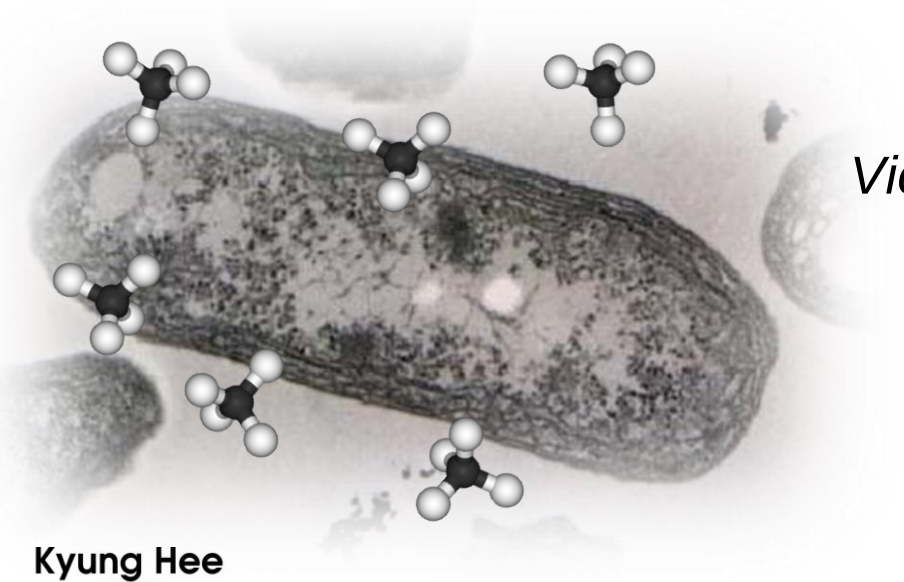
Metabolic Engineering of Methanotrophs as Cell Factory for Methane-to-Chemicals Conversion



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1. Introduction to C1 gas refinery R&D program in Korea
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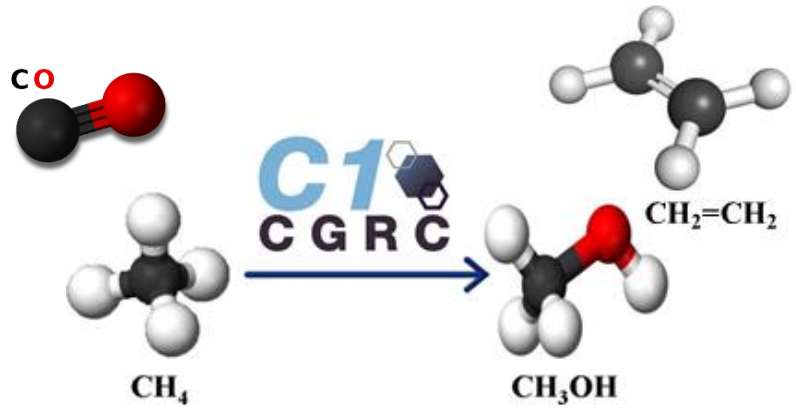
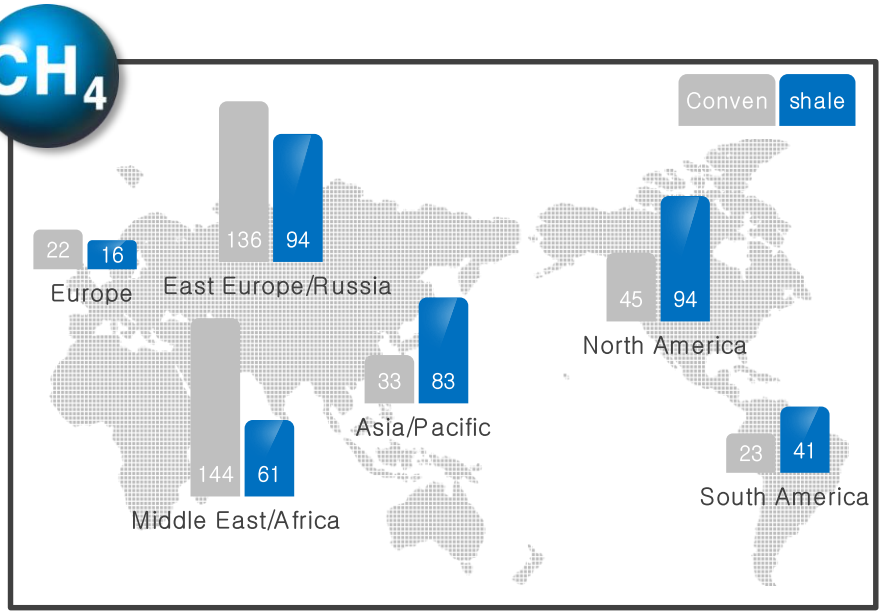
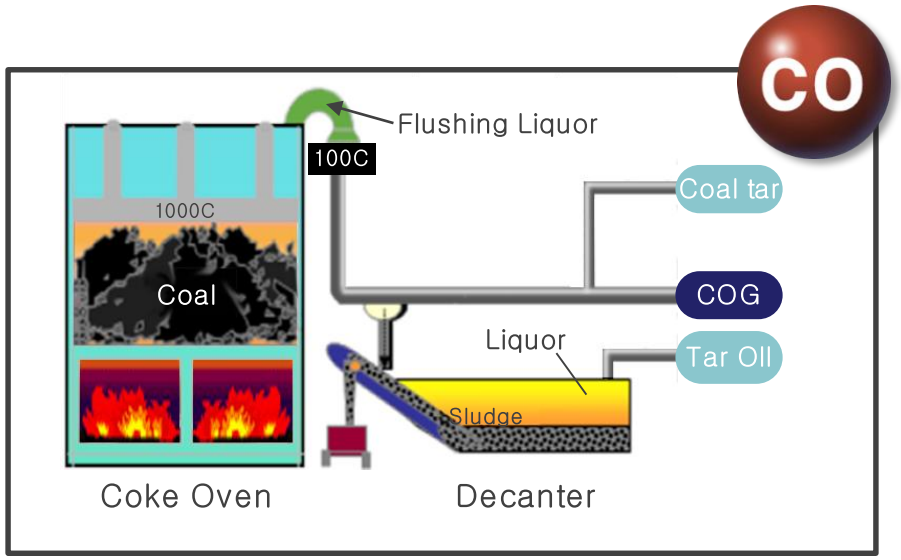


Abundance of C1 gas, methane and carbon monoxide as a low-priced carbon feedstock

- Gas is most abundant resources and it is easy to supply in Korea and overseas.





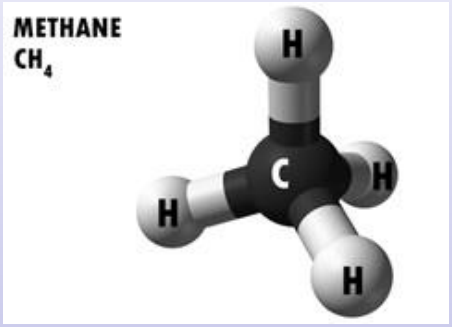
Source	Confirmed Reserves[1)]	Reserves/ Production
Crude Oil	188	46 year
Coal	419	118 year
Conventional Gas	168	59 year
Shale Gas	582	203 year

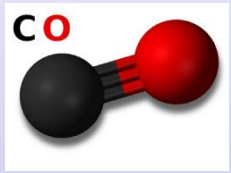
1) Unit : Billion TOE

[C1 Gas Production at Domestic and Overseas]

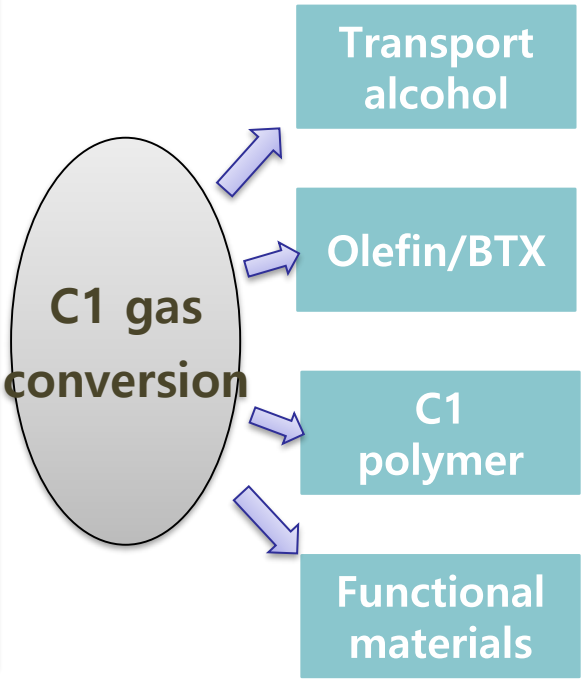
	Source	Production	
		Korea	World
CO	Steel mill, Petrochemical plant, Gasifier	16.8 mil. ton/year	0.4 bil. ton/year
CH_4	Biogas, Natural gas, Shale gas	2 mil. ton/year	0.6 bil. ton/year

C1 gas refinery R&D program (CGRC center)





C1 Gas Feedstock
- Valorization of waste gas
- Mitigation of GHG

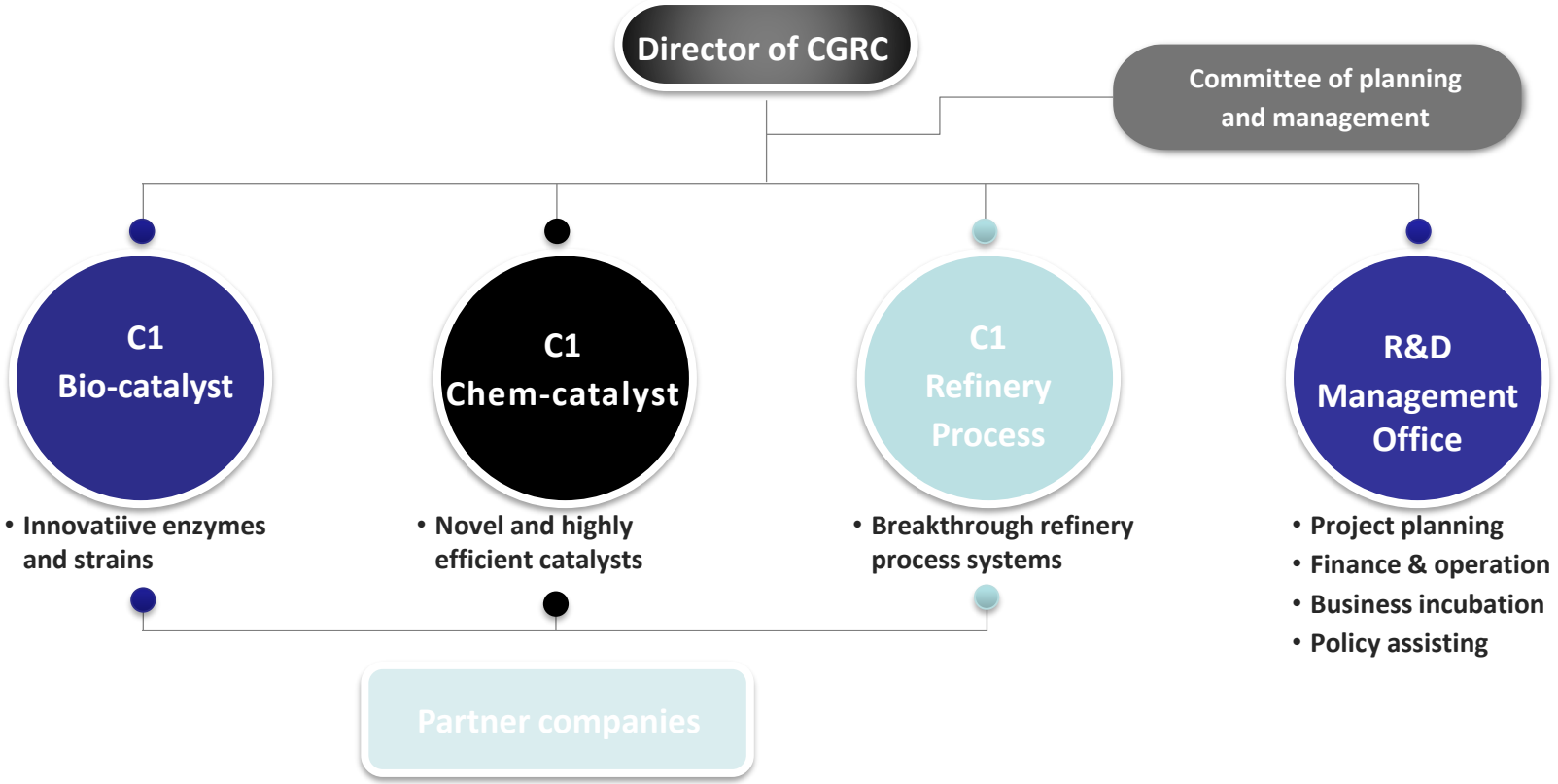


Budget
9 years(2015-2023)
125 M US$

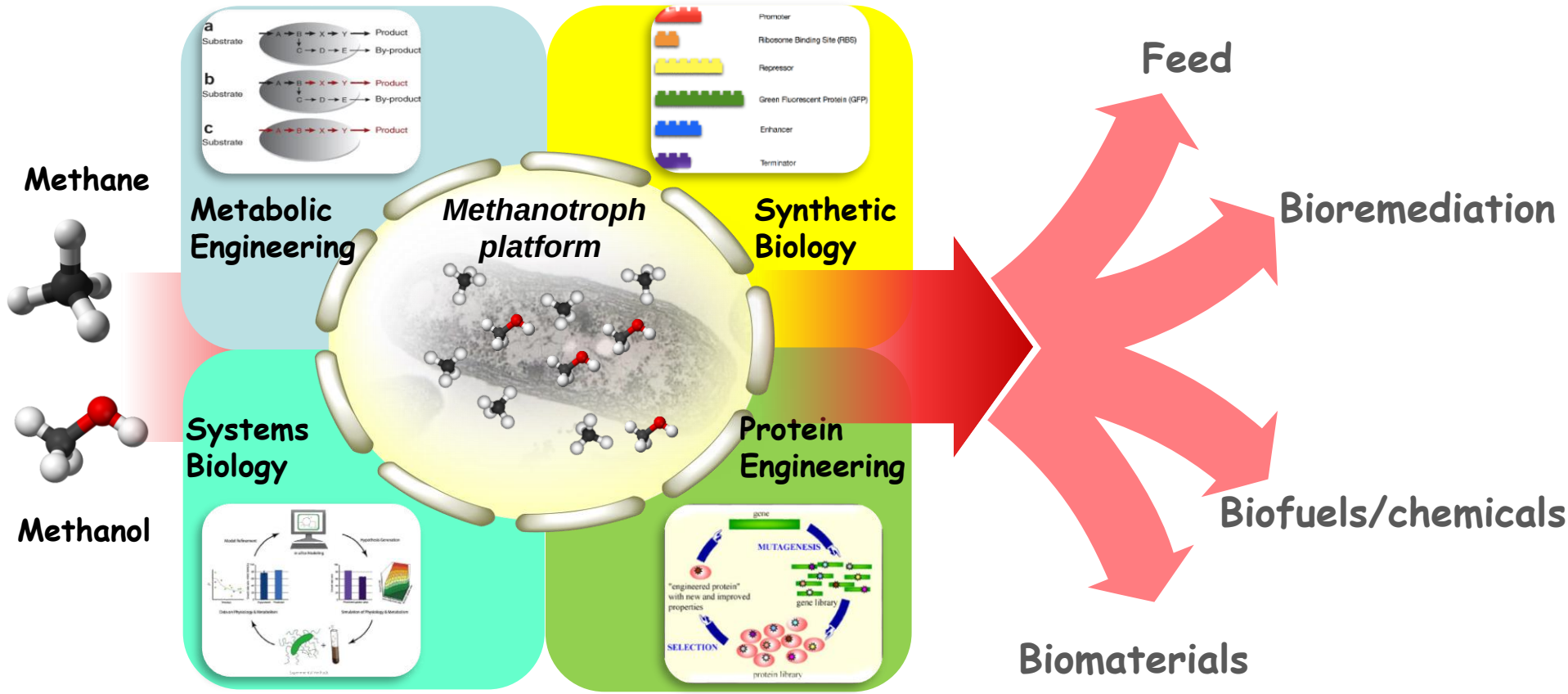
CGRC center Organization

CGRC consists of three major research groups, R & D strategy office, partner company association

Participants : 46 Universities, 9 National Institutes



Methanotrophs as cell factory platform



Isolation and characterization of *Methylomonas* sp. DH-1

- Morphological and biochemical analysis; methane-to-methanol conversion

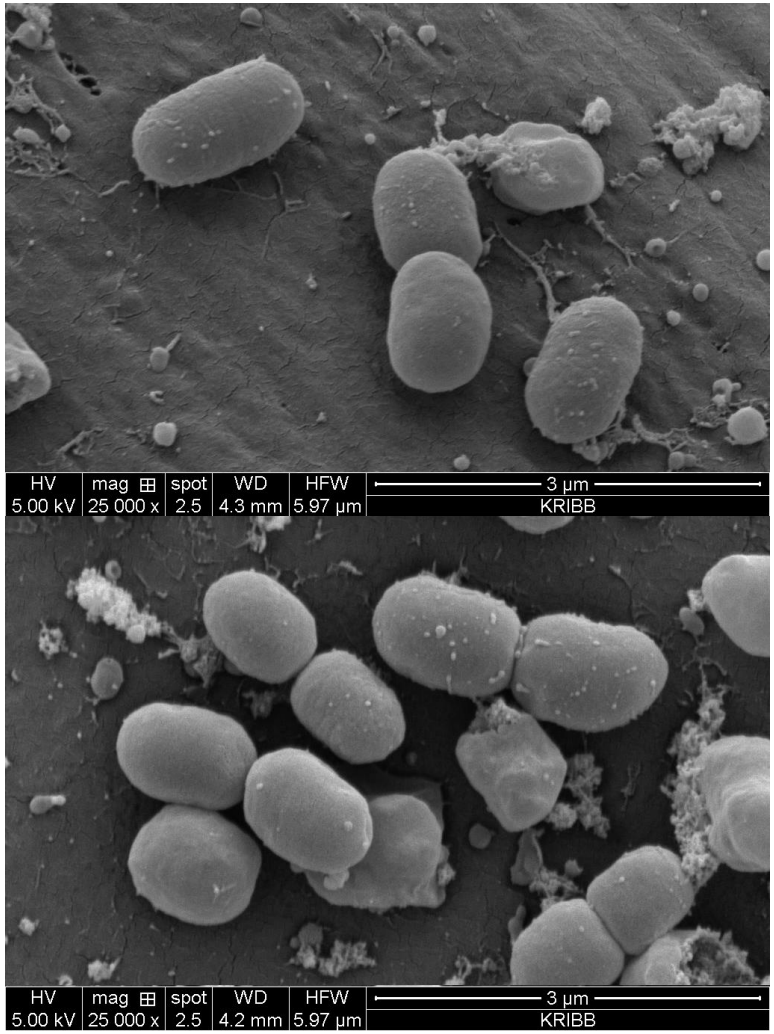


Fig. Scanning electron micrograph of the isolate.

Table. Morphological and biochemical properties of the strain DH-1.

Characteristics	Strain DH-1
Morphology	Rod
Gram reaction	Negative
Size	1 × 1.5 µm
Colony color	Yellow → Orange → Red
Growth temperature	30°C
Headspace gas ratio	Air : methane = 7 : 3
Copper ion concentration	10 µM
Antibiotic resistance	Amp, Tet, Cam

Table. Comparison of methanol productivity between previously reported values and the values of this study.

	Previously reported value		This study
Vol. productivity	28 mg/L/h	11 x →	**332 mg/L/h**
Specific productivity	1.6 mg/g cell/h	47 x →	**75.15 mg/g cell/h**

Advantages

1) High growth rate → high productivity
2) Methanol tolerance 7% (55.4 g/L) → high alcohol product titer

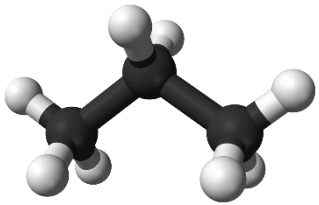
Kyung Hee University

infoGEN Diversity into Desirability

Propane-to-acetone bioconversion

Feedstock

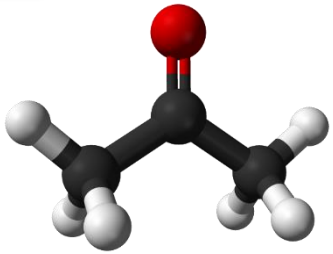
Propane, C_3H_8



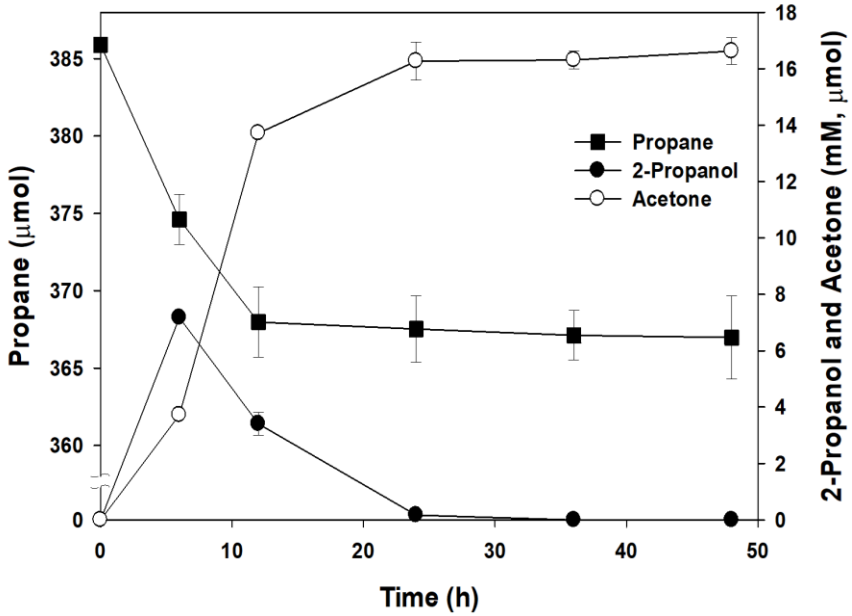
- Component of shale/natural gas
- Generated from petroleum refining
- Used as LPG

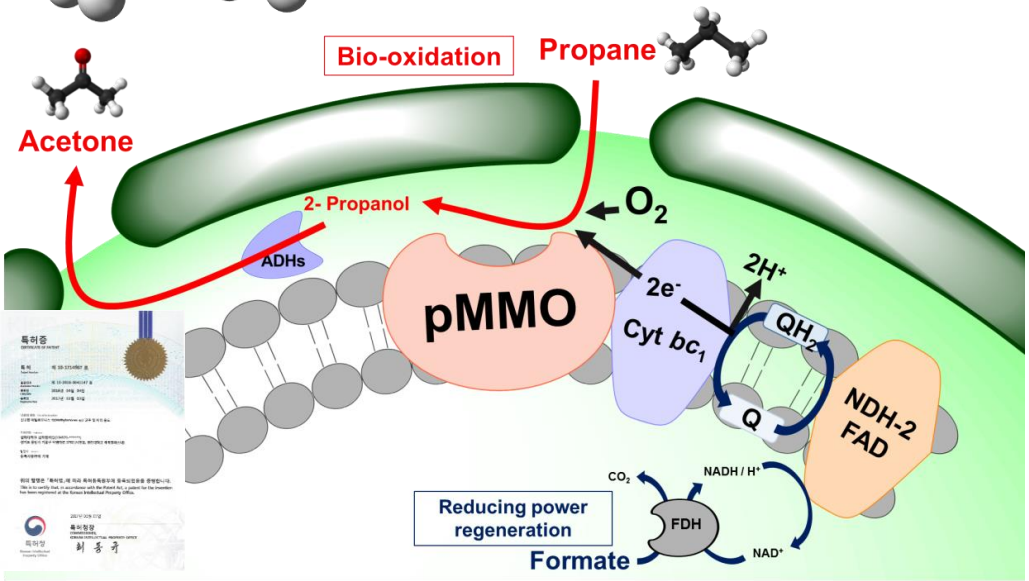
Target product

Acetone, $(CH_3)_2CO$



- Universal solvent
- Chemical intermediate
- Produced from propylene



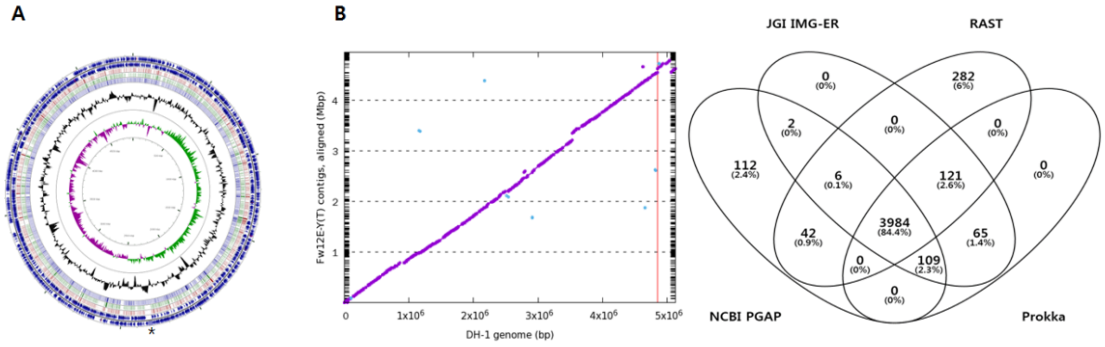


Propane-to-acetone

Acetone concentration: 16.27 mM
Conversion yield: 86 %
Average productivity: 0.68 mM/h
Specific productivity: 0.14 mmol/g cell/h

infoGEN
Diversity into Desirability

Methylomonas sp. DH-1 - Genomics



- ***Methylomonas* sp. DH-1 genome annotation**
- **Methane oxidation pathway (pMMO PQQ-MDH, H4F, H4MPT), No sMMO**
- **NAD$^+$-dependent MDH of G+**
- **RuMP cycle together with serine cycle**
- **PEP/pyruvate/AC carboxylase**

Fig. Genome annotation of *Methylomonas* sp. DH-1 by intergrating IMG, RAST, PROKKA and PGAP .

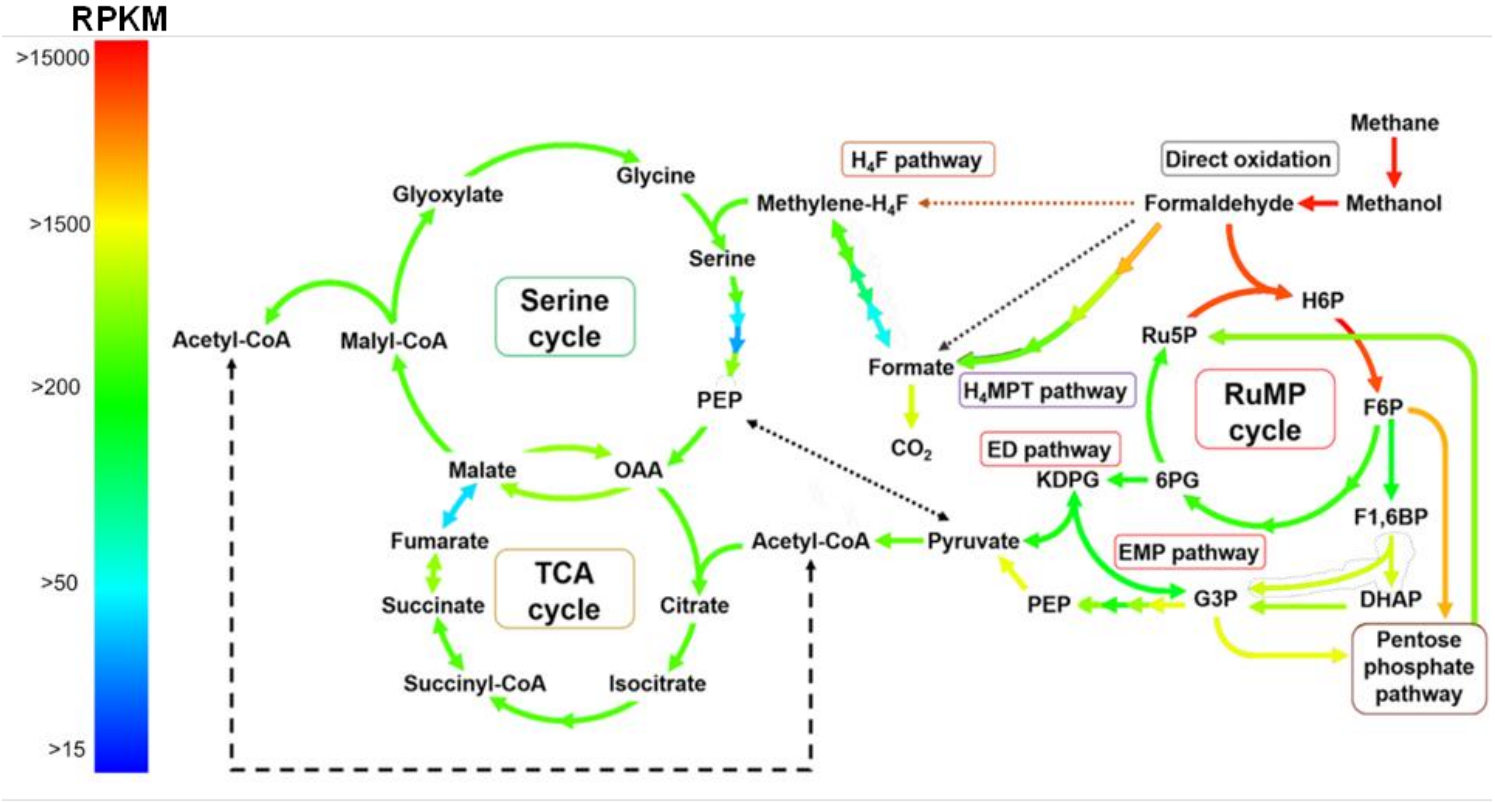
Table. General genome features of *Methylomonas* sp. DH-1

Feature	Chromosome	Plasmid
Size (bp)	4,849,532	277,875
G+C content (%)	56.47	51.66
Protein coding genes	4,441	228
Pseudogenes	85	13
tRNAs	47	0
rRNAs	3, 3, 3 (16S, 23S, 5S)	0
ncRNAs	4	0
CRISPR arrays	4	0
GenBank accession	CP014360	CP014361

Table. Specific genome features of *Methylomonas* sp. DH-1.

Gene	Locus tag	
PQQ-MDH	AYM39_03800	Alcohol dehydrogenase
NAD$^+$-MDH	AYM39_13885	
Propanol-preferring ADH	AYM39_01005	
Zn-ADH	AYM39_07405	
3-hexulose-6-phosphate synthase	AYM39_02470	RuMP pathway
3-hexulose-6-phosphate isomerase	AYM39_02475	
Serine-glyoxylate transaminase	AYM39_08355	Serine pathway
Serine hydroxymethyltransferase	AYM39_08865	
Phosphoenolpyruvate carboxylase	AYM39_12335	
Glycerate kinase	AYM39_08870	
Methylenetetrahydrofolate dehydrogenase	AYM39_08875	H_4F pathway
Methylenetetrahydrofolate reductase	AYM39_20685	
Formylmethanofuran-tetrahydromethanopterin N-formyltransferase	AYM39_03060	
Methenyltetrahydrofolate cyclohydrolase	AYM39_14560	
Methenyltetrahydromethanopterin cyclohydrolase	AYM39_08540	H_4MPT pathway

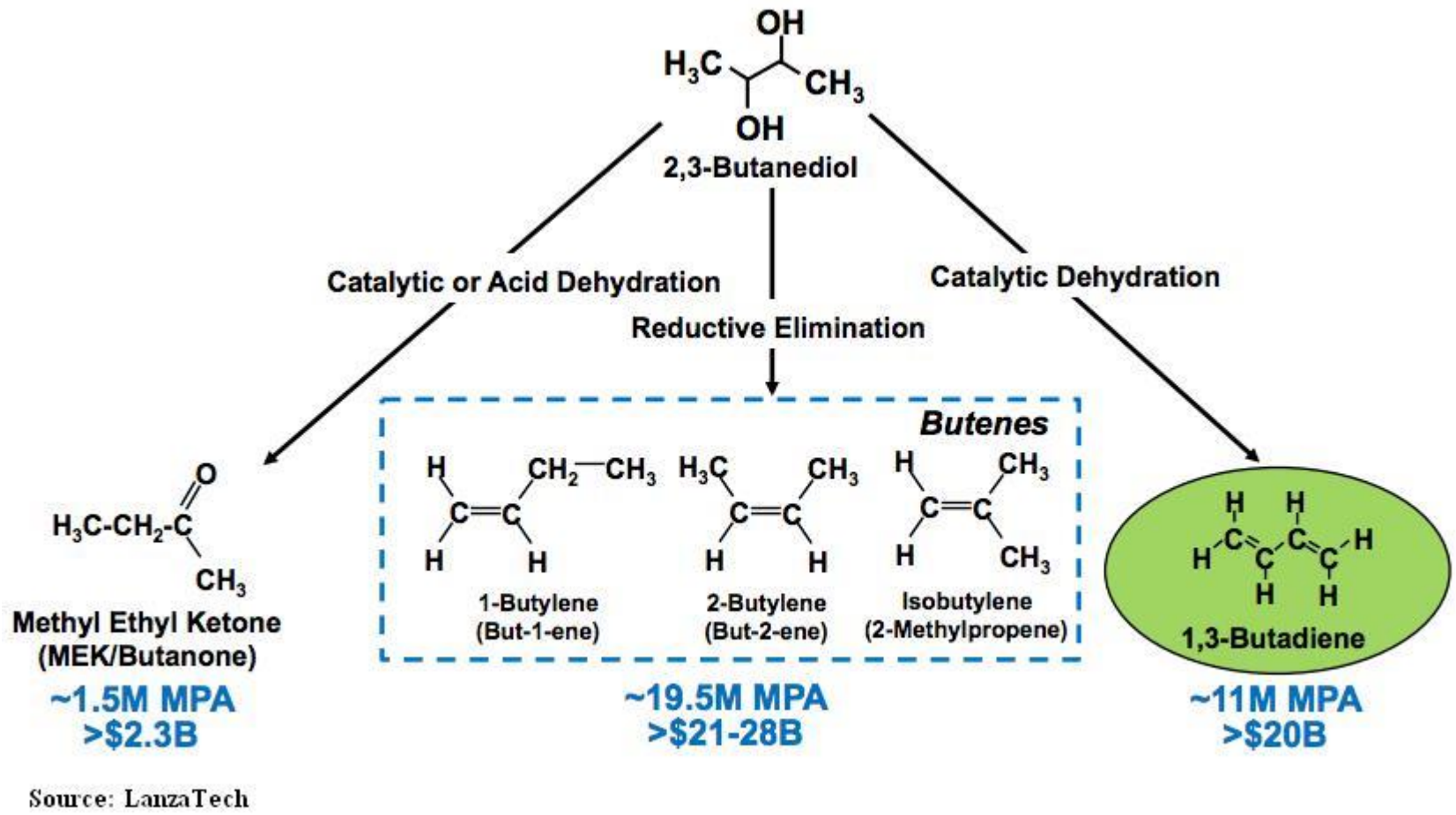
Methylomonas sp. DH-1 – Transcriptomics



Description of expression Level	RPKM range
Very high	>15000
High	1500-15000
Intermediate	200-1500
Low	50-200
Very low	15-50
Not expressed	<15

Fig. Overview of central metabolic pathways in *Methylomonas* sp. DH-1 predicted from genomic and transcriptomic data; Color indicates level of relative gene expression. Ru5P: ribulose 5-phosphate, H6P: hexulose 6-phosphate, F6P: fructose 6-phosphate, KDPG: 2-keto-3-deoxy 6-phosphogluconate, F1,6BP : fructose 1,6-bisphosphate, DHAP: dihydroxyacetone phosphate, G3P: glyceraldehyde 3-phosphate, PEP: phosphoenolpyruvate, OAA: Oxaloacetic acid.

Methane-to-Alcohol: 2,3-Butanediol



Source: LanzaTech

Metabolic engineering of methanotrophs for production of 2,3-BDO

❖ Scheme of 2,3-BDO production from methane

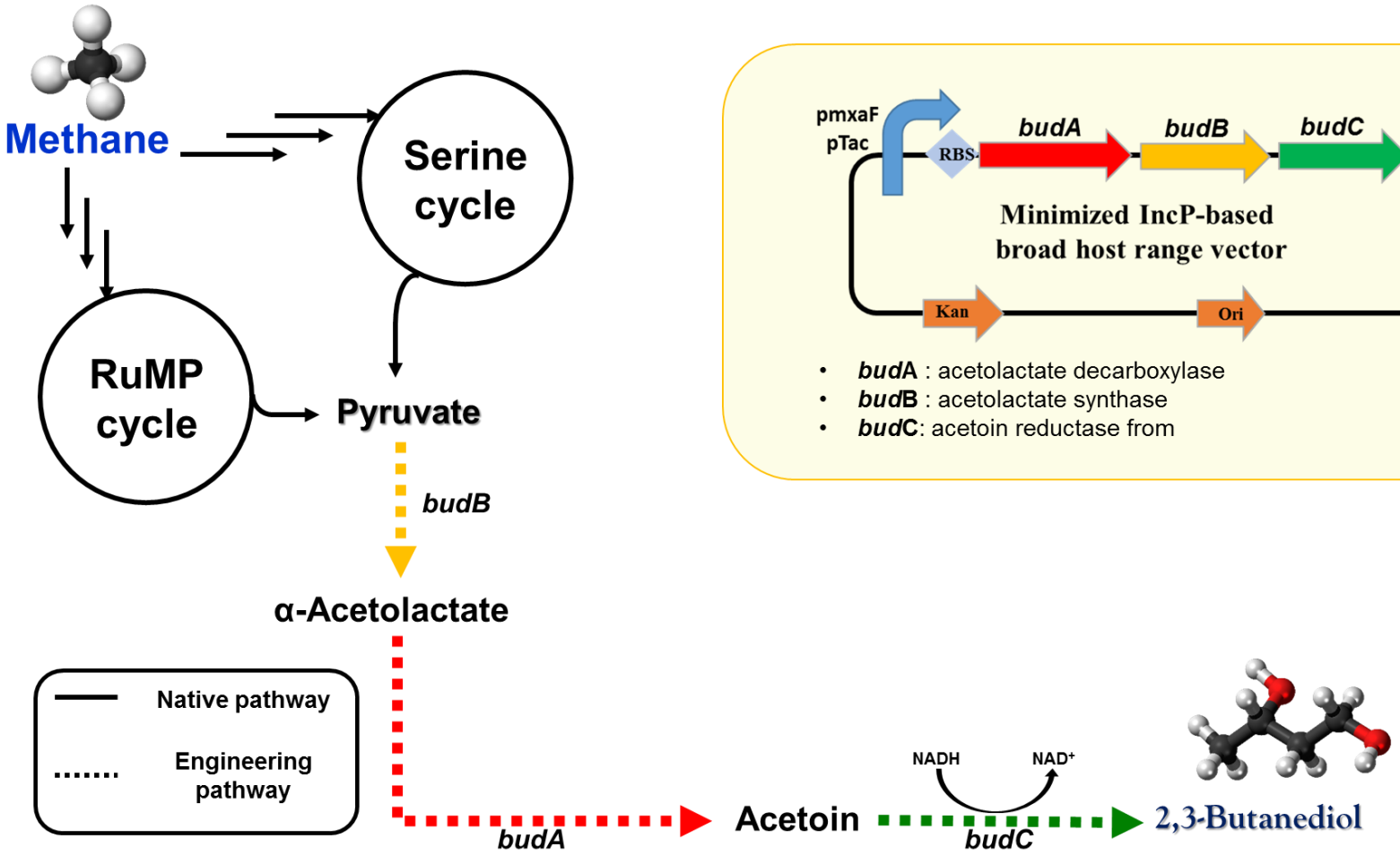


Fig. Engineering strategy for 2,3-BDO production.

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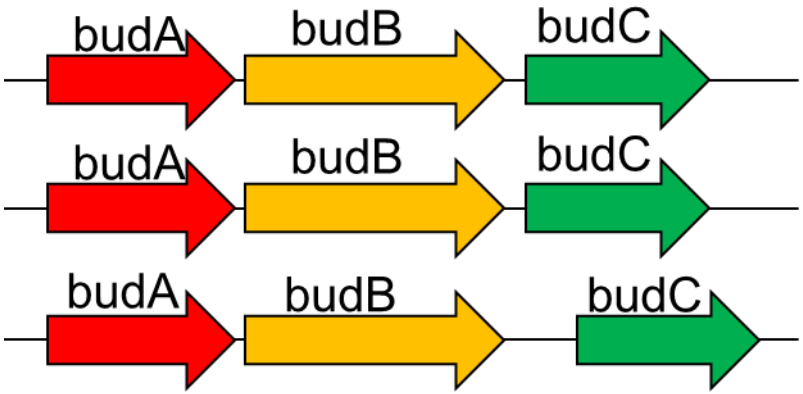
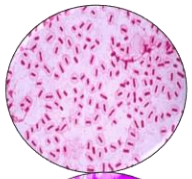
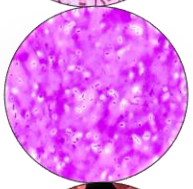
Gene cluster screening



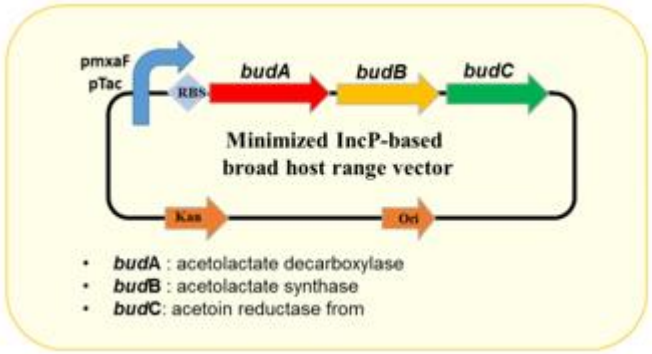


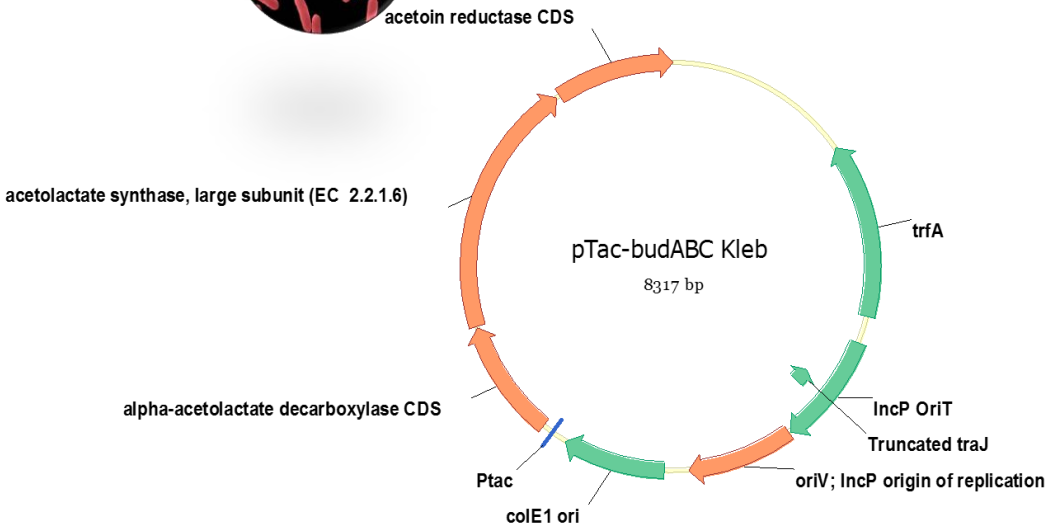
Klebsiella pneumoniae



Enterobacter aerogenes

Bacilus subtilis





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Engineering of methanotrophs for 2,3-BDO production

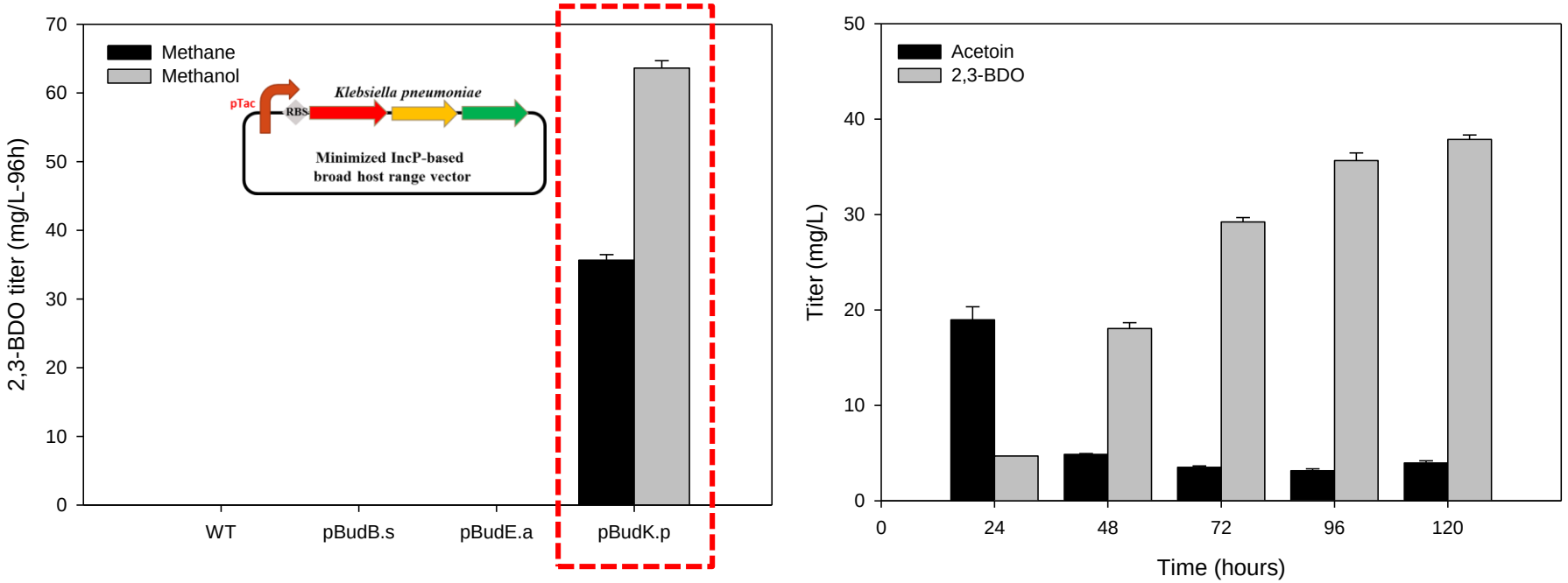


Fig. 2,3-BDO accumulation of engineered *M. alcaliphilum* 20Z harboring 2,3-BDO genes from *K. pneumonia* grown on 50% methane (v/v) and 1% methanol after 96h (**A**); acetoin and 2,3-BDO accumulation of 20Z/pBudK.p strain grown on 50% methane (v/v) (**B**).

In silico model-guided engineering for enhancing 2,3-BDO production

Model-derived knockout strategies to couple growth and 2,3-BDO

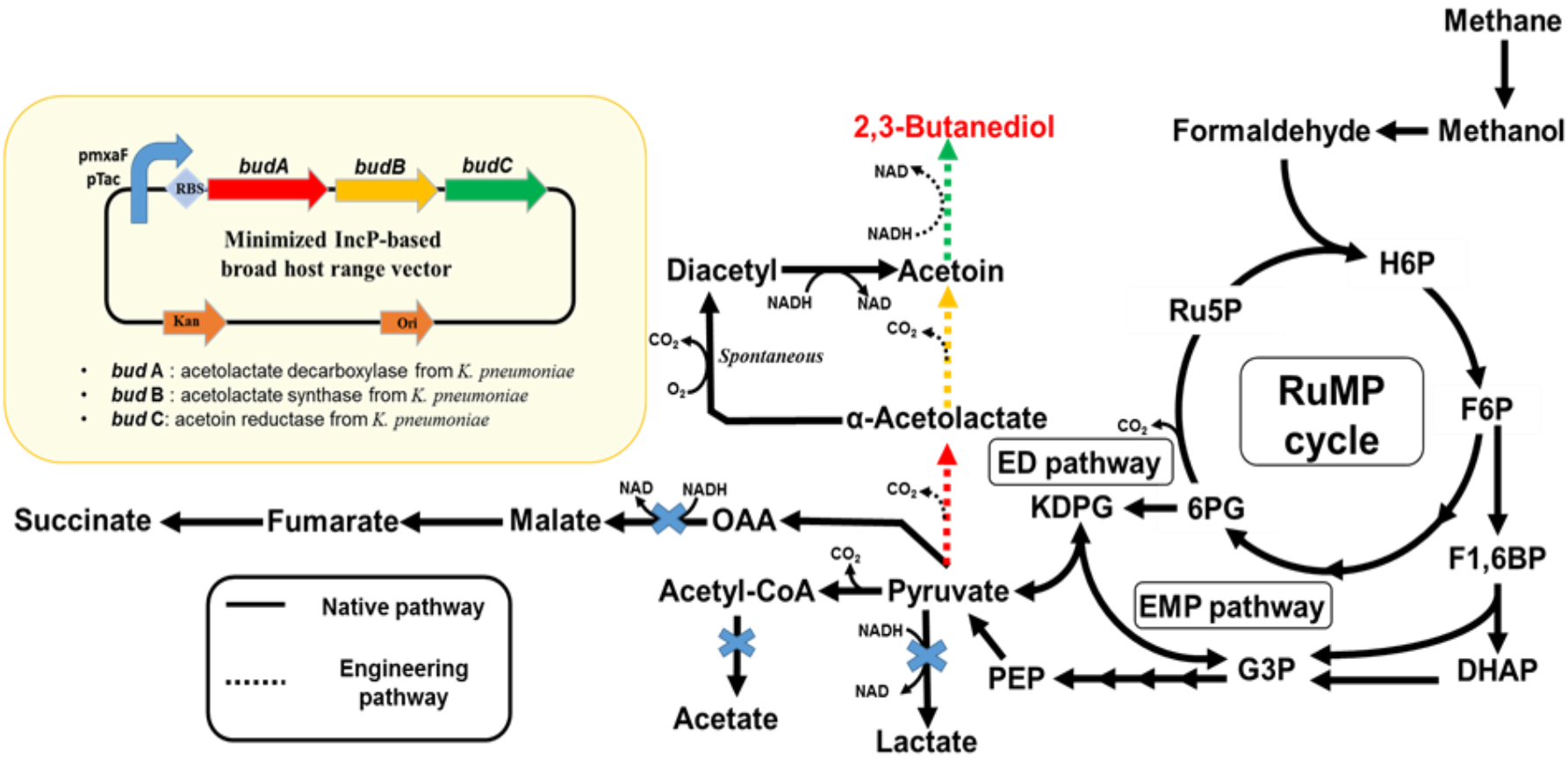
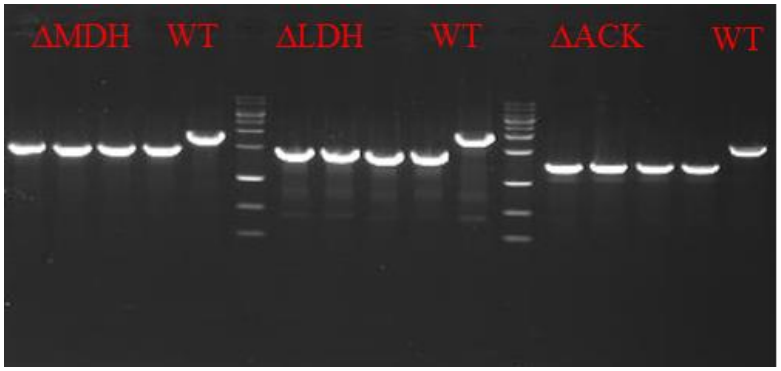
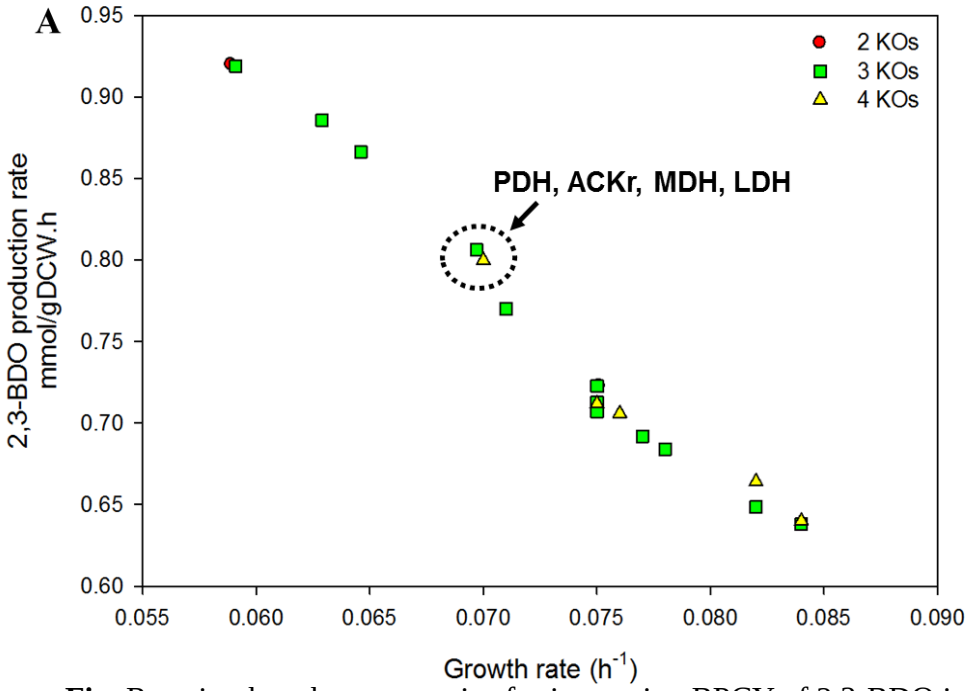


Fig. The development of the strain for production of 2,3-BDO by *in silico* genome-scale simulation.

In silico model-guided engineering for enhancing 2,3-BDO production



➢ ΔMDH,ACK,LDH strain improved 2,3-BDO production by enhancing precursor as well as reducing power availability.



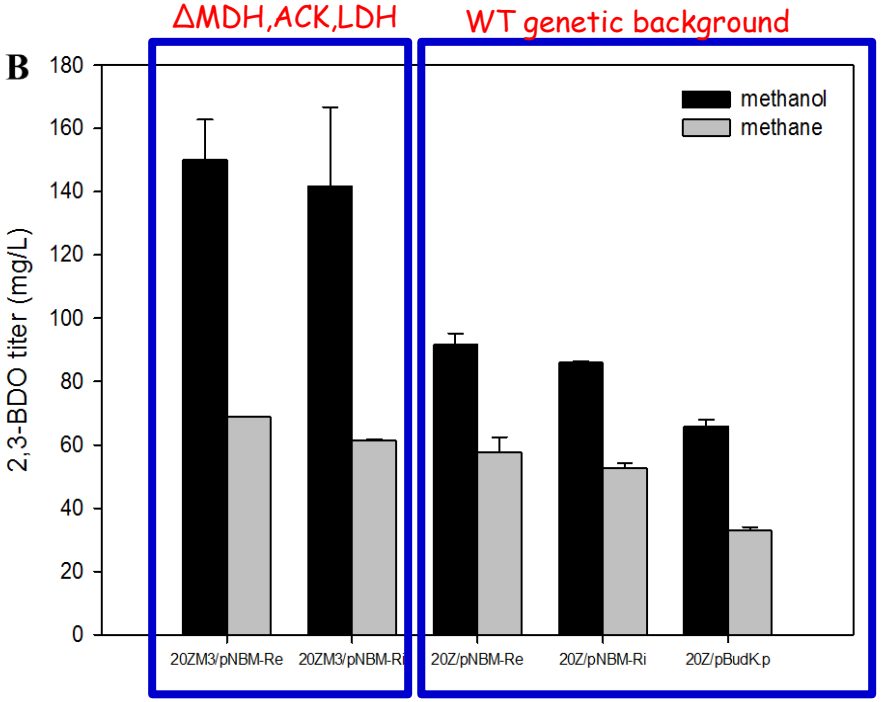


Fig. Reaction knockout strategies for improving BPCY of 2,3-BDO in *M. alcaliphilum* 20Z (A) and confirmation of 2,3-BDO improvement compared to WT genetic background (B).

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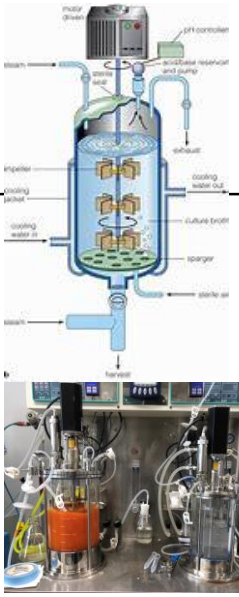
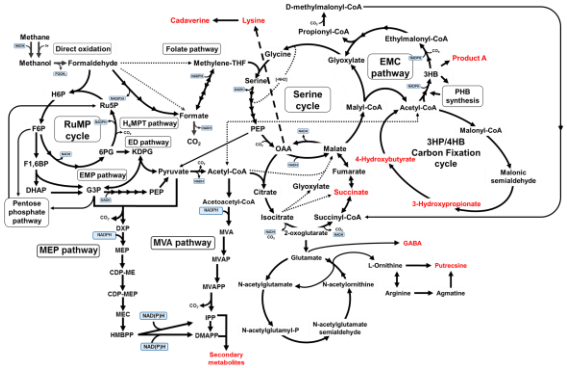
Technical issues: Low productivity

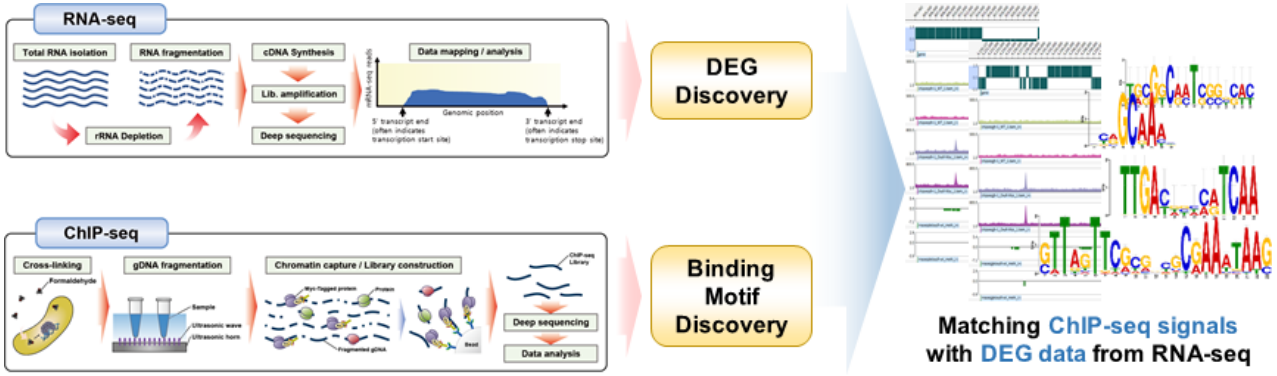


1. **Low mass transfer rate due to low solubility**
   → Gas fermentation systems with high k_{la}

2. **Low carbon flux in methanotrophs**
   → Enhance C1 assimilation efficiency of RuMP, serine cycle using Protein and Metabolic Engineering



3. **Lack of knowledge on metabolic regulation in methanotrophs**
   → Multi-omics analysis like ChIP-seq and etc.



Conclusion

- Methane has attracted much attention as next-generation carbon feedstock due to its abundance, cheap price and high degree of reduction. Methane is the major component of abundant shale/natural and bio-gas.
- We isolated and characterized *Mehtylomonas* sp. DH-1 for methane-to-chemical bioconversion. Multi-omics understanding and GSM were conducted for knowledge-based metabolic engineering.
- Methane-to-methanol and propane-to-acetone was conducted for biological gas-to-liquid conversion.
- Platform chemicals such as succinate, 2,3-BDO and etc. were synthesized from methane using metabolically engineered methanotroph strains.
- MEP pathway-based isoprenoid related products could be produced from methane using engineered methanotrophs.

→ **Metabolic engineering of methanotrophs will play a key role in methane-based C1 gas refinery.**