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Full Paper

Screening system for MAA-CoA productivity using 2-methylcitrate biosensor

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Abstract

Methyl methacrylate (MMA), the primary raw material of acrylic resin, is an important polymeric material due to its increasing demand and ease of recycling. The most promising biosynthetic route for MMA involves the condensation of methanol with methacrylyl-CoA (MAA-CoA), an intermediate in the valine degradation pathway. The toxicity of MAA-CoA, poor stability and low activity of the heterologous pathway enzymes make this biosynthetic pathway less feasible. For enabling the evolutionary engineering of this pathway and its components (enzymes), we constructed a biosensor system in which the cellular level of key intermediate MAA-CoA can be evaluated in a high-throughput manner. With the aid of this MAA-CoA sensory system, we could establish the functional pathway from isobutyric acid to MAA-CoA. The sensor described in this paper should be valuable tool in the design-build-test-learn cycle for optimizing and breeding this MMA pathway.

Keywords: methyl methacrylate; metabolic engineering; genetically-encoded biosensor; 2-methylcitrate; *Escherichia coli*

INTRODUCTION

Methyl methacrylate (MMA) is the primary raw material for acrylic resins, which are used as resin modifiers, paints, and paper coatings (Brydson, 1999; Lebeau et al., 2020). In recent years, its applications have expanded to include transparent ABS resins and MS resins, and the global market size is expected to exceed USD 8 billion by 2025, growing at an annual rate of 8~9% (https://www.grandviewresearch.com/press-release/global-polymethyl-methacrylate-pmma-industry). In addition, poly-methyl methacrylate (PMMA) resin is easily decomposed into MMA by thermal decomposition, making it easy to recycle, and some recycling plants have been put into practical use (Nagai & Ui, 2004). PMMA resin is thus an important polymer material for a sustainable society. MMA is currently produced from several petrochemical sources. The acetone cyanohydrin method is the most common, but use of toxic substances such as Hydrogen Cyanide (HCN) and concentrated sulfuric acid is necessary (Nagai & Ui, 2004). The direct oxidation of isobutylene has been commercialized in Asia and other regions, and MMA is produced from various feedstocks such as ethylene, propane, and isobutane (Nagai & Ui, 2004). But again, these methods use olefins as feedstock for synthesis, which is not a sustainable production method.

Fermentation-based production of MMA from more sustainable raw materials is attracting attention as a promising alternative to petrochemical synthesis. Bio-based processes may enable the production of MMA or its precursor methacrylic acid (MAA) via various intermediates, including acetyl CoA, pyruvate, succinyl CoA, 4-hydroxybutyryl CoA, valine, and 2- or 3-hydroxyisobutyrate (Burk et al., 2012; Lebeau et al., 2020). Several biosynthetic methods have been proposed for the consolidated biosynthesis of MMA (Lebeau et al., 2020), but the most promising synthetic pathway is the production via the intermediate methacrylyl-CoA (MAA-CoA) of the valine degradation pathway (**Fig. 1**). The pathway begins with the formation of α-keto acids, followed by oxidative decarboxylation and double bond formation, lead to the formation of MAA-CoA. This MAA-CoA can then be further metabolized via the TCA cycle through 3-hydroxyisobutyrate (3-HIB), propionyl-CoA, and 2-

methylcitrate (2-MC). Blocking this pathway and instead expressing alcohol acyltransferase (AAT) is expected to lead to the synthesis of MMA.

However, enabling this pathway and achieving titers sufficient for practical applications involve several technical challenges. First, the acyl-CoA dehydrogenase (ACD) enzymatic step requires several accessory proteins, and the optimal conditions for enabling this reaction are not yet fully understood. Eastham et al. used ACX4, an acyl-CoA oxidase from *Arabidopsis thaliana*, as an alternative to ACD and successfully generated MAA-CoA using *Escherichia coli* as a host (Eastham, 2016). However, ACX4 has low structural stability and lacks sufficient catalytic performance for industrial-scale MMA synthesis. In addition, MAA-CoA depletes redox mediators such as glutathione via Michael addition, significantly disrupting cellular metabolism (Plaga et al., 2000; Speir & Barnsley, 1971; Zhang et al., 2005). Therefore, the biosynthetic pathway should be carefully designed to prevent abnormal accumulation of this toxic intermediate. Finally, AAT is known to fold poorly in heterologous hosts (Yu & Mizunashi, 2017), and efforts to improve its expression and activity is still ongoing (Souleyre et al., 2005; Yu & Mizunashi, 2017).

In order to overcome the above problems and construct an efficient MMA heterologous production system, it is necessary to improve the activity of several enzymes constituting this pathway (especially ACD and AAT), optimize the expression balance of heterologous pathway components, and to engineer host strains better suited to this process. To this end, it is desirable to establish a high-throughput screening methods to evaluate enzyme performance and/or potential for high-titer MMA production. The most straightforward approach is to directly screen the production of the product (MMA). Developing a genetically-encoded biosensor for the target end product would enable fluorescence-based screening by linking the accumulation of intracellular products, such as flavonoids, plastic precursors, etc., to the expression levels of fluorescent proteins (Eggeling et al., 2015; Hwang et al., 2023; Rogers et al., 2015). However, since the final product accumulates at high concentrations within the cells, it can easily exceed the response concentration of the biosensor. As a result, while

biosensors may be used in the initial stages for poor to fair pathway improvements, it becomes difficult to use them for further optimization once the pathway reaches a certain level of performance.

In this paper, to establish a high-throughput method for evaluating the cellular supply capability of MAA-CoA, a key precursor for MMA synthesis but is a highly cytotoxic metabolite, we employed a strategy in which cell-generated MAA-CoA is continuously metabolized into the non-toxic and detectable metabolite 2-MC through a 5-step enzymatic reaction. We demonstrate that by placing a fluorescent protein under the control of a 2-MC-responsive transcription factor, the introduction of this 5-step pathway enables the high-throughput scoring of *E. coli*'s MAA-CoA production capability with a high detection dynamic range. With the aid of this system, functional pathway from isobutyric acid to MAA-CoA was established through the cooperation of microorganism-derived ACD and acyl-CoA ligase (ACL).

Materials and Methods

Strains, media and growth conditions

E. coli strain XL10-Gold (Agilent Technology, Inc., Santa Clara, CA) was used for standard molecular cloning. *E. coli* strain BW25113 was used for functional evaluation (Datsenko & Wanner, 2000). Unless otherwise noted, LB medium (2.0% (w/v) LB lennox, Nacalai Tesque, Kyoto, Japan) or LB agar plates (2.0% (w/v) LB, 1.5% (w/v) agar) were used for the cultivation of *E. coli*. Antibiotics were added at the following concentrations: 100 μg/mL ampicillin, 20 μg/mL kanamycin and 30 μg/mL streptomycin.

Plasmid construction

Plasmids used in this study are listed in **Table 1**. These were mostly constructed using Golden Gate DNA assembly (Engler et al., 2008) of PCR products amplified using KOD Plus (Takara Bio Inc., Shiga, Japan). Genes *prpR* and P_{prpRB} were amplified from *E. coli* BW25113 genome. The other genes (Gene Bank accession No.), which are *acl* (WP013415696), *acd* (NC002516), *etfa* (NC002516), *etfb* (NC002516), *etf-qo* (NC002516), *ech* (NC002516), *hch* (NC002516), *mmsA* (NC002516), *mmsB* (NC002516) were amplified from corresponding genomic DNA.

Functional analysis of 2-MC biosensors

Functional evaluations of 2-MC biosensors were performed on 96-well deep-well plates. Pre-culture was performed at 37°C and 800 rpm (MBR-034P, TAITEC, Saitama, Japan) overnight. Then, the main culturing was performed in LB + phosphate buffer medium [2.0% (w/v) LB, KH_2PO_4 2.31 g/L, K_2HPO_4 12.54 g/L] at 37°C, 800 rpm and an inoculum rate of 1.0% (w/v) from the pre-culture for 14 hours. As inducers, IPTG was added for genes on pathway plasmids expression. Propionic acid, 3-HIB, MAA or isobutyric acid were also added to the medium, depending on the experiment. The culture medium was diluted 10-fold with saline and the cell density and fluorescence intensity of sfGFP were

measured using FilterMax F5 (Molecular Devices) under the following conditions: sfGFP (485 nm excitation, 535 nm emission) and optical density (595 nm).

Evaluation of the MAA-CoA Producing Activity

The cellular performance of ACL mutants was compared based on their booster effect on the esterification of MAA into Benzyl methacrylate. To this end, BW25113 cells harboring pACL for the expression of ACL were transformed with AAT_{10m} expressing plasmid pAAT. The resultant transformants were grown as pre-culture in LB medium at 37°C and 800 rpm on 48-well deep-well plates overnight. The main culturing was performed in 10 mL of TB medium (0.4% (v/v) glycerol) at 30°C, 200 rpm and an inoculum rate of 1.0% (w/v) from pre-culture in a flask. Each culture was induced by adding 0.1 mM IPTG and added any concentration of MAA and 10 mM benzyl alcohol after being incubated for 3 hours. Immediately after the induction, 1mL (10% (v/v) of the original culture) of dodecane was overlaid onto the cultures and shaken for an additional 24 hours.

Then, 50 μL of dodecane fraction was collected and diluted in 450 μL of hexane. Thus, obtained samples were analyzed by GC-FID (Shimadzu GC-2014, Shimadzu, Kyoto, Japan) with an RTX-5MS capillary column (30 m × 25 mm ID and 0.25 mm film thickness, Restek Corporation, Bellefonte, PA, USA). Splitless injections (1 μL) were performed with an injector and an FID detector temperature of 250 °C and separated with a GC oven-temperature program starting at 40 °C for 6 min, which was increased by 20 °C min^{-1} up to 230 °C, and held at 230 ℃ for 9 min. For quantification, the calibration curve was drawn with a Benzyl methacrylate (TCI, Tokyo, Japan).

Results

Construction of MAA-CoA biosensor

In the valine metabolic pathway of many organisms including humans, MAA-CoA is hydrolyzed to hydroxyisobutyryl-CoA by enoyl CoA hydratase (**ECH**) and then to 3-HIB by 3-hydroxyisobutyric CoA hydrolase (**HCH**). Subsequently, 3-HIB is oxidized to methylmalonate semialdehyde and propionyl CoA by 3-hydroxyisobutyrate dehydrogenase (**mmsB**) and then methylmalonate semialdehyde dehydrogenase (**mmsA**). Propionyl CoA is further converted to 2-MC by endogenous enzyme PrpC. By heterologously expressing four enzymes—ECH, HCH, mmsB, and mmsA—MAA-CoA can be converted to 2-MC.

E*scherichia coli* possesses a 2-MC-responsive transcriptional activator PrpR, which regulates chromosomal genes related to propionate metabolism (Palacios & Escalante-Semerena, 2004; Plassmeier et al., 2013). We investigated whether the ability to supply MAA-CoA could be evaluated using a PrpR-based biosensor by introducing a plasmid into *E. coli* that encoding four heterologous enzymes for conversion of MAA-CoA to 2-MC.

First, a 2-MC sensor plasmid was constructed by cloning the *prpR* gene and its corresponding promoter region from the *E. coli* genome to amplify PrpR expression, and the *sfGFP* gene was placed downstream of the PrpR-responsive promoter (**Fig. 2a**).

E. coli cells transformed with this plasmid exhibited strong fluorescence upon addition of propionic acid to the culture medium (**Fig. 2b**). Propionic acid is converted to propionyl CoA by endogenous PrpE, which is converted to 2-MC by another endogenous enzyme PrpC. Although 2-MC is eventually catabolized to pyruvate and succinate by endogenous enzymes (PrpD, ACN, PrpB), a portion of it binds to PrpR. Since this strain lacks the genes required to convert 3-HIB or MAA to 2-MC, it did not respond to either of these substrates (**Fig. 2b**).

When ECH, HCH, mmsA, and mmsB from *Pseudomonas aeruginosa* PAO1, along with ACL from *Rhodococcus hoagii*, were introduced, GFP fluorescence increased in response to MAA, as well

as to propionic acid and 3-HIB (**Fig. 2d**). In contrast, when mmsA and mmsB were omitted, the response to MAA abolished, while the response to 3-HIB remained (**Fig. 2c**). These results clearly demonstrate that 2-MC is produced from MAA only via the pathway we designed. The fluorescence leakage under substrate-free conditions is likely due to basal levels of intracellular 2-MC.

We found that in cells transformed with pNull and pHIB, the growth of the cells was significantly increased upon the addition of propionic acid (**Fig. 3**). This is likely due to the entry of propionic acid into the TCA cycle via 2-MC, thereby supporting biomass production. On the other hand, the strain harboring pMAA did not exhibit propionate-dependent growth. It is possible that propionyl-CoA is converted into methacrylate by the heterologous pathway instead of being channeled into the TCA cycle. Furthermore, this strain showed reduced growth when cultured with high concentration of MAA or 3-HIB, likely due to the accumulation of MAA-CoA. MAA-CoA is highly reactive with glutathione and other substances, leading to its depletion and thus high toxicity (Plaga et al., 2000; Speir & Barnsley, 1971; Zhang et al., 2005).

Visualizing the titer of the two-step pathway from MAA to MMA-CoA.

To further confirm that PrpR can be used as an indicator of MAA-CoA supply, we investigated the relationship between biosensor output and the activity of CoA-ligase, which converts MAA into MAA-CoA. To this end, we added various concentrations of MAA to the culture medium of *E. coli* harboring the two plasmids pSensor and pMAA and measured the resulting cellular fluorescence. This plasmid encodes an ACL from *Rhodococcus hoagii*, which catalyzes the conversion of MAA to MAA-CoA (**Fig. 4a**). The titer of MAA-CoA is expected to increase in proportion to the concentration of MAA in the medium. Consisting with this, the biosensor output increased nearly proportionally with MAA concentration ranging from 0 to 25 mM (**Fig. 2d, 4c**). However, at 50 mM MAA, the fluorescence signal decreased, which is likely attributed to MAA-induced cytotoxicity (**Fig. 3c**) (Plaga et al., 2000; Webb et al., 2018).

To determine whether the sensor output accurately reflects the intracellular MAA-CoA production, we evaluated MAA-CoA productivity by quantifying the accumulation of benzyl methacrylate (Benzyl-MA), which is produced through alcoholysis with supplemented benzyl alcohol. For this purpose, we additionally expressed an engineered variant of *Malus domestica*-derived AAT, carrying ten adaptive mutations (hereafter referred to as AAT_{10m}) (**Fig. 4b**) (Yu & Mizunashi, 2017). Benz-MA was measured instead of MMA because AAT_{10m} exhibits higher specificity for benzyl alcohol than for methanol. The amount of Benzyl-MA accumulated in the culture supernatant was quantified by GC-FID after feeding the cultures with varying concentrations of MAA and a fixed concentration (10 mM) of benzyl alcohol. As expected, Benzyl-MA production increased proportionally with MAA concentration in the range of 0-25 mM.

Identification of the minimal factors required for the dehydrogenation of isobutyryl-CoA to MAA-CoA.

ACD is an enzyme that catalyzes the dehydrogenation at the α-β positions of fatty acyl-CoAs, a reaction involves in amino acid metabolism and the β-oxidation cycle. During this desaturation process, ACD transfers a hydride ion from C_β position to abound FAD, generating $FADH_2$. This reduced $FADH_2$ is then reoxidized by electron transfer flavoprotein (ETF) (Henriques et al., 2021). The electrons transferred to ETF are subsequently passed to electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO), which ultimately feeds them into the respiratory chain (**Fig. 5c**) (Ghisla & Thorpe, 2004; Watmough & Frerman, 2010). However, the specific cofactors required for the transfer of electrons from isobutyryl-CoA via ACD to the respiratory chain have not been fully elucidated, hindering the reconstruction of the activity in heterologous system.

Recently, Bannister et al. demonstrated that activity of the ACD of *Pseudomonas putida* KT2440 can be reconstituted in *E. coli* when three additional genes (PP4201-4203) are co-expressed (Bannister & Prather, 2024). We hypothesized that other bacteria may exhibit a similar genetic

arrangement, and that our MAA-CoA detection system developed in this study could facilitate the identification of a minimal sufficient and necessary set of genes capable of reconstituting microbial ACD activity in heterologous hosts.

In addition to the genes encoding enzymes that convert MAA-CoA to propionyl-CoA, we cloned the ACD gene from *Pseudomonas aeruginosa* PAO1 (PA0746) into a plasmid (**Fig. 5b**). Based on the PAO1 genome, we identified three adjacent genes—PA2951 to PA2953—that appear to encode the complete set of ETF components: PA2951 and PA2952 are presumed to encode the α- and β-subunits of ETF, respectively, while PA2953 is predicted to encode ETF-QO (**Fig. 5b**) (Winsor et al., 2016). In addition to the pMAA and pSensor, a third plasmid expressing these putative ETF-related genes was co-transformed into the cells (**Fig. 5a, b**).

Under conditions in which all three ETF-related genes were expressed, the cellular fluorescence increased proportionally with isobutyric acid concentration, suggesting that the reaction from isobutyric acid-CoA to MAA-CoA is fully activated (**Fig. 5d**). The maximum fluorescence observed in response to isobutyric acid was comparable to that observed for propionic acid (**Fig. 2, 5d**), suggesting that the ACD-mediated supply of MAA-CoA was at least as efficient as the endogenous conversion from propionic acid to 2-MC. When any one of the three genes was deleted, the sensor response was abolished (**Fig. 5d**). This result clearly shows that the three genes (PA2951-PA2953) encoded the minimal but sufficient set of ETFs required to support ACD activity.

Discussion

In this study, we developed a gene-encoded biosensor that can evaluate intracellular MAA-CoA production by translating it into fluorescent output by coupling 2-MC-responsive transcription factor PrpR with a 5-step enzymatic pathway from MAA-CoA to 2-MC. When a heterologous valine degradation pathway was introduced into *E. coli*, the sensor response increased with the concentration of propionic acid, 3-HIB, and MAA. Furthermore, this biosensor system enabled the identification of a minimal yet sufficient set of genes that can shuttle electrons from ACD to the respiratory chains, thereby facilitating the heterologous reconstruction of a fully functional ACD derived from *P. aeruginosa*.

The PrpR-based sensor was previously tested as possible induction system using propionate as an inducer (Lee & Keasling, 2005), but it suffered from the limited change in output signal. The PrpR-based sensor was also tested as possible sensory system for 3-hydroxy propionate (3-HP), but they ended up with only 4-fold change in fluorescence (Rogers & Church, 2016). Our PrpR-based sensor showed an approximately 12-fold fluorescence response to MAA-CoA by co-expressing four biosynthetic enzymes to metabolize MAA-CoA to propionyl-CoA, all without optimization process. The sensor's signal-to-noise ratio could be further increased by redesigning the PrpR promoter and RBS sequence. Additionally, it may be possible to further increase the signal-to-noise ratio by using transcription interference (Watanabe et al., 2024).

To improve the overall titer of the valine to MMA pathway, enhancing the activity of ACD (or ACX) remains the key objective. Our results demonstrate that our PrpR-based biosensor system is a powerful tool for visualizing MAA-CoA production capacity in *E. coli*. Using this system, we were able to rapidly reconstitute fully functional ACD activity. Because one can detect the MAA-CoA with the setup described herein, one can possibly screen for better ACD variants by searching for variants exhibiting higher sensory signals. In addition, we are currently exploring the use of this sensor platform to screen for enhanced AAT variants, as AAT represents another bottleneck step in this biosynthetic

pathway.

Acknowledgments

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Author Contribution

SS and MT constructed the plasmids for the pathway from organic acids to propionyl-CoA. TO and TM constructed the ETF plasmids. RK and DI established the GC/FID protocol for evaluating the production level of benzyl methacrylate. All experimental data was taken by SH. SN and DU supervised the project. SH and DU wrote the manuscript.

Declaration of Conflicting Interests

The authors declare no competing financial interest.

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Figure Legends

Fig. 1 |

Schematic representation of the engineered pathway for the production of the MMA and components required for biosensing MAA-CoA, the direct and key precursor for MMA.

Fig. 2 |

Extension of responsiveness of 2-MC sensors.

Fig. 3 |

Effect of organic acids on the growth of *E. coli* cells harboring plasmids (a) pNull, (b) pHIB, (c) pMAA.

Fig. 4 |

Evaluation of the titer of MAA to MAA-CoA conversion using 2-MC sensor couple and Benzyl-MA production.

Fig. 5 |

Dehydrogenation of isobutyryl-CoA to MAA-CoA by co-expression of ACD and ETF electron transfer proteins.

Table-1 | Plasmids used in this study

Name	Description	Ori	Marker	Source
pNull	pJ404-lacI-$P_{T5/lacO}$-φ	pMB1	Amp^R	This study
pHIB	pJ404-lacI-$P_{T5/lacO}$-mmsAB-	pMB1	Amp^R	This study
pMMA	pJ404-lacI-$P_{T5/lacO}$-mmsAB-$P_{T5/lacO}$-acl-$P_{T5/lacO}$-ech-hch	pMB1	Amp^R	This study
pIBA	pJ404-lacI-$P_{T5/lacO}$-mmsAB-$P_{T5/lacO}$-acd-acl-$P_{T5/lacO}$-ech-hch	pMB1	Amp^R	This study
pAAT	pJ404-lacI-$P_{T5/lacO}$-aat_{10m}	pMB1	Amp^R	This study
pETF's-Full	pJ212-PJ23119-etfa-etfb-etfqo	p15A	Kan^R	This study
pETF's-*Δetfa*	pJ212-PJ23119-etfb-etfqo	p15A	Kan^R	This study
pETF's-*Δetfb*	pJ212-PJ23119-etfa-etfqo	p15A	Kan^R	This study
pETF's-*Δetf-qo*	pJ212-PJ23119-etfa-etfb	p15A	Kan^R	This study
pACL	pJ212-PJ23116-acl	p15A	Kan^R	This study
pSensor	pCDF-prpR-P_{prpRB}-sfgfp	CloDF13	$Strep^R$	This study

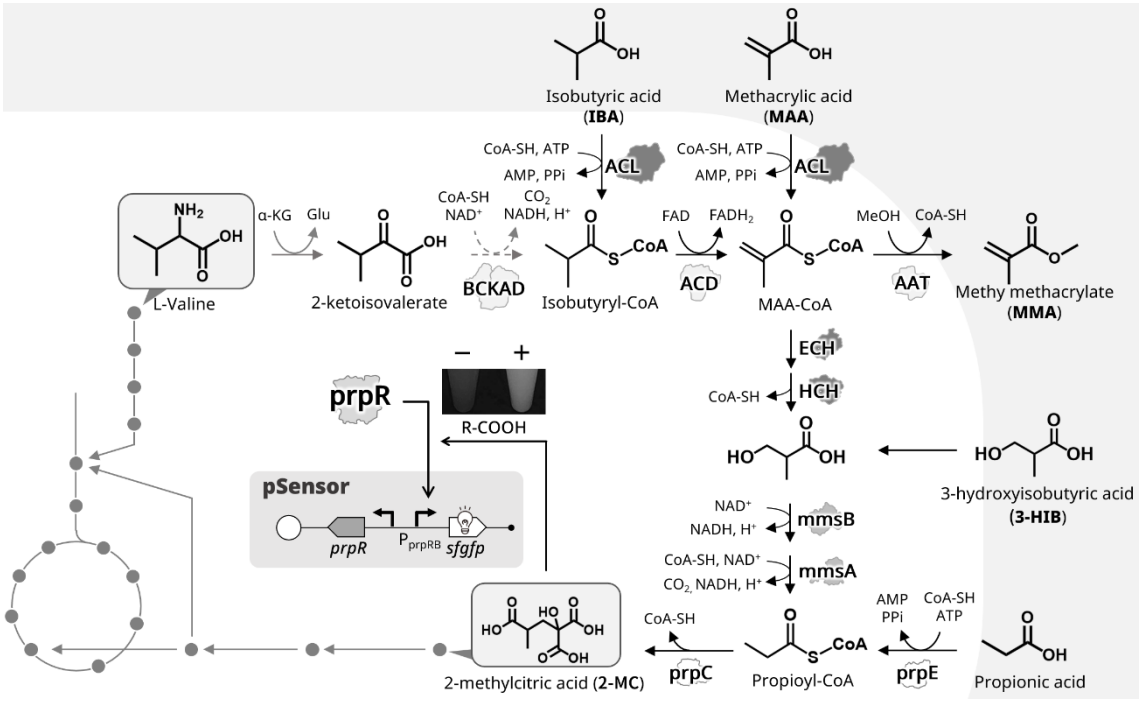
Isobutyric acid
(IBA)
Methacrylic acid
(MAA)
CoA-SH, ATP
AMP, PPi
ACL
CoA-SH, ATP
AMP, PPi
ACL
L-Valine
α-KG
Glu
2-ketoisovalerate
CoA-SH
NAD+
CO2
NADH, H+
BCKAD
Isobutyryl-CoA
FAD
FADH2
ACD
MAA-CoA
MeOH
CoA-SH
AAT
Methy methacrylate
(MMA)
ECH
CoA-SH
HCH
3-hydroxyisobutyric acid
(3-HIB)
prpR
R-COOH
pSensor
prpR
PprpRB
sfgfp
NAD+
NADH, H+
mmsB
CoA-SH, NAD+
CO2, NADH, H+
mmsA
2-methylcitric acid (2-MC)
CoA-SH
prpC
Propioyl-CoA
AMP
PPi
CoA-SH
ATP
prpE
Propionic acid

Fig.1

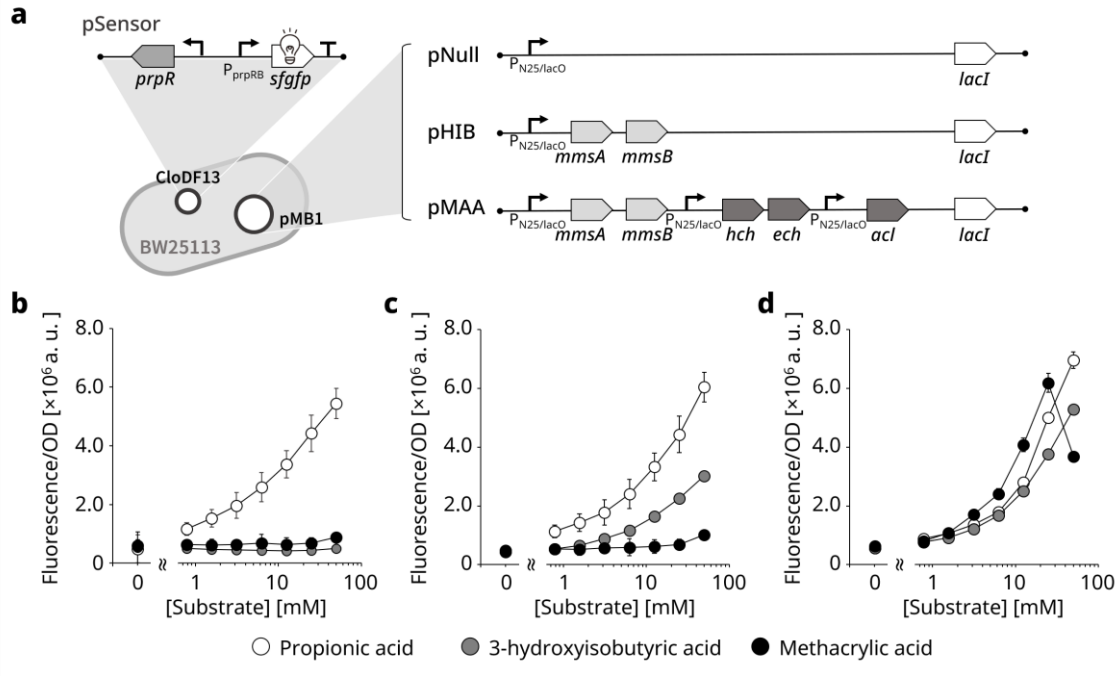
a
pSensor
prpR
P prpRB
sfgfp
CloDF13
pMB1
BW25113
pNull
P N25/lacO
lacI
pHIB
mmsA
mmsB
pMAA
hch
ech
acl
b
c
d
Fluorescence/OD [×10^6 a. u.]
[Substrate] [mM]
Propionic acid
3-hydroxyisobutyric acid
Methacrylic acid

Fig.2

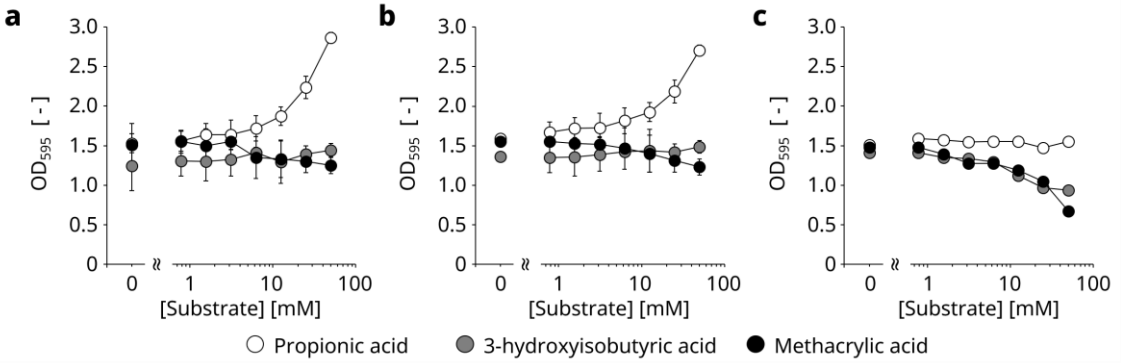
a
b
c
OD595 [-]
[Substrate] [mM]
Propionic acid
3-hydroxyisobutyric acid
Methacrylic acid

Fig.3

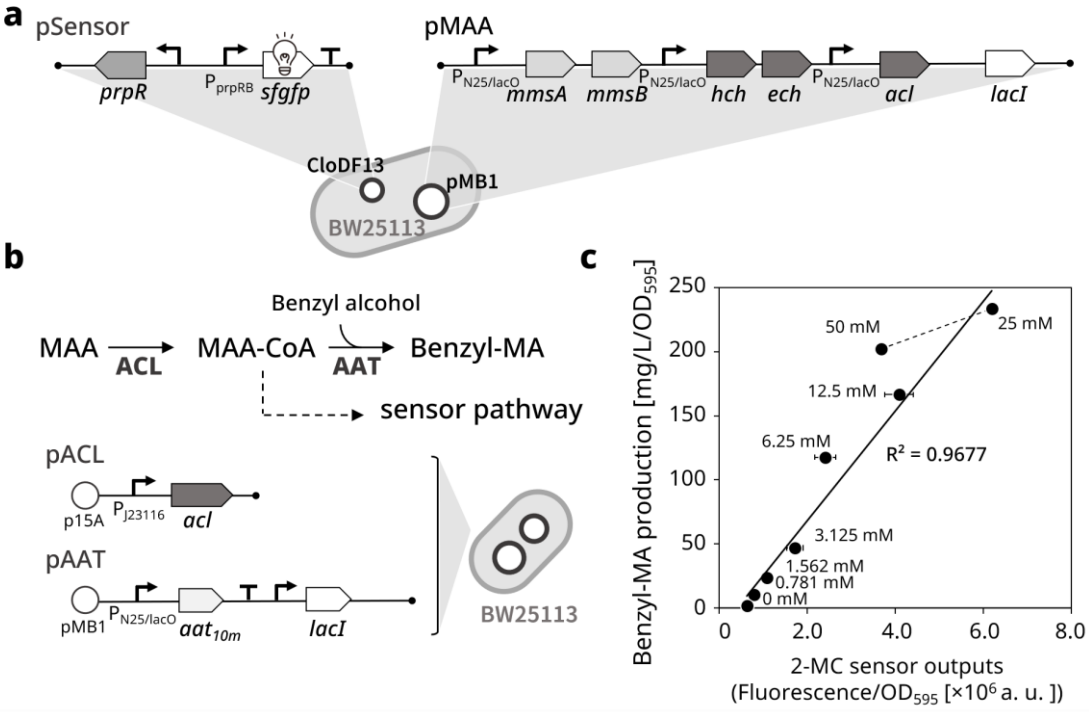
a
pSensor
prpR
P prpRB
sfgfp
pMAA
P N25/lacO
mmsA
mmsB
hch
ech
acl
lacI
CloDF13
pMB1
BW25113
b
Benzyl alcohol
MAA
ACL
MAA-CoA
AAT
Benzyl-MA
sensor pathway
pACL
p15A
P J23116
acl
pAAT
pMB1
aat10m
lacI
BW25113
c
Benzyl-MA production [mg/L/OD595]
2-MC sensor outputs
(Fluorescence/OD595 [×10^6 a. u.])
50 mM
25 mM
12.5 mM
6.25 mM
R² = 0.9677
3.125 mM
1.562 mM
0.781 mM
0 mM

Fig.4

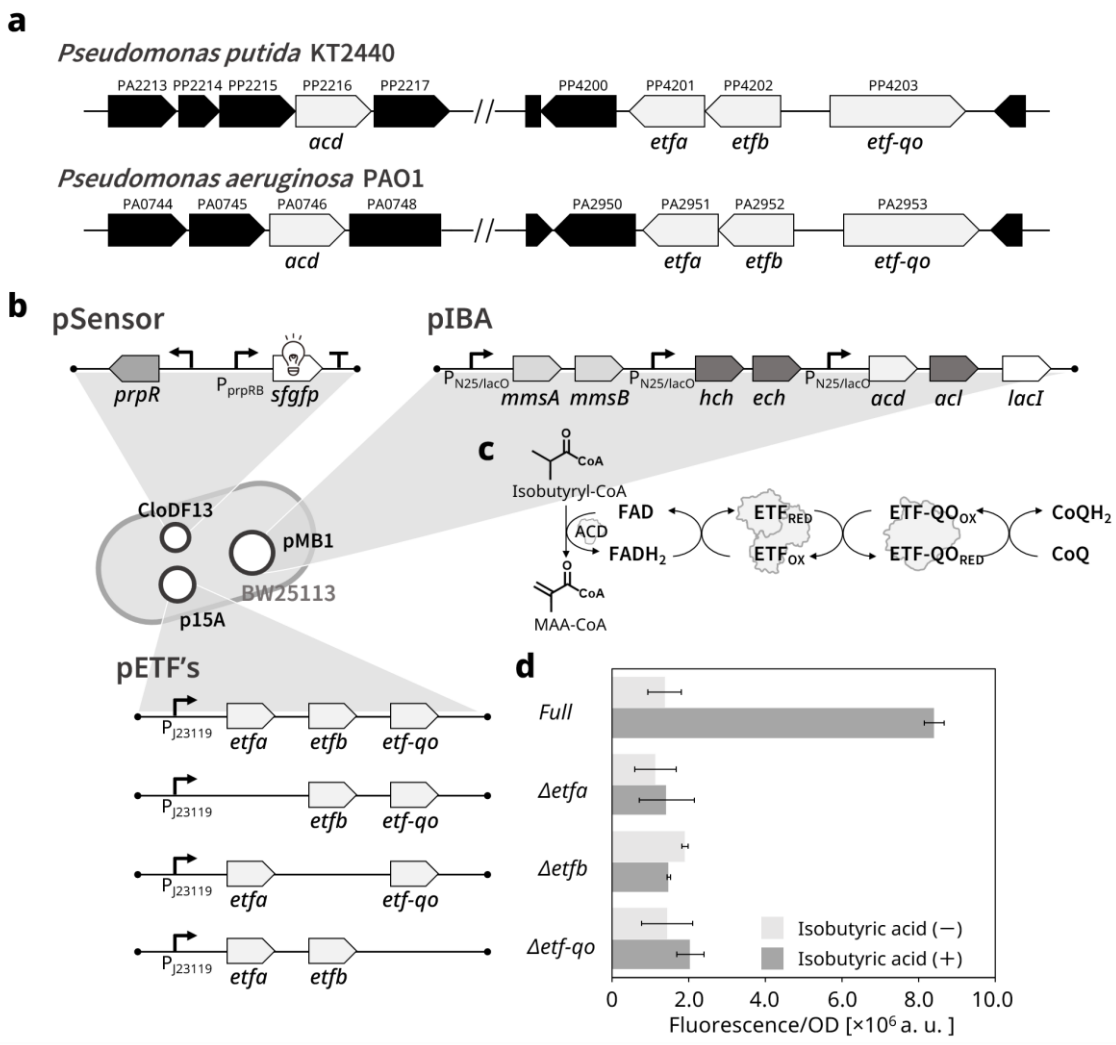
a
Pseudomonas putida KT2440
PA2213 PP2214 PP2215 PP2216 PP2217
acd
PP4200 PP4201 PP4202 PP4203
etfa etfb etf-qo
Pseudomonas aeruginosa PAO1
PA0744 PA0745 PA0746 PA0748
acd
PA2950 PA2951 PA2952 PA2953
etfa etfb etf-qo
b
pSensor
prpR
P prpRB sfgfp
pIBA
P N25/lacO mmsA mmsB
P N25/lacO hch ech
P N25/lacO acd acl lacI
CloDF13
pMB1
BW25113
p15A
pETF's
P J23119 etfa etfb etf-qo
P J23119 etfb etf-qo
P J23119 etfa etf-qo
P J23119 etfa etfb
c
CoA
Isobutyryl-CoA
ACD
FAD
FADH2
ETFRED
ETFOX
ETF-QOOX
ETF-QORED
CoQH2
CoQ
CoA
MAA-CoA
d
Full
Δetfa
Δetfb
Δetf-qo
Isobutyric acid (−)
Isobutyric acid (+)
0 2.0 4.0 6.0 8.0 10.0
Fluorescence/OD [×10^6 a. u.]

Fig.5