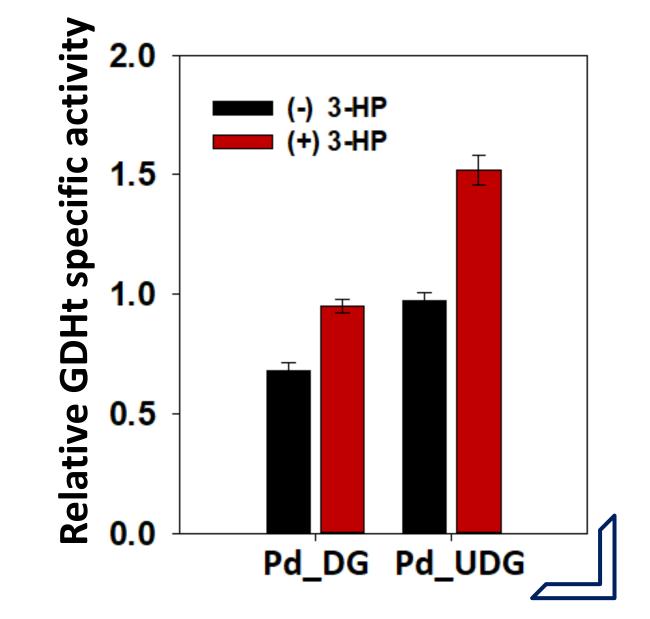
Results

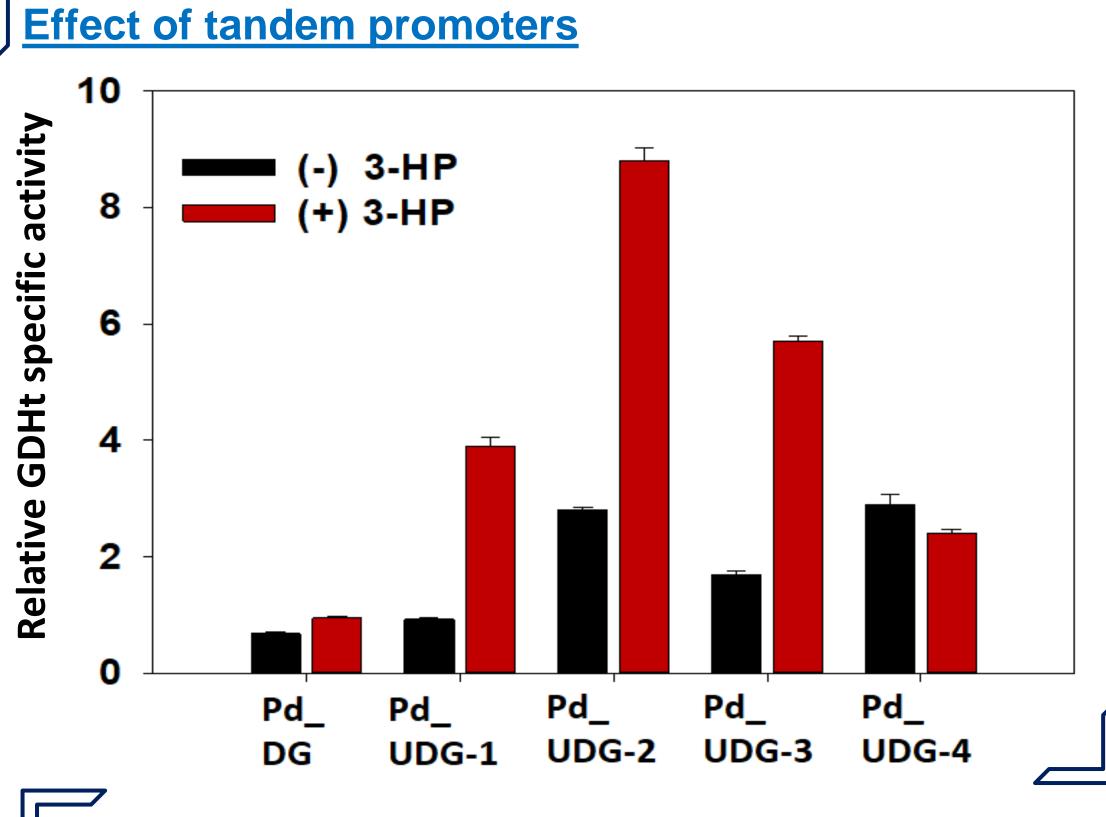
The control of the inducible PhpdH promoter



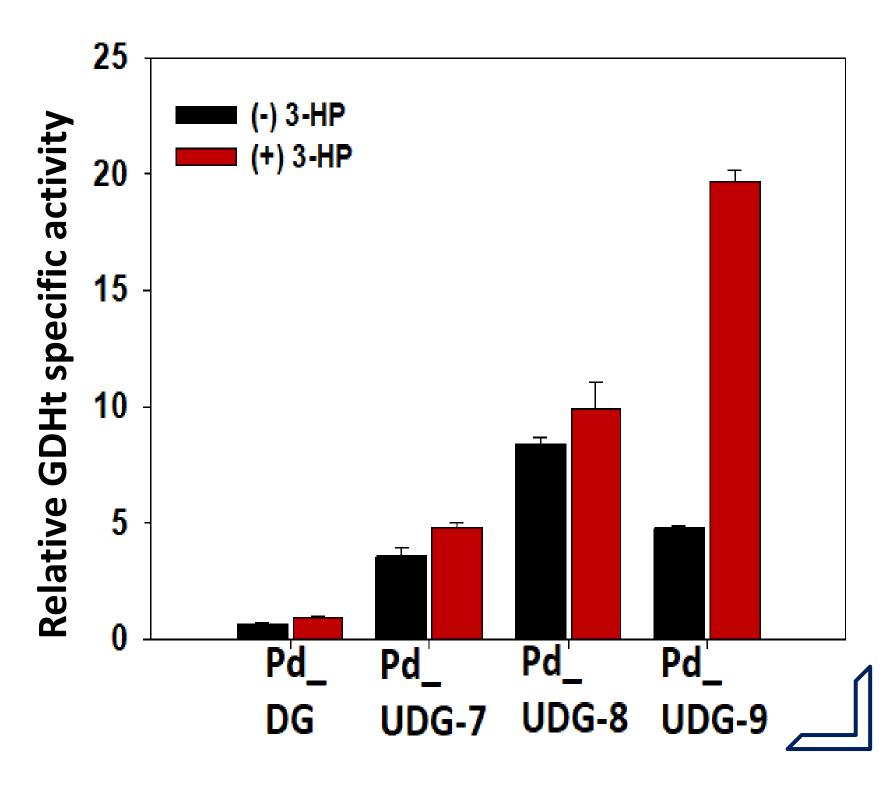
Effect of 5'UTR engineering

Genes	Expression level (5'UTR strength)		
	Native	Modified	Fold
dhaB1	101650	529526	5.2
dhaB2	322625	1650921	5.1
dhaB3	72550	302020	4.2
gdrA	86753	477676	5.5
gdrB	5546	25822	4.6

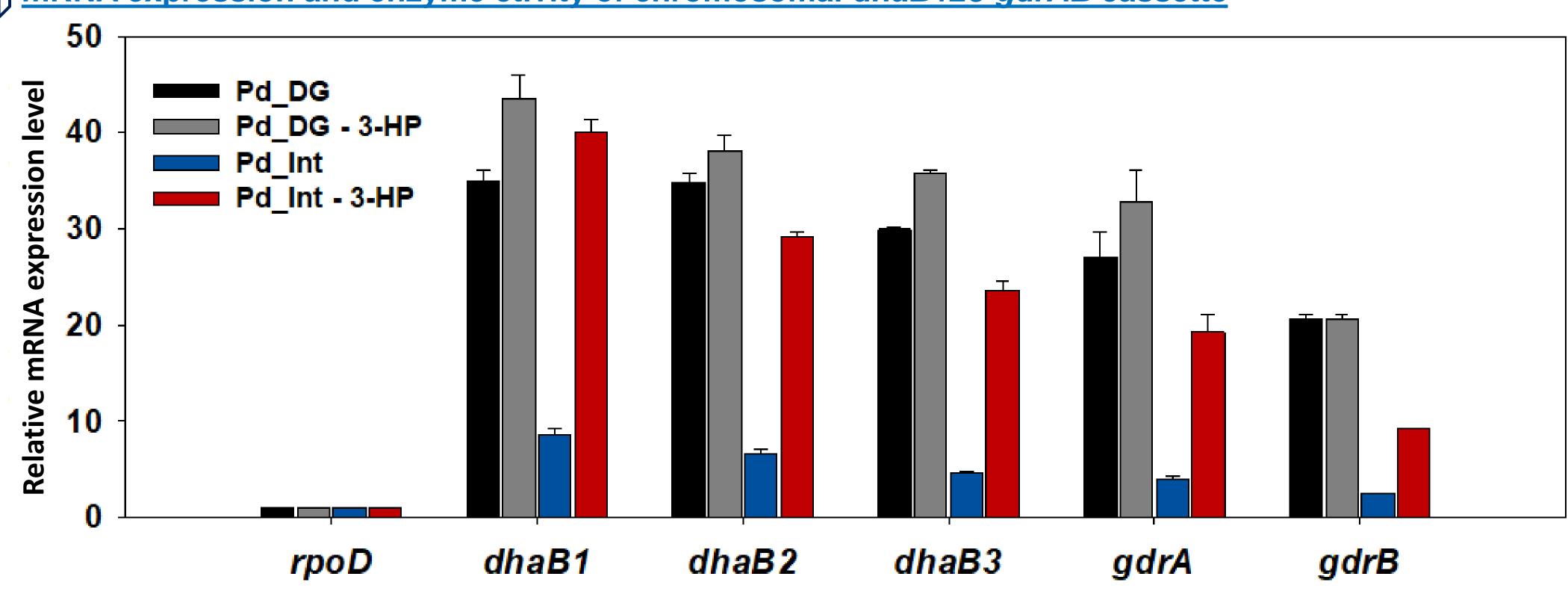


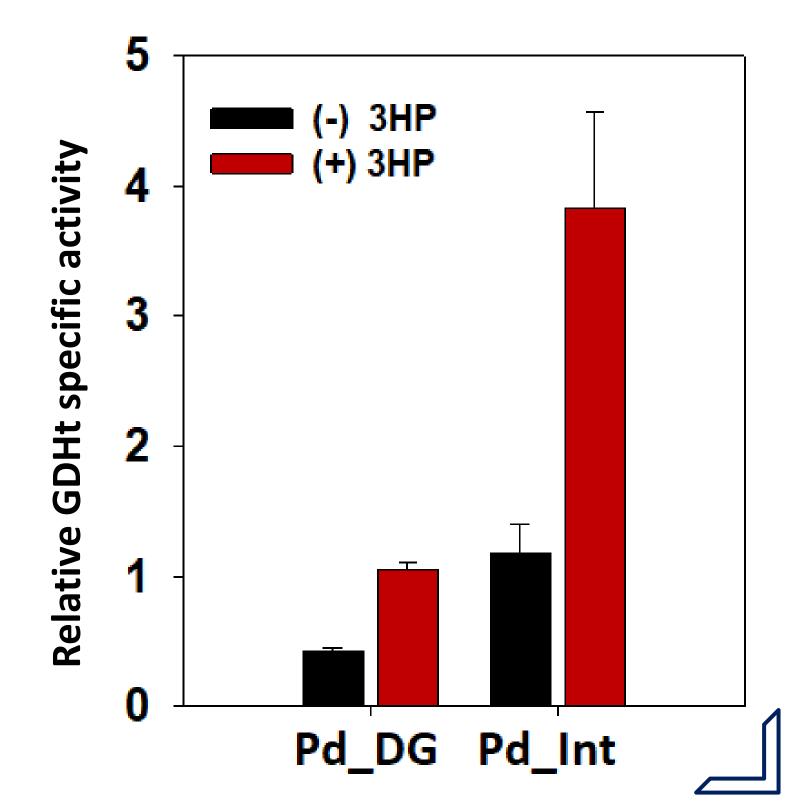


Effect of promoter engineering 18 16 (-) 3-HP (+) 3-HP 12 10 Pd_ Pd_ Pd_ Pd_ DG DG UDG-5 UDG-6



mRNA expression and enzyme ctivity of chromosomal dhaB123-gdrAB cassette





Conclusions

- ☐ Inducible expression systems giving a wide range of GDHt activity,
- ☐ The best construction of each approach (tandem promoter and 5'UTR engineering) showing the highest GDHt activity.
- ☐ High expression of GDHt was observed when integrating into chromosome

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