Engineered *Pseudomonas putida* monoculture system for green synthesis of 7-methylxanthine

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TABLE OF CONTENTS

1.0 Supplemental materials and methods

1.1 Production of 7-MX from green tea hydrothermal dissolution (HD) samples

Green tea HD sample obtained from Thermaquatica Inc. (Carbondale, IL, USA) was filter sterilized, pH adjusted to neutral, and stored at 4 °C. Sample of 9 mL green tea was added to 250 mL shake flasks separately to obtain 90% (v/v) HD cultures, and the 2XM9 media was supplemented with 40 mM glycerol and antibiotics (10 mL total volume). Overnight cultures were prepared as described in 2.4, and OHD test cultures were inoculated for an initial OD_{600} of 1.0.

1.2 Sample preparation for RNA-seq

Shake flask cultures were prepared in 250 mL flasks (culture volume 25 mL) supplemented with 40 mM glycerol and 2 g/L glucose separately (n=6). 10 mM of caffeine was added to three glycerol-containing flasks and three glucose-containing flasks (n=3). LJBJ500 culture was inoculated to all flasks at an initial OD_{600} of 0.1, and the cultures were grown at 30 °C, shaking at 225 rpm for 3 h. Growth was measured (OD_{600}), and cultures were centrifuged at 4500 rpm for 10 min (4 °C). Cell pellets were then washed twice with 10 mL of 2XM9, and the cell pellets were transferred to 1.5 mL Eppendorf tubes, labeled, and frozen by immersing in liquid N_2 for 2 min. All samples were stored in dry ice and shipped to the RNAseq facility [1].

1.3 Microscopic observation of cells expressing GFP.

Microscopic images were obtained using a Leica DM4000B fluorescent microscope (Leica Microsystems, USA) equipped with a QIClick digital CCD camera (QImaging). LJBJ693 was grown in M9 media containing 40 mM glycerol, 10 mM caffeine, and 2 g/L glucose- 10 mM caffeine separately in 5 mL volume (in 15 mL culture tubes) for 3 h (30 ˚C, shaking at 225 rpm). 1 mL culture samples were then centrifuged, cells were added onto glass slides, and specimens were prepared for imaging. The acquired images were assembled, color-balanced, and analyzed with Velocity® software (Velocity Software Inc., CA, USA).

1.4 Measurement of the percentage of cells expressing GFP using flow cytometry.

Separate cultures were prepared in 5 mL volumes (in 15 mL tubes) of strain LJBJ693 for 3 h (30 ˚C, shaking at 225 rpm) in M9 glycerol (40 mM) media and glucose (2 g/L) media with and without caffeine (10 mM) (n=2). One milliliter of sample from each treatment was then diluted 10X before injecting into the FACSymphony™ A1 Cell Analyzer (BD Biosciences, USA). The instrument parameters were adjusted for GFP and captured $1X10^7$ events.

1.5 Determination of Ki value

The inhibition constant, K_i, represents the equilibrium dissociation constant for the binding of an inhibitor to the enzyme-substrate complex. Based on experimental data, uncompetitive inhibition was proposed for the N-demethylase NdmB in the presence of 7-MX. As per the following equation, K_i was determined from K_m or $V_{max.}$

$$Ki = \frac{[I]}{(\frac{Vmax}{Vmax,app}-1)} = \frac{[I]}{(\frac{Km}{Km,app}-1)} \quad \text{(Eq1)}$$

Where [I] is the inhibitor concentration, V_{max} and K_m as the true kinetic parameters without inhibitor, and $V_{max, app}$ and $K_{m, app}$ represent the apparent kinetic parameters in the presence of 7-MX. The K_i values determined through both kinetic parameters are given in **Table S7**.

1.6 Quantification of endotoxin content in extracted 7-MX powder.

Endotoxin and lipopolysaccharides (LPS) detection and quantification were done using Genscript ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, detected at a concentration of < 20 EU/mg (Genscript Biotech Corp., Piscataway, NJ, USA) designed to quantify an In Vitro end-point endotoxin test for human and animal parenteral drugs, biological products, and medical devices according to manufacturer's instructions. In brief, nearly 1 mg of extracted 7-MX powder was dissolved in 1 mL of LAL reagent water, in endotoxin-free vials in triplicates. Calibration standards, blank and test samples were processed as instructed by the vendor and the absorbance at 545 nm was measured in Tecan Infinite M PLEX microplate reader (Tecan Trading AG, Switzerland). The endotoxin concentrations were then calculated using the calibration curve and expressed in EU/mL (R^2 0.9898, Fig. S9).

1.7 Removal of lipopolysaccharides (LPS) from the extracted 7-MX.

Removal of LPS was carried out using GenScript ToxinEraser™ (Genscript Biotech Corp., Piscataway, NJ, USA), according to manufacturer instructions. ToxinEraser™ is a high-efficiency endotoxin removal resin based on the affinity matrix of modified polymyxin B, which allows highly efficient endotoxin removal. In brief, 10 mg of extracted 7-MX powder was first dissolved in 5 mL of DMSO and then volume up to 50 mL with water with vigorous vortexing. The ToxinEraser™ eraser column was prepared and equilibrated using the reagents provided in the kit. 7-MX solution was then passed through the column at a rate of 10 drops per minute. Once 50 mL was completely filtered, the column was again regenerated and equilibrated using the regenerating reagent and equilibrating reagent provided. The sample was filtered through the column for the second time at the same rate, and the filtered solution (collected in pre-weighed Falcon tubes) was evaporated in a vacuum evaporator (LABCONCO, Centrivap Concentrator, Marshall Scientific) at 60 °C for 8 h.

2.0 Supplemental tables

Table S1: Plasmid construction details.

Plasmid Constructed	Forward Primer	Reverse Primer	Gene/vector	Remarks
pBTL-2-P$_{tac}$-*ndmA-ndmB-ndmD*	GAGGAATCTTACTACATGGAACAGGCAATCATTAATGATGAAC	TTCTAAACCAAAATTATATGTAGCTCCTATCGCTTTCAATGACT	*ndmA* from *P. putida* CBB5	*ndmCD* was amplified together and later *ndmC* was removed by mutegenesis. Used to express *N*-demethylase enzymes.
	TTCAGCTTATCTAACCAGACATCTCAAAATACGCGT	AGCTACATATAATTTTGGTTTAGAACAATAAGACCAGCAATCC	*ndmB* from *P. putida* CBB5	
	CCTGAATGATATCATCTCTTCAAATGTAGCACCTGAAGTCAG	GAGATGTCTGGTTAGATAAGCTGAAAGGCCAGAGGGGATTATAATGT	*ndmCD* from *P. putida* CBB5	
	ATTTGAAGAGATGATATCATTCAGGACGAGCCTCAGAC	CCTGTTCCATGTAGTAAGATTCCTCCTCTAGAGTGTGAAATTG	pBTL-2 vector fragment with *tac* promoter	
pBTL-2-P$_{lac}$-*fmdEFGH-fdhA*	GAGGAGGAATCTTACATGCCTGATGAATTGCTCC	ATTTTTATCTAGACCAACGCCTCCC	*fmdEFGH* fragment from *P. putida* EM42	Used to overexpress *fdhA* and *fmdEFGH*
	GAGGAGGAATCTTACATAAAAATCAAGCGAGGTAAGAGC	GGTCTAGATAAAAATCAAGCGAGGTAAGAG	*fdhA* fragment from *P. putida* EM42	
	GATATCATTCAGGACGAGCC	GTAAGATTCCTCCTCTAGAGTGT	pBTL-2 vector fragment with tac promoter	
pK18sB-P$_{tac}$-*ndmABD*	GAGCCTCAGGATATCATTCAGGACGAGCC	CTCTAGAGTGTGTAAGATTCCTCCTCTAGAGTGTG	pK18sB vector	Used to genome integrate *ndmABD* genes in *vanAB* locus of *P. putida* EM42
	GGAATCTTACACACTCTAGAGGAGGAATCTTAC	GAATGATATCCTGAGGCTCGTCCTGAA	*ndmABD* fragment from pBTL-2-P$_{tac}$-*ndmABD* plasmid	

	ACGAATTCGAGCTCGGTACCCGGGGATCCTAAGCCGAATGTCGATGATAT	GGCTTTTGACTTGAGAAAAAACCGCACCTGG	Homologous arm 1 (up arm)	
	TGTTCCGCGGCCACCCATGGATGCCTGAAAGG	CGGCCAGTGCCAAGCTTGCATGCCTGCAGGGCCCCTCTGGAGAATCG	Homologous arm 2 (down arm)	
pK18sB-*glpR*Δ	XhoI	SalI	pK18sB vector was enzyme digeted	Used to delete *glpR* repressor from *P. putida* EM42 gemone
	CGAATTCGAGCTCGGTACCCCGCTGGTCTTCATTCTGC	TGTCCGGCAGCTCGAGACTAGTGTCGACCATGCCGCCAGACTTTTG	Upper homologous arm to *glpR* locus	
	TAGTCTCGAGCTGCCGGACAAATTCTGC	CAAGCTTGCATGCCTGCAGGTTCAACATCCACAGCCTG	Down homologous arm to *glpR* locus	
pBTL-2-P_{tac}-*fdhA*	GCCTCAGGAGGAGGAATCTTACATAAAAATCAAG	GTCCTGAATGATATCTTACGCCGCACCCC	PCR amplify pBTL-2-P_{tac} vector	To overexpress *fdhA* gene
	GATATCATTCAGGACGAGCC	GTAAGATTCCTCCTCTAGAGTGT	*fdhA* gene from *P. putida* EM42	
pK18sB-*glpR*Δ-P_{tac}-*fdhA-P51*	XbaI	SmaI	pK18sB-*glpR*Δ vector	To genome integrate *fdhA* gene in *glpR* locus of *P. putida* EM42
	AATTTGTCCGGCAGCGCCAGCCGGTGGCCGCG	GTCCTGAATGATATCTTACGCCGCACCCC	P_{tac}-*fdhA* fragment from pBTL-2-*tac-fdhA*	
	TTTACAACGTCGTGACCGGTAGCAAATGGGG	CGATCCTCATCCTGTGATTCAATCCTTATTCAGCTTGC	To add *P51* promoter amplified from *P. putida* EM42	
pBTL-2-P_{tac}-*ndmBD*	ATTTTGGTTTAGAACAATAAGAC	TGTAGTAAGATTCCTCCTC	*ndmA* was removed from pBTL-2-P_{tac}-*ndmA-ndmB-ndmD* by mutagenesis	

pBTL-2-P*tac*-*ndmAD*	AGAGTCCGCCAAGTG	ATTATATGTAGCTCCTATCGC	*ndmB* was removed from pBTL-2-P_{tac}-*ndmA-ndmB-ndmD* by mutagenesis.	
pBTL-2-P_{lac}-*fmdEFGH*	GAGGAGGAATCTTACATGCCTGATGAATTGCTCC	GTCCTGAATGATATCCTAGACCAACGCCTCCC	PCR amplify *fmdEFGH* fragment from *P. putida* EM42 genome	To overexpress *fmdEFGH* genes
	GATATCATTCAGGACGAGCC	GTAAGATTCCTCCTCTAGAGTGT	PCR amplify vector from pBTL-2-P_{lac}-GFP plasmid	
pK18sB-P_{51}-*PP_18750*∆	SalI	XbaI	pK18sB-P_{51} vector was enzyme digested	
	GTACCCGGGGATCCTTACGCCCAGCAGCCG	TACCTTGGTCAGCGCCCTGGTTGACTG	Upstream homologous arm was PCR amplified from *P. putida* EM42 genome	
	GCGCTGACCAAGGTATCGATGGCAACCAT	TTGCATGCCTGCAGGGGCCTCAGTAGCCGGC	Downstream homologous arm was PCR amplified from *P. pu-tida* EM42 genome	
pBTL-2-P_{tac}-*PP_RS18750*	GCGGCTTTTTACCCGGTAAGATTCCTCCTCTAGAGTG	GAGGAGGAATCTTACCGGGTAAAAAGCCGCG	pBTL-2-P_{tac} vector was PCR amplified	Used to overexpress *PP_RS18750* gene
	ATCTTACTACATGGAACAGGCCTTGGATGAGGACTGCC	ATGATATCATCTCTTCAAATGTCAACCAGGGCGCTGATC	*PP_RS18750* gene was PCR amplified from *P. putida* EM42 genome	

pK18sB-*PP_5043*-P_{tac}-*fmdEFGH*-P_{51}	AAAAACGCCTCACACAGGAAACAGCTATGACATGATTACGCGGAACAGCCCGTGCT	CCGACCGGAGGCTTTTGACTTCGCCACAGAATCGAGCG	Upstream homologous arm was PCR amplified from *P. putida* EM42 genome	Used to genome integrate P_{tac}-*fmdEFGH* in *PP_5043* locus
	CCTTGTGGCGCTTTAGTTTTGGCATGAAGCACTGAACCCACC	TGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTGACCTCACAGACCTGGTCG	Downstream homologous arm was PCR amplified from *P. putida* EM42 genome	
	GACATGATTACGAATTCGAGCTCGGTACCCATGGGTTTTGAACAACTAGCT	CAGGCAGGGTCCGTGTCAAGTCAAAAGCCTCCGG	*fmdEFGH* gene cluster was PCR amplified from pBTL-2-P_{tac}-*fmdEFGH*	
	XbaI	SalI		
pBTL-2-P_{tac}-*PP_RS18750*-GFP	CTTGCTCATCCAAGGTATCGATGG	TACAAGTAGTCAGCGCCCTGGTTG	pBTL-2-P_{tac}-*PP_RS18750* vector was PCR amplified	Used to express DMT family transporter (PP_RS18750) with GFP with a flexible linker
	AGCTCCTCGCCCTTGCTCATAGAACCTCCTCCACCTCCGTTTTCTTGCGCCTTCAC	TGAGGGCGGATCCCCCTCAACACCCCAGGCTTGACAATTAATC	GFP gene fragment was amplified with Glycine (X4) and serine (X1) linker	
pBTL-2-P_{tac}-*cafP*	CACTCTAGAGATTGTTTTTCTCTCTTAGACGGAAT	CTTATTAGTTTAGGTGCTTCTCATGCA	PCR amplify *cafP* from *P. putida* CBB5	Used for heterologous expression of *cafP* gene
	GTAACTGGACTGAAAACAAACTAAAG	GACCTCCATAGCAGAAAG	PCR amplify pBTL-2-P_{tac} vector from pBTL-2-P_{tac}-GFP plasmid	

Table S2: Sequencing primers used in sequencing of constructed plasmids.

Primer number	Primer sequence	Genes sequenced	Plasmid
oLJ710	CACCCCTGAGCGCCT	*fdhA* and *fmdEFGH* gene cluster	Used to sequence confirm pBTL-2-P_{lac}-*fdhA*-f*mdEFGH* plasmid
oLJ711	CGTGGTGTGCAACGC		
oLJ712	CAGTGGCCGCCCGCT		
oLJ713	ACCAGCATCCCCAAAC		
oLJ714	TGGTCGACTCGCCGC		
oLJ715	AGGACATCGCCCAGAG		
oLJ716	GCCTGGAGATCCACCC		
oLJ717	GCCCGGTCGGCCTGGC		
oLJ641	TTTTTCTGAGGGCGG	*ndmABD* gene cluster	Used to sequnce varify pBTL-2-P_{tac}-*ndmABD* plasmid
oLJ642	TGACTGCACTGAAATTC		
oLJ643	GAAGCTCATAAAGAAGGTTC		
oLJ644	AATCCCGACATGCAG		
oLJ647	AGAGGATTGCGGTTG		
oLJ648	AGCATTACAGATATCAGC		

Table S3: Description of the strains developed/used in the study.

Strain ID	Plasmid used for integration/deletion	Genotype of the strain	Strain description
LJBJ110		*P. putida* KT2440:: pBTL-2-P_{tac}-*ndmABCDE*$_{(kan)}$	pBTL-2plasmid with *ndmA, ndmB, ndmC, ndmD,* and *ndmE* genes along with *tac* promoter and *tonB* terminator. Kanamycin resistant
LJBJ113		*P. putida* KT2440:: pBTL-2-P_{tac}-*ndmABD*$_{(kan)}$	pBTL-2plasmid with *ndmA, ndmB* and *ndmD* genes along with tac promoter and *tonB* terminator. Kanamycin resistant
LJBJ115		*P. putida* KT2440:: pBTL-2-P_{tac} *ndmABD*$_{(kan)}$ + pBTL-2$_{(chl)}$	*P. putida* KT2440 containing pBTL-2 plasmid with *ndmA, ndmB, ndmD*, and pBTL-2 plasmid with chloramphenicol resistance
LJBJ116		*P. putida* KT2440:: pBTL-2-P_{tac} *ndmABD* + pBTL-2-P_{tac} *cafP*$_{(chl)}$	*P. putida* KT2440 containing PBLT-2 plasmid with *ndmA, ndmB, ndmD* and pBLT-2 plasmid with *cafP* gene driven under *tac* promoter.
LJBJ120		*P. putida* EM42:: pBTL-2-P_{tac} *ndmABD*	pBTL-2 plasmid with *ndmA, ndmB* and *ndmD* genes along with *tac* promoter and *tonB* terminator. Kanamycin resistant
LJBJ121		*P. putida* EM42:: pBTL-2-P_{tac} *ndmABD* + pBLT-2$_{(Chl)}$	pBTL-2 plasmid with Chloramphenicol resistance added in LJBJ120
LJBJ220	pK18sB-*glpRΔ*	*P. putida EM42*::*ΔglpR*	*glpR* gene was deleted from *P. putida* EM42 genome
LJBJ221	pK18sB-*glpRΔ*	*P. putida* KT2440::*ΔglpR* pBTL-2-P_{tac}-*ndmABD*$_{(kan)}$	*glpR* gene was deleted from *P. putida* KT2440 genome and pBTL-2 plasmid with *ndmA, ndmB* and *ndmD* genes along with *tac* promoter and *tonB* terminator. Kanamycin resistant.
LJBJ222		*P. putida* EM42:: *glpRΔ* + pBTL-2$_{(Kan)}$	pBTL-2plasmid with kanamycin resistant added

LJBJ223		*P. putida* EM42:: *glpRΔ*- pBTL-2-P_{tac}-*ndmABD*$_{(kan)}$	pBTL-2 plasmid with *ndmA, ndmB,* and *ndmD* genes along with *tac* promoter and *tonB* terminator. Kanamycin resistant
LJBJ224		*P. putida* EM42:: *glpRΔ*-pBTL-2-P_{tac}-*ndmABD* + pBTL-2$_{(Chl)}$	pBTL-2 plasmid with chloramphenicol resistant added to LJBJ122
LJBJ230		*P. putida* EM42:: *glpRΔ*- pBTL-2-P_{tac}-*ndmABD* + pBTL-2-P_{lac}-*fdhA-fmdEFGH*$_{(chl)}$	pBTL-2 plasmid with *fdhA* and *fmdEFGH* genes along with *lac* promoter and *tonB* terminator added into LJBJ223. Kanamycin and chloramphenicol resistant
LJBJ231		*P. putida* EM42:: *glpRΔ*:pBTL-2-P_{tac}-*ndmABD* + pBTL-2- P_{tac}-*fdhA*	pBTL-2 plasmid with *fdhA* gene added along with *tac* promotor and *tonB* terminator. Chloramphenicol resistant
LJBJ260		*P. putida* EM42:: *glpRΔ*- pBTL-2-P_{tac}-*ndmBD* + pBTL-2-2-P_{lac}-*fdhA-fmdEFGH*	pBTL-2-P_{tac}-*ndmBD* plasmid and pBTL-2-P_{lac}-*fdhA-fmdEFGH* added to LJBJ 220
LJBJ500	pK18sB::P_{tac}-*ndmABD*	*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*	Genome integrated *ndmA, ndmB,* and *ndm*D with a *tac* promotor in *vanAB* intergenic locus
LJBJ501		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2$_{(Kan)}$	pBTL-2 plasmid with kanamycin resistant added to the strain LJBJ500
LJBJ502		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2	Two pBTL-2 plasmids with kanamycin and chloramphenicol resistance added to the LJBJ 500
LJBJ503		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2-P_{tac}-*fdhA* + pBTL-2 $_{(Kan)}$	pBTL-2 plasmid with *fdhA* gene and empty pBTL-2 plasmid with kanamycin resistance added to the strain LJBJ500
LJBJ504		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2$_{(chl)}$	Two pBTL-2 plasmid with chloramphenicol resistance added to LJBJ500
LJBJ550		*P. putida* EM42::*glpR*Δ:P_{tac}	pBTL-2 plasmid with *fdhA* and *fmdEFGH* genes along with *lac* promoter and *tonB*

		ndmABD + pBTL-2-P_{lac}-*fdhA*-*fmdEFGH*	terminator added into LJBJ 500. Chloramphenicol resistant
LJBJ551		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2-P_{tac}-*fdhA*-*fmdEFGH* + pBTL-2 (Kan)	pBTL-2 plasmid with kanamycin resistant added to LJBJ 550
LJBJ552		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2-P_{tac}-*fdhA*-*fmdEFGH* + pBTL-2-P_{tac}-*ndmAD*	An additional copy of *ndmA* and *ndmD* added in a pBTL-2 plasmid (*tac* promoter and *tonB* terminator) with kanamycin resistance to LJBJ550
LJBJ553		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD* + pBTL-2 (Chl)-P_{lac} -*fmdEFGH* + pBTL-2 (Kan)	pBTL-2-P_{lac}-*fmdEFGH* added with chloramphenicol resistance and pBLT-2 plasmid with kanamycin resistance added
LJBJ600	pK18sB-P_{51}::*glpR*Δ-P_{tac}-*fdhA*	*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA*	*fdhA* genome integrated with a *tac* promoter at *glpR* locus
LJBJ601		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA* + pBTL-2(Kan)	pBTL-2 plasmid with kanamycin resistance added
LJBJ602		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA* + pBTL-2(Kanl)+ pBTL-2 (Chl)	Two pBTL-2 plasmids with kanamycin and chloramphenicol resistance added
LJBJ603		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA* + pBTL-2(Chl)-P_{lac}-*fdhA*	pBTL-2 plasmid with lac driven *fdhA* gene added. Chloramphenicol resistance.
LJBJ604		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA* + pBTL-2(Chl)	pBTL-2 plasmid with chloramphenicol resistance added

LJBJ606		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA* + pBTL-2-P_{tac}-n*dmBD*$_{(Kan)}$	pBTL-2-P_{tac}-n*dmBD*$_{(Kan)}$ added to LJBJ600
LKBJ690	pK18sB-P_{51}::*PP_RS18750*Δ	*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA:PP_RS18750* Δ	*PP_RS18750* gene deleted in LJBJ600
LJBJ691		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA:PP_RS18750* Δ + pBTL-2$_{(kan)}$	pBTL-2 plasmid with kanamycin resistance added to LJBJ690
LJBJ692		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA:PP_RS18750* Δ + pBTL-2-P_{tac}-*PP_RS18750*$_{(kan)}$	pBTL-2-P_{tac}-*PP_RS18750$_{(kan)}$* added to LJBJ690
LJBJ693		*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac}-*fdhA:PP_RS18750* Δ + pBTL-2-P_{tac}-*PP_RS18750*-*GFP*$_{(kan)}$	pBTL-2-P_{tac}-*PP_RS18750*-GFP$_{(kan)}$ added to LJBJ690
LJBJ700	pK18sB-P_{51}::*PP_5043*-P_{lac}-*fmdEFGH*	*P. putida* EM42::*glpR*Δ:P_{tac} *ndmABD*::P_{tac} *fdhA*::P_{lac} *fmdEFGH*	*lac* promotor driven *fmdEFGH* cluster genome integrated at *PP_5043* locus.

Table S4: Primers used in confirming genome integration/deletion of genes.

Primer number	Primer sequence	Added or deleted genes/gene cassette	Integrated/deleted locus
oLJ599	GATCAAGGTGCATACGCGGTAG	P_{tac}-*ndmABD*	*fpva* locus
oLJ602	CCAGCAATACGCCACGGT		
oLJ812	AAGCGGTCCAGATACGG	P_{tac}-*fdhA*	*glpR*
oLJ813	GAAGGTTCACGTCACCG		
oLJ266	GGTACTGTCGGCGGGGT	P_{tac}-*fmdEFGH*	*PP_5043*
oLJ267	GCTGGCTCTTGCTTTGGAGC		
oLJ1202	GGAAAGGCCCATCAAAATACT	*PP_RS18750*	*PP_RS18750*
oLJ1203	ACCTCGCGCTGACCT		

Table S5: qRT-PCR primers used in the study.

Primer number	Primer sequence	Gene
oLJ1000	GCAAGGTCGAAGTACAGAAGAT	*fdhA*
oLJ1001	TCAGAACCGCAGATGTTGG	*fdhA*
oLJ1002	CCTGCGCCCTGTTTACTG	*fmdE*
oLJ1003	GGCAACCGTTGACCAAGT	*fmdE*
oLJ1188	GAGGACTGCCATGTTCAGTAA	*PP_RS18750*
oLJ1189	ACCAATCCACAACCACCAA	*PP_RS18750*

Table S6: Enzymatic parameters for NdmB and NdmD under multiple concentrations of 7-MX

	0mM 7-MX	0.1mM 7-MX	0.25mM 7-MX	0.5mM 7-MX
K_m (uM)	15.36	14.10	10.90	8.27
V_{max} (mM/min)	0.00890	0.00752	0.00617	0.00436

Table S7: Inhibition constant for NdmB and NdmDP1 under Multiple concentrations of 7-MX

[7-MX] (mM)	K_i form V_{max} (µM)	K_i from K_m (µM)
0.1	545 ± 30	1119 ± 19
0.25	565 ± 21	611 ± 96
0.5	480 ± 76	583 ± 49

Table S8: Breakdown of estimated capital costs incurred in setting up the 7-MX production facility.

Capital Costs			
	Quantity	**Unit cost**	**Total cost**
Preculture reactor (3000 L)	2	$150,000	$300,000
6 Bioreactors (100000 L)	6	$5,000,000	$30,000,000
Centrifuge	6	$50,000	$300,000
Drying	6	$150,000	$900,000
Column Separation	4	$200,000	$800,000
Resin Regeneration	1	$500,000	$500,000
Solvent Extraction	1	$1,800,000	$1,800,000
Capital Equipment Subtotal			$34,600,000
ISBL			$164,004,000.00
OSBL			$65,601,600.00
Total Capital Expenses			**$229,605,600**
Per ton of product			**$114.80**

Table S9: Estimated cost breakdown for bioreactor production and purification, including fixed costs and operational costs (energy and personnel costs).

Bioreactor	**Price per Unit**		**Total amount needed per year**		**Costs per year**
Caffeine	20	$/kg	226.86	MT	**$4,537,205**
Disodium phosphate	1.5	$/kg	307.62	MT	$461,434
Monopotassium phosphate	1.7	$/kg	136.12	MT	$231,397
Sodium chloride	60	$/ton	22.69	MT	$1,361
Ammonium chloride	1.3	$/kg	45.37	MT	$58,984
Iron sulfate	1	$/kg	0.01	MT	$0
Magnesium sulfate	1	$/kg	0.02	MT	$16
Calcium chloride	400	$/ton	0.01	MT	$4
Water	4	$/ton	28130.67	MT	$112,523
Glycerol	2	$/kg	97.19	MT	$194,374
Other Media Cost					**$1,060,089**
Purification					
Sodium hydroxide	700	$/ton	18.15	MT	$12,704
Hydrochloric acid	300	$/ton	3.16	MT	$947
Silicone oil	10	$/kg	2.72	MT	$26,407

Ethanol	2.5	$/kg	178.99	MT	$447,482
Polymyxin B column consumable	2000	$/L	1000.00	L	$2,000,000
Ethyl acetate	1734	$/ton	1634.48	MT	$2,834,193
Purification costs					**$5,321,733**
Materials cost					**$10,919,027**
Energy			Per reactor		Cost per year
Preculture energy (heating, 30 °C for 18+3 hours)	0.1702	$/kWh	87.17	kWh	$742
Preculture stirring, 250 rpm	0.1702	$/kWh	22.21	kWh	$189
Bioreactor cooling energy (cooling, 30C, 140 hours)	0.1702	$/kWh	17888.89	kWh	$913,407
Bioreactor stirring and heating	0.1702	$/kWh	1526.65	kWh	$3,897,531
Centrifuge energy	0.1702	$/kWh	3023.46	kWh	$154,378
Column pumping energy	0.1702	$/kWh	39300.00	kWh	$334,443
Ethyl acetate distillation	0.1702	$/kWh	14156.44	kWh	$14,156
Total energy cost					**$5,314,846**
Fixed Costs	Number of Staff			Salary	Cost per year
Operators	5			$40,000	$200,000
QC	2			$60,000	$120,000
Maintenance	2			$60,000	$120,000
Managers	2			$100,000	$200,000
Packaging	2			$60,000	$120,000
Compliance	1			$80,000	$80,000
Support	1			$80,000	$80,000
Personnel					**$920,000**
Insurance, etc.					$460,000
Maintenance					$1,730,000
Overhead					$2,021,500
Total Fixed Costs					**$5,131,500**

Cost Breakdown		**Cost per kg**
Caffeine	$4,537,205	$45.37
Other Bioreactor Reagents	$1,060,089	$10.60

Purification consumables	$5,321,733	$53.22
Energy	$5,314,846	$53.15
Other Fixed Costs	$5,131,500	$51.32
Capital Costs per year, 20-year useful life	$11,480,280	$114.80
Cost per kg of 7-methylxanthine		**$328.46**

Table S10: Environmental impacts of the production of 1 kg of 7-methylxanthine

Fine particulate matter formation	0.0548	kg PM 2.5 eq
Fossil resource scarcity	8.6427	kg oil eq
Freshwater ecotoxicity	1.3002	kg 1,4-DCB
Freshwater eutrophication	0.0160	kg P eq
Global warming	34.074	kg CO_2 eq
Human carcinogenic toxicity	3.8767	kg 1,4-DCB
Human non-carcinogenic toxicity	21.705	kg 1,4-DCB
Ionizing radiation	6.7868	kBq Co-60 eq
Land use	6.2032	m^2a crop eq
Marine ecotoxicity	1.8889	kg 1,4-DCB
Marine eutrophication	0.0329	kg N eq
Mineral resource scarcity	0.1756	kg Cu eq
Ozone formation, Human health	0.0505	kg NO_x eq
Ozone formation, Terrestrial	0.0537	kg NO_x eq
Stratospheric ozone depletion	3.9E-05	kg CFC11 eq
Terrestrial acidification	0.1194	kg SO_2 eq
Terrestrial ecotoxicity	38.323	kg 1,4-DCB
Water consumption	0.6907	m^3

Table S11: E-factor and The Process Mass Intensity (PMI) calculation and comparison with the recent study on the biocatalytic production of 7-MX (A), current study (B), and the chemical process (C).

The current study outperforms the *E. coli*-based biocatalytic production of 7-MX by nearly 63% when water is recovered and recycled. The chemical process parameters and inputs were extracted from a Chinese patent (patent no: CN118047777A) granted in 2024[8].

A.

Input materials	Mass (g) per L of culture
Caffeine	11.65
LB powder	4.16
Arabinose (0.2%)	2.00
IPTG	0.0024
Tryptone	10
Water	1000
Yeast extract	5.00
Na_2HPO_4	6.70
K_2HPO_4	3.40
Na_3SO_4	0.71
NH_4Cl	2.68
Glycerol	20.00
Trace elements	0.22
Glucose	2.00
Mg_2SO_4	4.93
Cell dry weight	40.00
Ethyl acetate	16.34
Ethanol	0.79
Total mass input	1113.45
7-MX produced	8.37
cEF	**135.58**
sEF	**13.55**
Total PMI	**134.7**
PMI substrate, reagents, and solvents	**15.2**
PMI substrate and reagents	**13.1**
PMI Solvent	**2.0**
PMI water	**119.5**

B.

Input materials	Mass (g) per L of culture
LB powder	2.29
Caffeine	11.65
M9 salt mix	22.70
$FeSO_4$	1.67E-06
$MgSO_4$	0.0006
$CaCl_2$	2.33E-06
Water	1000.00
Glycerol	3.57
10 N NaOH	2.20
HCl	0.19
Anti-foam	0.09
Cell dry weight	2.73
Ethyl acetate	16.34
Ethanol	0.79
Total mass input	1062.56
7-MX produced	9.21
cEF	**115.37**
sEF	**4.93**
Total PMI	**115.4**
PMI substrate, reagents, and solvents	**6.8**
PMI substrate and reagents	**4.9**
PMI Solvent	**1.9**
PMI water	**108.6**

C.

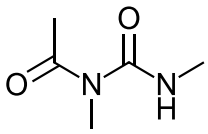
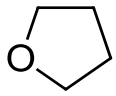
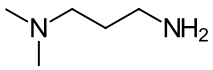
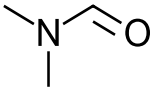
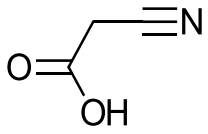
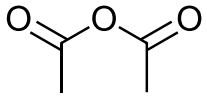
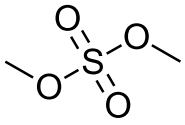
Input materials	Mass (g)
Guanosine	311.58
N, N- dimethylacetamide	562.20
Dimethyl sulfate	277.48
Water	2754.15
$NaNO_2$ (30% solution)	151.77
Acetone	940.80
Total mass input	4998.00
7-MX produced	84.70
cEF	**59.15**
sEF	**26.55**
Total PMI	**59.2**
PMI substrate, reagents, and solvents	**26.6**
PMI substrate and reagents	**15.4**
PMI Solvent	**11.1**
PMI water	**32.6**

Note: In the E-factor calculation, cEF includes both solvent and water without recycling, while E-Factors, sEF exclude these components[2]. The proposed process in this study aims to recycle the water and solvents.

For the E-factor and PMI calculations of the biological process, solvent extraction using ethyl acetate and ethanol was adopted as the final purification step.

Table S12: Toxicities of chemical synthesis relevant compounds as predicted by the EPA Toxicity Estimation Software.

Values are reported for numerical predictions. Developmental toxicants are highlighted in red, and the developmental non-toxicants are highlighted in green.

Chemical compound		Developmental toxicity	Oral rat LD50 (mg/kg)	Bioconcentration factor (log10)	Mutagenicity
	Acetyl-dimethyl urea	0.74	718.30	0.34	+
	Tetrahydrofuran	0.48	1259.03	2.67	-
	Dimethylaminopropylamine	0.46	1155.73	1.28	-
	N, N-dimethylformamide	0.94	3464.05	0.68	-
	Cyanoacetic acid	0.75	439.82	0.62	-
	Acetic anhydride	0.61	1778.38	N/A	-
	Dimethyl sulfate	0.92	259.23	N/A	+

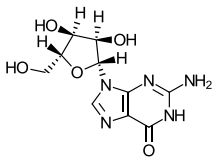
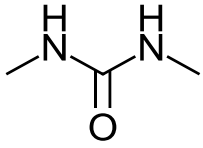
	Guanosine	0.57	N/A	N/A	-
	1, 3-dimethyl urea	0.83	N/A	0.35	-

Table S13: Toxicities of biological production relevant compounds as predicted by the EPA Toxicity Estimation Software.

Values are reported for numerical predictions. Developmental toxicants are highlighted in red, and the developmental non-toxicants are highlighted in green.

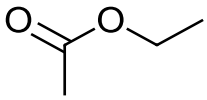
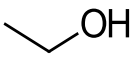
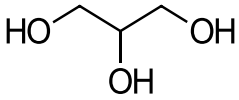
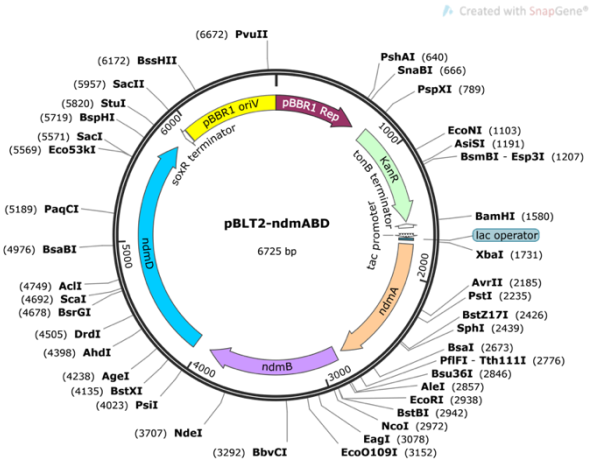
Chemical compound		Developmental toxicity	Oral rat LD50 (mg/kg)	Bioconcentration factor	Mutagenicity
	Ethyl acetate	0.59	5479.60	2.61	-
	Ethanol	0.46	7055.25	1.26	-
	Glycerol	0.47	10488.70	0.57	-

Table S14: Current market values of methylxanthines according to reported literature and chemical vendors.

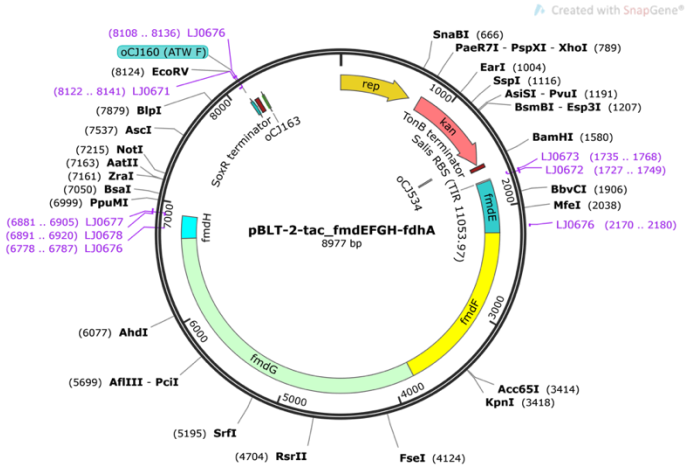
Methylxanthine	Price per gram (USD)	Reference
1-methylxanthine	900 – 1100	[3, 4], chemicalbook.com, medchemexpress.com
3-methylxanthine	230 - 450	[3], sigmaldrich.com
7-methylxanthine	600 - 750	[3, 5], sigmaldrich.com
Paraxanthine	1450 - 1600	[5], Medchemexpress.com
Theophylline	0.2 – 6.5	[4], Thermofisher.com
Theobromine	0.6 – 1.1	Tcichemicals.com

3.0 Supplementary figures



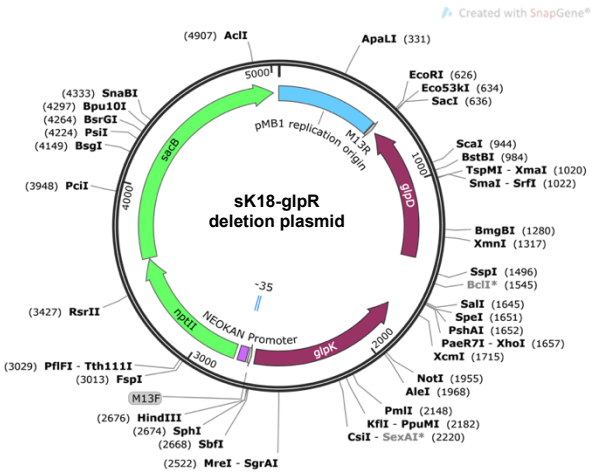
Supplementary Fig. S1: Plasmid map of pBTL-2-P$_{tac}$-*ndmABD*

Plasmid was constructed for expressing *ndmA*, *ndmB*, and *ndmD* genes taken from *P. putida* EM42. The plasmid was constructed in pBLT-2 expression vector with kanamycin antibiotic selection. The genes were driven by a *tac* promotor.



Supplementary Fig. S2: Plasmid map of pBTL-2-f*mdEFGH-fdhA*

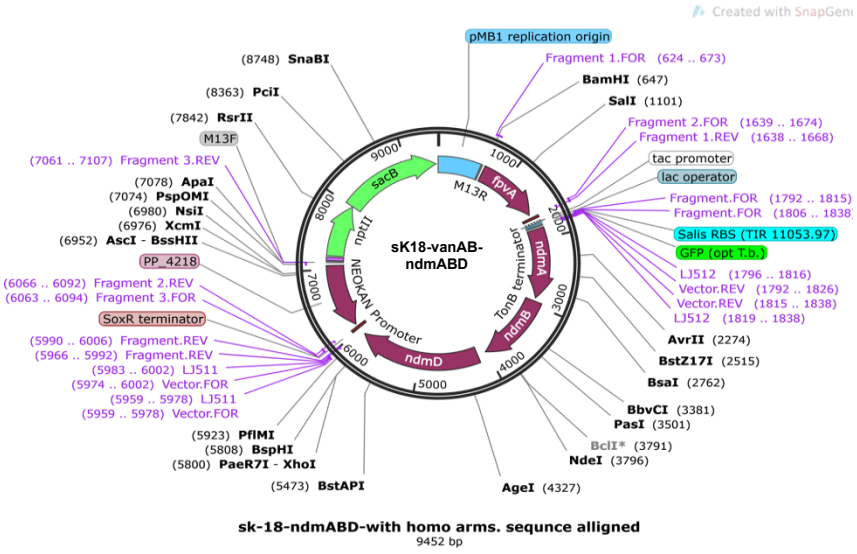
Plasmid was constructed for over-expressing formaldehyde dehydrogenase gene (*fdhA*) and formate dehydrogenase gene cluster (*fmdEFGH*) in *P. putida* EM42. *fdhA* and *fmdEFGH* genes were amplified from *P. putida* EM42 genome. The genes were driven by a *lac* promotor in a pBTL-2 expression vector.



Supplementary Fig. S3: Plasmid map of sK18-*glpR*Δ

Plasmid constructed for the genome deletion of *glpR* (repressor gene of *glp* genes). The deleted region was substituted by a multiple cloning region with three restriction sites (XhoI, SalI, and SpeI). The homologous arms and the substituted gene sequence is provided.

Integrated gene sequence in *glpR* locus, 5'-3'
TCGGTACCCCGCTGGTCTTCATTCTGCAGAATGTAGGCATGTTCGCCTTCATACAGGCGCGGCACGATAATGTGGCTGCC CTGGATCAGGCGGATGCCGTAAGGCGCATCGAGCTTGAGGTCGTCCTTGATGAAGCTGGCCACCCACGGGCCGGCGG CGTTGACCAGCGCGCGGGCATGGATCGTCTGGAGGCTGCCATCGGCGTGCTGTAGCTCTACCTGCCACAAGCCCTCTAC ACGTTCGGCGCGCAAGCAGCGGGTGCGGGTGCGGATATGGGCGCCAAGCTCACGGGCCGCCATGGCGTTGAGTACTA CCAGGCGGGCGTCATCGACAGCGCAGTCGGCGTATTCGAAGCCGCGGGTGATTGCCGGCTTCAGCGGGTAGCCCGGG CCAAAGCGCAGGCTGCGCGAGGCGCCAAGGCGCTTGCGCTTGCCCAGGTGGTCGTAGAGGAACAGGCCGGCGCGGA TCATCCATGCAGGGCGCAGGTGCGGGCGGTGCGGAAGCACGAAGCGCATAGGTTTGACGATGTGGGGCGCCTTCGCC AGCAGCACTTCGCGTTCAGCCAGGGCCTCGCGTACAAGACGAAATTCGTAGTGCTCCAGGTAGCGCAGGCCACCGTGA ATCAGTTTGCTGCTGGCTGACGACGTGTGTTGGGCCAGGTCGTCTTTTTCGCAAAGGAAGACCTTCAAGCCGCGACCA GCGGCGTCGGCAGCGATGCCCACGCCATTGATGCCACCGCCGATTACGGCAAGGTCATAGCAGTTGGCTGTTGGGGGC TGGGACGAAACTGGCTGGGACACGGGCAAGGCCTCCTGAGGCTGTTCACATCGAATGTGAACATTGATGTTCGTTTGC GAAAATATTAGCGCAACAGAACGCCGGTCGCCAGTCAGTCTCTATAGAAAAAACTGATCAAATGACAATGAAGTGAACA TTTACGAAAATTCAGGGGGTACGGCTTGGGTGAGGGGGCGCTTGCTCACGCAGACACCAAAAGTCTGGCGGCATGGT CGACACTAGTCTCGAGCTGCCGGACAAATTCTGCAATGATGCCGTAGGAAGGGGATGACGGCCAGTAGACGATTGGG GCCGCATGGCGGCCCCAACAGCTATCATGCCTCGTGATCTTCCCAGTCACGGGTACGCTCGACCGCCTTCTTCCAGCCG GCATACAGCTTTTCTTTCTGCGTTTCGTCCAGTTGCGGGCTGAACTCACGCTCGATGATCGCCTTGTCGCGCAGCTCGTC CAGGCCACTCCAGAAGCCACAGGCCAGGCCTGCCAGATAGGCGGCGCCCAGCGCCGTGGTTTCGCGCATCTGCGGCC GCTCCACGCAGGTGCCGAGGATGTCGGCCTGGAACTGCATGAGGAAGTTGTTGGCCACGGCACCACCGTCCACGCGC AGCTCGGACAGGCGCTGGCCACAGTCCTGCTGCATGGCGTCGAGGACATCGCGGGTCTGGTAGGCGATGGACTCCAG TGCGGCGCGGATGATGTGGTCTACCTTCACGCCACGTGTCAGGCCGAACAACGCACCACGGGCATACGGGTCCCAGTA CGGGGCACCCAGGCCGGTAAAGGCTGGCACCAGGTACACGCCATTGCTGTCCTTGACCTTGCTGGCGAAATACTCGGT ATCCAGGGCGTCGTTGACGATCTTCAGTTCGTCACGCAGCCATTGCACGGTAGAGCCACCATTGAATACCGCGCCTTCC AGTGCATAGGCCACCTCACCACGCGGGCCGCAGGCAATGGTCGTGAGCAGGCCGTGGGACGACTTCACCGCCTGGTC GCCCGTGTTCATCAACAGGAAGCAGCCAGTGCCGTAGGTATTCTTGGCTTGGCCCGGCTCCACGCACATCTGGCCGAA CAGTGCCGATTGCTGGTCGCCGGCGATACCGGCGATGGCTATGCCGCTCTTGGTGTGGCCGTACACTTCCGAGGATGG GCGCACCTCAGGCAGCATCTGCCGCGGGATGCCCAGGATTTCCAGCAGCTTGTCGTCCCACTGCAGGCTGTGGATGTT GAA



Supplementary Fig. S4: Plasmid map of sK18-*vanAB-ndmABD.*

Plasmid constructed for the genome integration of *ndmA*, *ndmB*, and *ndmD* genes in *vanAB* locus of *P. putida* EM42 genome. The homologous arms and the introduced gene sequence is provided. The genome-integrated region was PCR amplified and sequenced verified.

Integrated gene sequence in *fpva* locus, 5'-3'
GGAAACAGCTATGACATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTAAGCCGAATGTCGATGATATCTACAACCTGAGCGAGC
ACGTCGAGCAGGAAGGGGCGTACGTCACCACCCGCCTGAACATCGCCGACCCGCTGAAGGTCATCCTGGGTGCACGCCTGGACTG
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GCCTCGGGCAAGGTACGCACCCACGGTATCGACCTGGAGTTGATGGGCGCACTGACCCCCAACTGGCAAGTCGGCGCAGGCTACAC
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TAGGCGATGAGTACGTCGCGATGCGTGATAGATGCGCTCATCGATCAGCTAAGCTTTCCTTGGGTACAGTAAGTGGAAACCGCCTACA
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CATGCCGTTTGCTATTAACCTTGAAGTATCCAAATACTCCAGCAGTTCGCTGCATGTGTTATTCAATGTGTCATGTCCGGTAGACAGCCA
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GACATGCAGGTAATCGTTGCCGATTCGTATATTTGGGAGACTGTTGCCGAGCGGCGGTGGGAGAACTTTACAGATTTTTCGCACTTT
GCCTTCGTACACCCAGGGACGCTCTATGATCCGTTTTTCGCTAGCCACCCAACTGTTTACGTGAATCGCGTTGATGGTGAGTTGCAATT
CAAACTCGCTCCGCCGCGTGAAATGAAAGGCATCCCGCCAGAAGCACCGATGGGTGACTTCACCTACCGCTGCACAATGCCGTATTC
AGTAAATCTTGAAATCAAATTGTGGAAAGATGACTCACGTTTCGTTCTTTGGACTACCGCTAGCCCTGTCGATAACAAGTCTTGCCGG
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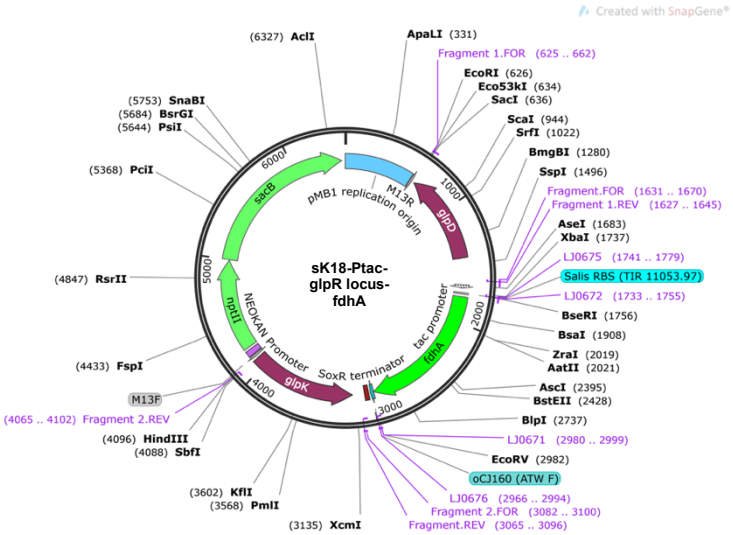

Supplementary Fig. S5: Plasmid map of sK18-*glpRΔ-P$_{tac}$-fdhA*.
Plasmid constructed for the genome integration of P_{tac} driven *fdhA* gene *in glpR* locus of *P. putida* EM42 genome. The homologous arms and the introduced gene sequence is provided below. The genome-integrated region was PCR amplified and sequenced verified.

Integrated sequence in *glpR* locus, 5'-3'
CACAGGAAACAGCTATGACATGATTACGAATTCGAGCTCGGTACCCCGCTGGTCTTCATTCTGCAGAATGTAGGCATGTT
CGCCTTCATACAGGCGCGGCACGATAATGTGGCTGCCCTGGATCAGGCGGATGCCGTAAGGCGCATCGAGCTTGAGGT
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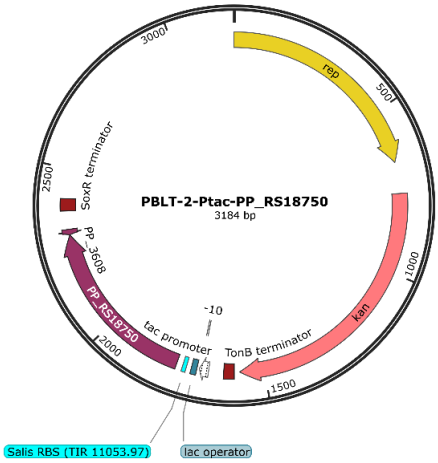
A.



Gene sequence of PP_RS18750 5'-3'
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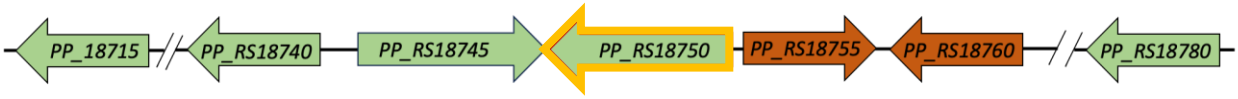
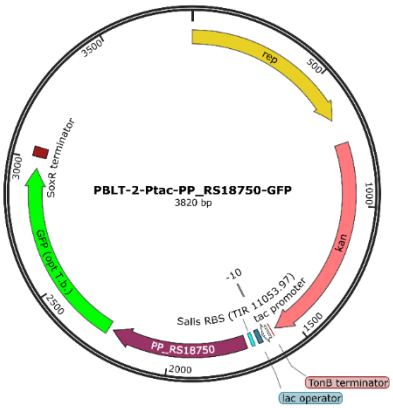
B.

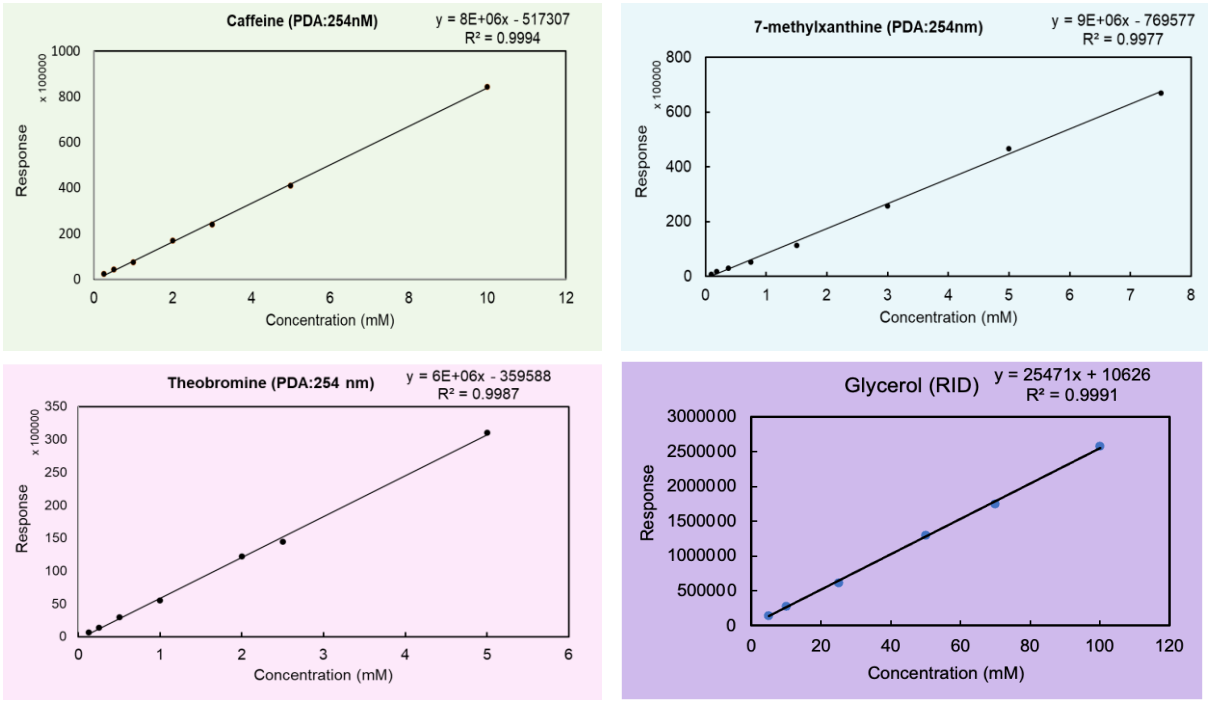


Supplementary Fig. S6: Plasmid map of pBLT-2-P$_{tac}$-*PP_RS18750* and gene map of genes significantly up/down-regulated in response to caffeine.

(A), Plasmid constructed for the overexpression of DMT family transporter (*PP_RS18750*) in LJBJ690 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$-*fdhA:PP_RS18750*Δ), the nucleotide sequence of *PP_RS18750*, and **(B),** the graphical illustration of the orientation of genes in the proximity of *PP_RS18750* (Outlined in yellow). Positively upregulated genes in response to caffeine are in green and the down-regulated genes are in red. *PP_RS18750* was upregulated by 5.6 folds, *PP_RS18745* (LysR family transcriptional regulator) in 3.9 folds, *PP_RS18740* (YeeE/YedE family protein) in 1.97 folds, *PP_RS18715* (FadR family transcriptional regulator) in 1.24 folds, *PP_RS18780* (Hypothetical protein) in 1.22 folds. *PP_RS18755* (Hypothetical protein) and *PP_RS18760* (Hypothetical protein) were downregulated in 1.11 and 1.78 folds, respectively.

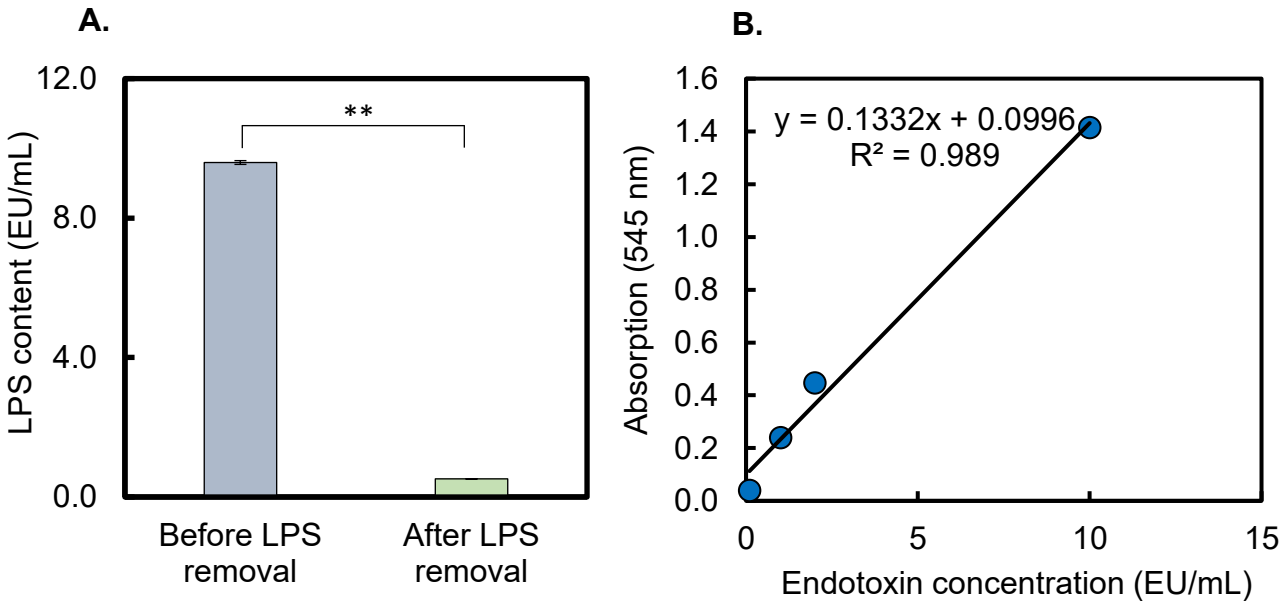


Supplementary Fig. S7: Plasmid map of pBTL-2-Ptac-*PP_RS18750*-GFP.
Plasmid constructed for the overexpression of DMT family transporter (*PP_RS18750*) linked with sfGFP in LJBJ690 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$-*fdhA:PP_RS18750*Δ).

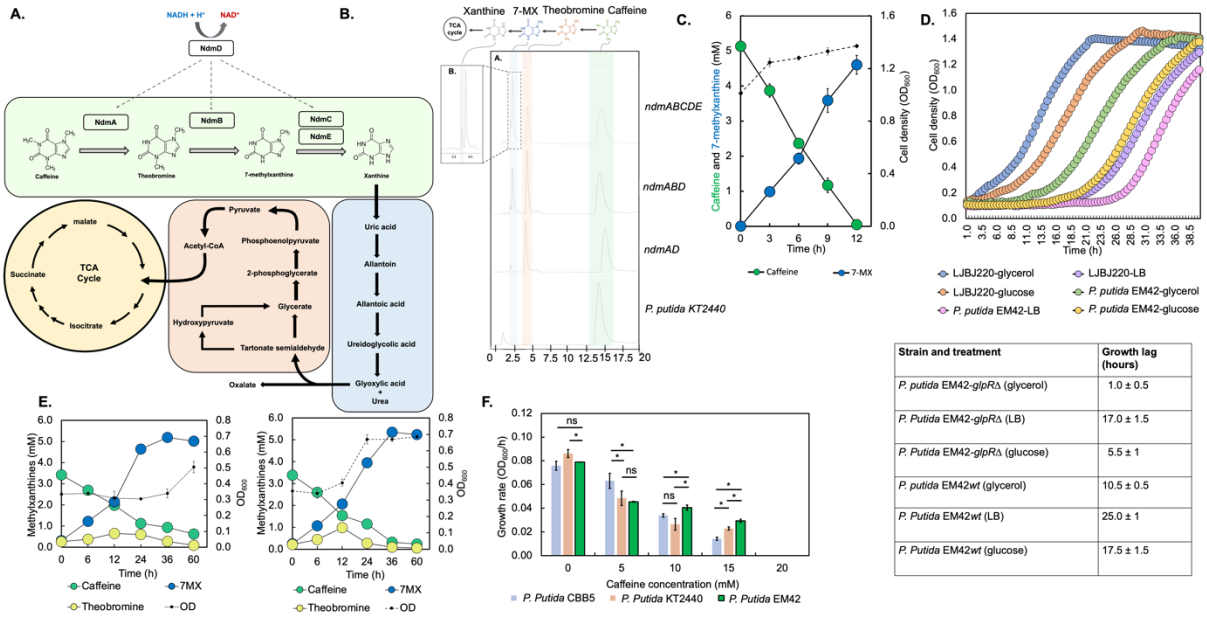


Supplementary Fig. S8: Calibration curves used in HPLC quantification of analytes.
Matrix-matched calibration curves were prepared in 2XM9 media to quantify methylxanthines and glycerol. Methylxanthines, including caffeine, theobromine, and 7-MX, were detected and quantified using a Photodiode array (PDA) detector at 254 nm wavelength, and glycerol was detected and quantified using a Refractive Index Detector (RID) coupled to a Shimadzu LC-2500 HPLC system. Linearity (R^2) for all analytes was above 0.9900, and methods were partially

validated (for linearity, repeatability, accuracy, precision, and matrix effect) according to European Medicine Agency guidelines for validation of analytical methods [6].



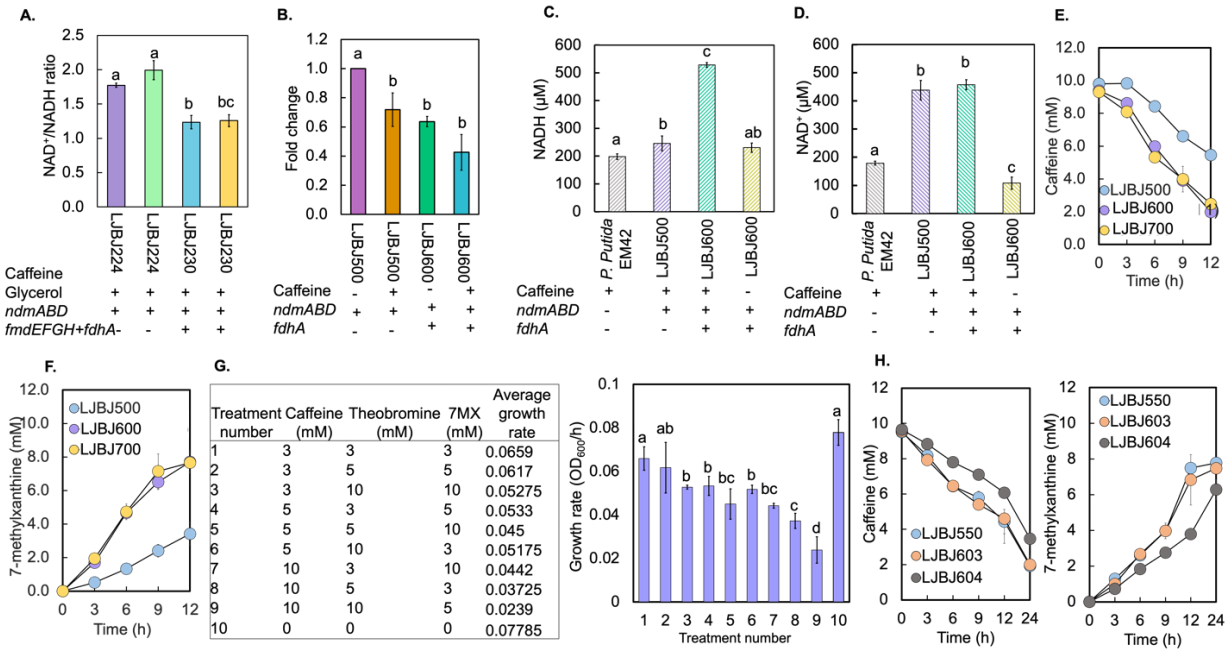
Supplementary Fig. S9: Quantifying lipopolysaccharides (LPS) in extracted 7-MX using Genscript ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit.
(A), Initial 9. 594 ± 0.054 EU/mL of LPS was reduced to 0.52 ± 0.003 (95% reduction) by LPS removal. **(B),** The calibration curve was constructed to quantify LPS according to manufacturer's instructions using the standard LPS solutions provided.



Strain and treatment	Growth lag (hours)
P. putida EM42-*glpR*Δ (glycerol)	1.0 ± 0.5
P. Putida EM42-*glpR*Δ (LB)	17.0 ± 1.5
P. Putida EM42-*glpR*Δ (glucose)	5.5 ± 1
P. putida EM42*wt* (glycerol)	10.5 ± 0.5
P. Putida EM42*wt* (LB)	25.0 ± 1
P. Putida EM42*wt* (glucose)	17.5 ± 1.5

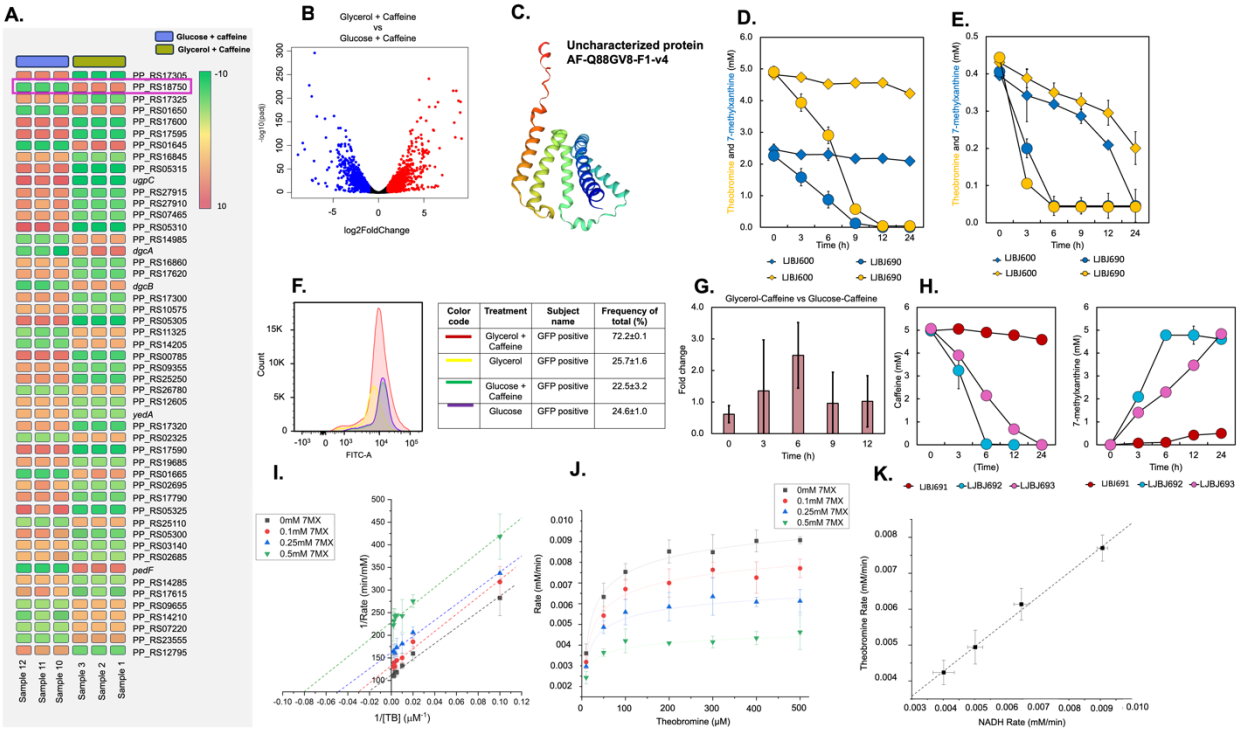
Supplementary Fig. S10: Heterologous expression of N-demethylase encoding genes from *P. putida* CBB5 in *P. putida* KT2440 and *P. putida* EM42.

(A), Schematic representation of the proposed pathway of caffeine metabolism in *P. putida* EM42 and *P. putida* KT2440 upon the heterologous expression of *ndmA*, *ndmB, and ndmD*. **(B),** Reconstruction of caffeine N-demethylation pathway of *P. putida* CBB5 in *P. putida* KT2440. Strain LJBJ110 (*P. putida* KT2440:: pBTL-2-P$_{tac}$-*ndmABCDE*$_{(kan)}$) could convert caffeine to xanthine. **(C),** Caffeine utilization and 7-MX production of LJBJ113 (*P. putida* KT2440::pBTL-2-P$_{tac}$-*ndmABD*$_{(kan)}$) in 40 mM of acetate**. (D),** Comparison of the growth lag of *P. putida* EM42 and LJBJ220 (*P. putida EM42*::*ΔglpR*) with different pre-culture media. **(E),** Comparison of caffeine utilization, product formation, and growth of LJBJ221 (*P. putida* KT2440::*ΔglpR*+pBLT-2-P$_{tac}$-*ndmABD*$_{(kan)}$) (left) and LJBJ223 (*P. putida* EM42:: *glpRΔ*+ pBTL-2-2-P$_{tac}$-*ndmABD*$_{(kan)}$*)* (right) with 5 mM of caffeine in M9 minimal medium supplemented with 40 mM of glycerol. **(F),** Comparison of the growth rates of *P. putida* CBB5, *P. putida* KT2440, and *P. putida* EM42 at varying concentrations of caffeine. The results are expressed as means ± SEM (n=3). The level of statistical significance is indicated for differences between the two strains (*p <0.05). Bars labeled with different symbols (a, b, and c) indicate statistical significance in the differences (p < 0.05; one-way ANOVA followed by Tukey's post hoc honest significance difference test). Bars labeled with the same symbol indicate no statistically significant difference (p > 0.05). ANOVA: analysis of variance.



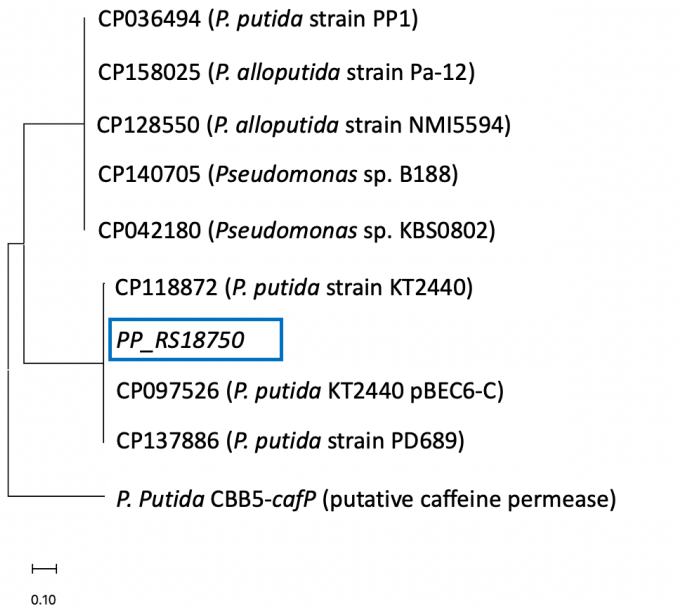
Supplementary Fig. S11: Establishment of redox co-factor regeneration system in LJBJ500

(A), Comparison of NAD^+/NADH ratio in response to the addition of caffeine in the presence and absence of redox engineering. **(B),** qRT-PCR results of the fold change of *fmdE* transcription in response to caffeine in the presence and absence of redox engineering. **(C),** NADH and **(D),** NAD^+ in response to the addition of caffeine in the presence and absence of an additional copy of *fdhA*. **(E,F),** Comparison of genome integration of an additional copy of *tac* promoter driven (formate dehydrogenase) *fmdEFGH,* LJBJ700 strain (*P. putida* EM42::*glpR*Δ:P_{tac}-*ndmABD*::P_{tac}-*fdhA*::P_{lac}-*fmdEFGH*) with the LJBJ600 (*P. putida* EM42::*glpR*Δ:P_{tac}-*ndmABD*::P_{tac}-*fdhA*) *and the* LJBJ500 (*P. putida* EM42::*glpR*Δ:P_{tac}-*ndmABD*] strains. **(G),** Taguchi orthogonal array layout (left panel) and the comparison of growth rates in the mix of methylxanthines (right panel). **(H),** Comparison of caffeine utilization and 7-MX production of LJBJ603 (*P. putida* EM42::*glpR*Δ:P_{tac}*ndmABD*::P_{tac}-*fdhA*+pBLT-2-P_{lac}-*fdhA*$_{Chl}$), LJBJ550 (*P. putida* EM42::*glpR*Δ:P_{tac}*ndmABD*+pBLT2-P_{lac}-*fdhA-fmdEFGH*$_{(chl)}$) and LJBJ604 (*P. putida* EM42::*glpR*Δ:P_{tac}*ndmABD*::P_{tac}-*fdhA* + pBTL-2 $_{(Chl)}$). The results are expressed as means ± SEM (n=3). The level of statistical significance is indicated for differences between the two strains (**p <0.05). Bars labeled with different symbols (a, b, and c) indicate statistical significance in the differences (p < 0.05; one-way ANOVA followed by Tukey's post hoc honest significance difference test). Bars labeled with the same symbol indicate no statistically significant difference (p > 0.05). ANOVA: analysis of variance**.**



Supplementary Fig. S12: Identification of putative caffeine/methylxanthine transporter in *P. putida* EM42

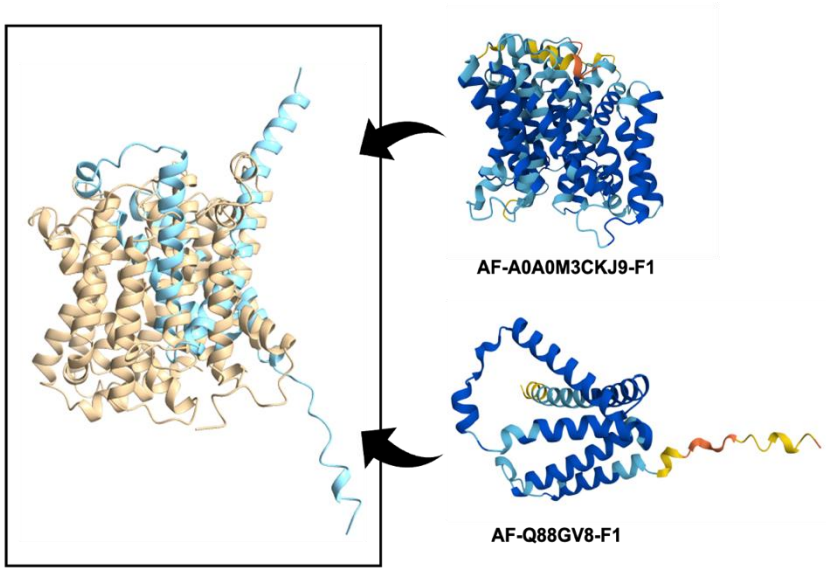
(A), Heat map of highly upregulated and downregulated genes in glycerol + caffeine and glucose + caffeine condition. The putative caffeine transporter *PP_RS18750* is marked with a pink outline. **(B),** The volcanic plot of glycerol + caffeine vs glucose + caffeine. **(C),** Structure predicted for *PP_RS18750* in alpha fold for the uncharacterized protein in source organism *P. putida* (strain ATCC 47054), Uniprot Q88GV8) and the catalytic activity is undefined. The theobromine and 7-MX intake at high concentration **(D)**,and lower concentration **(E),** LJBJ600 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA*) and LJBJ690 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA: PP_RS18750*Δ) strains. **(F),** Expression of GFP in LJBJ693 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA:PP_RS18750*Δ + pBLT-2-P$_{tac}$*-PP_RS18750-GFP*$_{(kan)}$) grown in different carbon sources and presence/absence of caffeine (in 3 h) as determined by flow cytometry. **(G),** Fold change of expression of mRNA (*PP_RS18750*) in strain LJBJ692 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA: PP_RS18750*Δ + pBTL-2-P$_{tac}$*-PP_RS18750*$_{(kan)}$) in glycerol caffeine condition compared to glucose caffeine condition. No significant (p>0.05) difference was observed when *PP_RS18750* was expressed under the constitutive tac promotor irrespective to the carbon source. **(H),** Comparison of caffeine utilization (left) and 7MX production (right) of LJBJ691 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA:PP_RS18750*Δ + pBTL-2 $_{(kan)}$), LJBJ692 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA:PP_RS18750*Δ + pBTL-2-P$_{tac}$*-PP_RS18750*$_{(kan)}$) and LJBJ693 (*P. putida* EM42::*glpR*Δ:P$_{tac}$*ndmABD*::P$_{tac}$*-fdhA:PP_RS18750*Δ + pBTL-2-P$_{tac}$*-PP_RS18750-GFP*$_{(kan)}$). **(I),** The change of 7-MX concentration effects the relationship between N-demethylation reaction rates versus Theobromine (TB) concentration was employed to determine the type of inhibition. Total of 7 different TB concentrations were fed to the fixed amount of NdmB and NdmD to carry the N-demethylation reaction, and such test involved 4 different concentration of 7-MX, respectively. NADH consuming rates then plotted versus multiple TB concentration to find the trends of N-demethylation reaction rates under the presence of the 7-MX. **(J),** Lineweaver-Burk Plot was applied to the experiment 3A data by plotting the inverse NADH consuming rate with inverse concentration of TB. Michaelis constants (K_m) and maximum reaction velocities (V_{max}) for each reaction was determined and reported in **Supplementary Data Table S6** from the slope and interception of each plot. By observing parallel correlation among all four studies, uncompetitive inhibition was suggested for the N-demethylase NdmB with the presence of 7-MX . **(K),** The experimental data plot suggested a linear correlation between NADH consuming rate and the TB degradation rate in the same reaction, indicating that measuring the concentration change of NADH overtime is a valid parameter for such an enzymatic study. The results are expressed as means ± SEM (n=2).



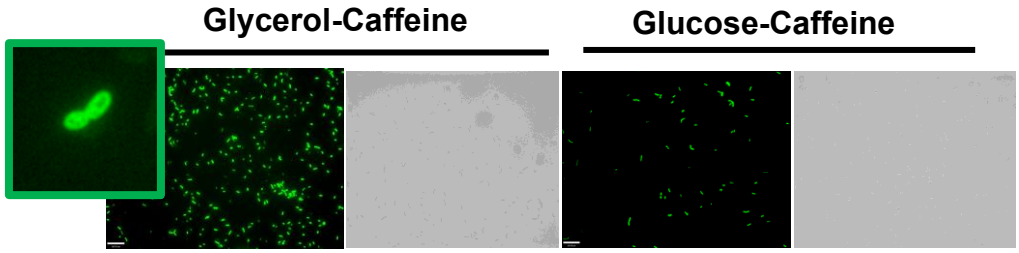
Supplementary Fig. S13: Maximum likelihood phylogenetic tree constructed based on the nucleotide sequence of *PP_RS18750.*

National Center for Biotechnology Information (NCBI) blast was used to retrieve sequences with E. value 0.00 and query cover 100% and aligned with MUSCLE (multiple sequence alignment tool) with DMT family transporter *PP_RS18750* (outlined in blue) along with putative caffeine permease sequence (*cafP*) from *P. putida* CBB5. An unrooted Maximum likelihood phylogenetic tree was generated by MEGA 11, with 1000 bootstraps.

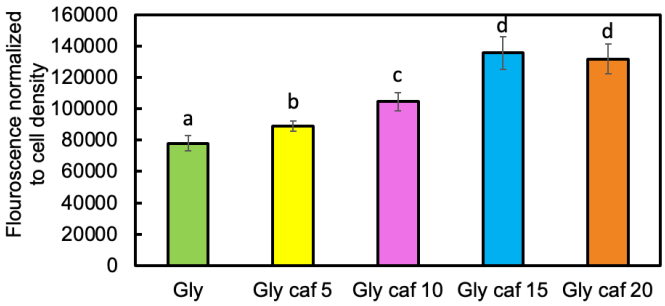
ChimeraX matchmaker parameters	
Chain pairing	bb
Alignment algorithm	Needleman-Wunsch
Similarity matrix	BLOSUM-62
SS fraction	0.3
Gap open (HH/SS/other)	18/18/6
Gap extend	1
SS matrix	HSO H 6-9-6 S -6-6 O 4
Iteration cutoff	2



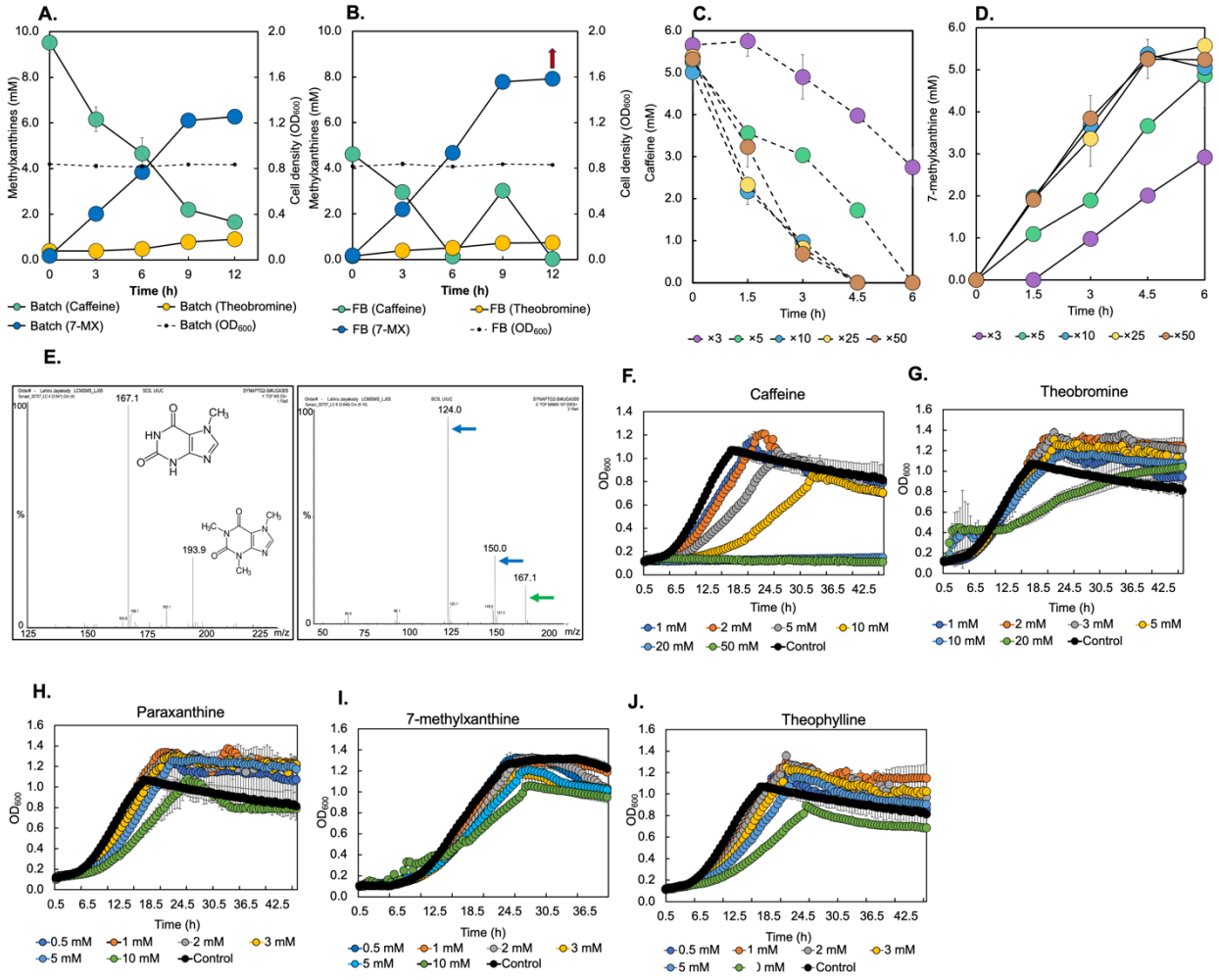
Supplementary Fig. S14: Comparison of the structure of putative methylxanthine permease, *cafP,* and putative methylxanthine transporter, *PP_RS18750.*
The AlphaFold protein structure database predicted protein structures were generated for *cafP* (AF-A0A0M3CKJ9-F1) and *PP_RS18750* (AF-Q88GV8-F1). The PBD files were then exported and aligned using ChimeraX 17.1, the matchmaker feature. The sequence alignment score was 161.6, RMSD between 10 pruned atom pairs is 1.440Å (across all 167 pairs: 24.593).



Supplementary Fig. S15: Microscopic images of GFP expression in glycerol and caffeine-containing media (Left) and glucose and caffeine-containing media (right).

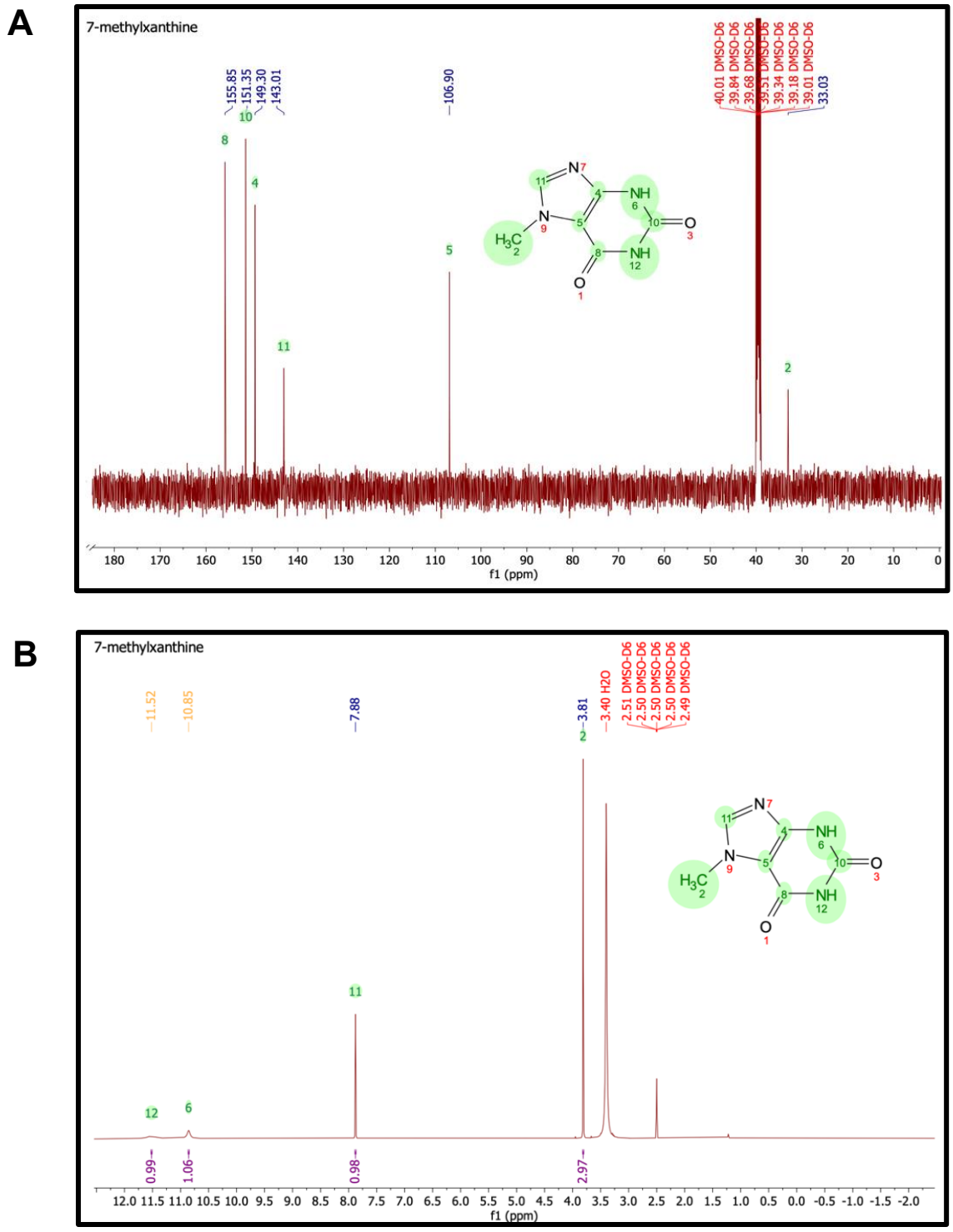


Supplementary Fig. S16: Statistical analysis of the cell density normalized fluorescence at 12 h with different concentrations of caffeine in glycerol-containing media. Different letters depict the significant differences between the treatments (ANOVA followed by Tukey's post hoc test $p < 0.05$). Error bars show the standard deviation of the replicates (n=3).



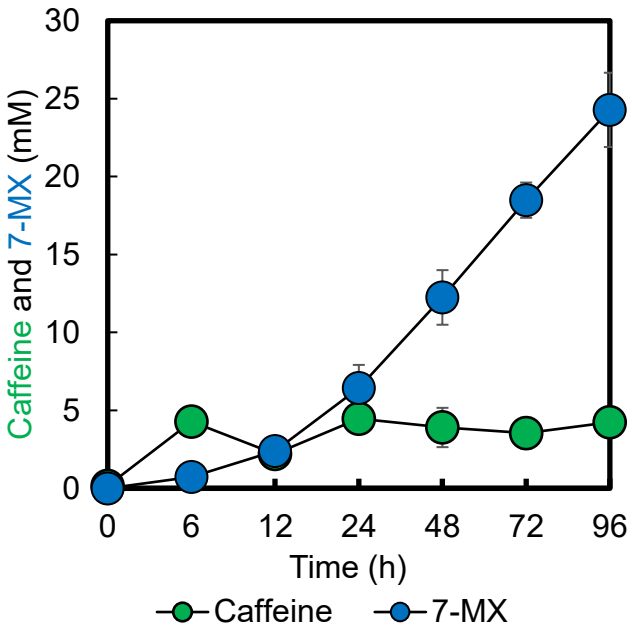
Supplementary Fig. S17: Culture optimization and toxicity tolerance of *P. putida* EM42 for different methylxanthines.

Comparison of batch **(A),** and fed batch **(B),** feeding at 10 mM total caffeine concentration. Complete conversion of 10 mM of caffeine to 7-MX was obtained during fed-batch feeding. **(C),** caffeine utilization and 7-MX production **(D),** at different flask volume/culture volumes. **(E)**, Initial confirmation of the production of 7-MX in cultures using HPLC-TOF MS/MS. The precursor ion (Green arrow) and the fragment ions (Blue arrow) are shown in the right panel. The results are expressed as means ± SEM (n=3). The level of statistical significance is indicated for differences between the two strains (***p* <0.05). Growth curves of *P. putida* EM42 in **(F),** caffeine **(G),** theobromine **(H),** paraxanthine **(I),** 7-MX, and **(J),** theophylline**.**

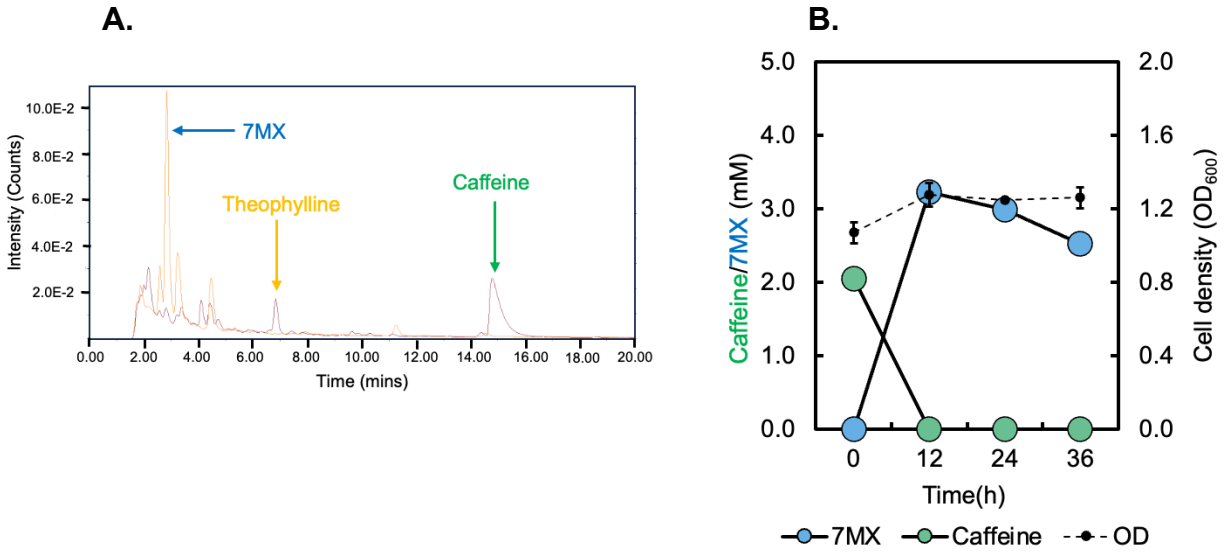


Supplementary Fig. S18: ^{13}C-NMR and ^{1}H-NMR spectrum of microbially synthesized and extracted 7MX powder.

(A), The NMR spectrum of extracted 7-MX recorded in DMSO-d_6 with a Bruker DRX 500 NMR spectrometer at 299 K, ^{13}C NMR (126 MHz, DMSO-D_6) δ 155.85, 151.35, 149.30, 143.01, 106.90, 33.03. **(B),** ^{1}H-NMR spectrum of the extracted 7-MX, ^{1}H NMR (500 MHz, DMSO-D_6) δ 11.52 (δ, 1H), 10.85 (δ, 1H), 7.88 (δ, 1H), 3.81 (δ, 3H)

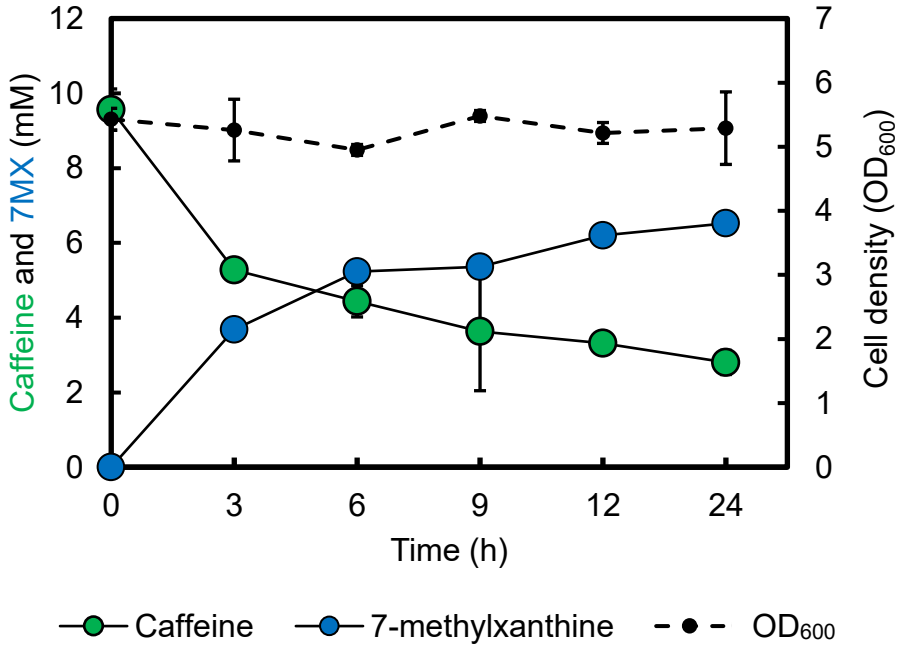


Supplementary Fig. S19: Synthesis of 7-MX with the addition of caffeine powder.
Caffeine was weighed and added to the culture in powdered form in increments for the biocatalytic conversion to 7-MX. Caffeine was added at 0 h, 6 h, 12 h, 24 h, and 72 h.



Supplementary Fig. S20: Synthesis of 7-MX by utilizing caffeine and theophylline in green tea hydrothermal dissolution (HD) samples.
(A), Chromatogram of hydrothermal dissolution samples (90% v/v) at 0 h and after 12 h of fermentation. HD samples were prepared with waste green tea samples using bench scale reactor at Thermaquatica Inc (Carbondale, IL, USA), following the methodology described in

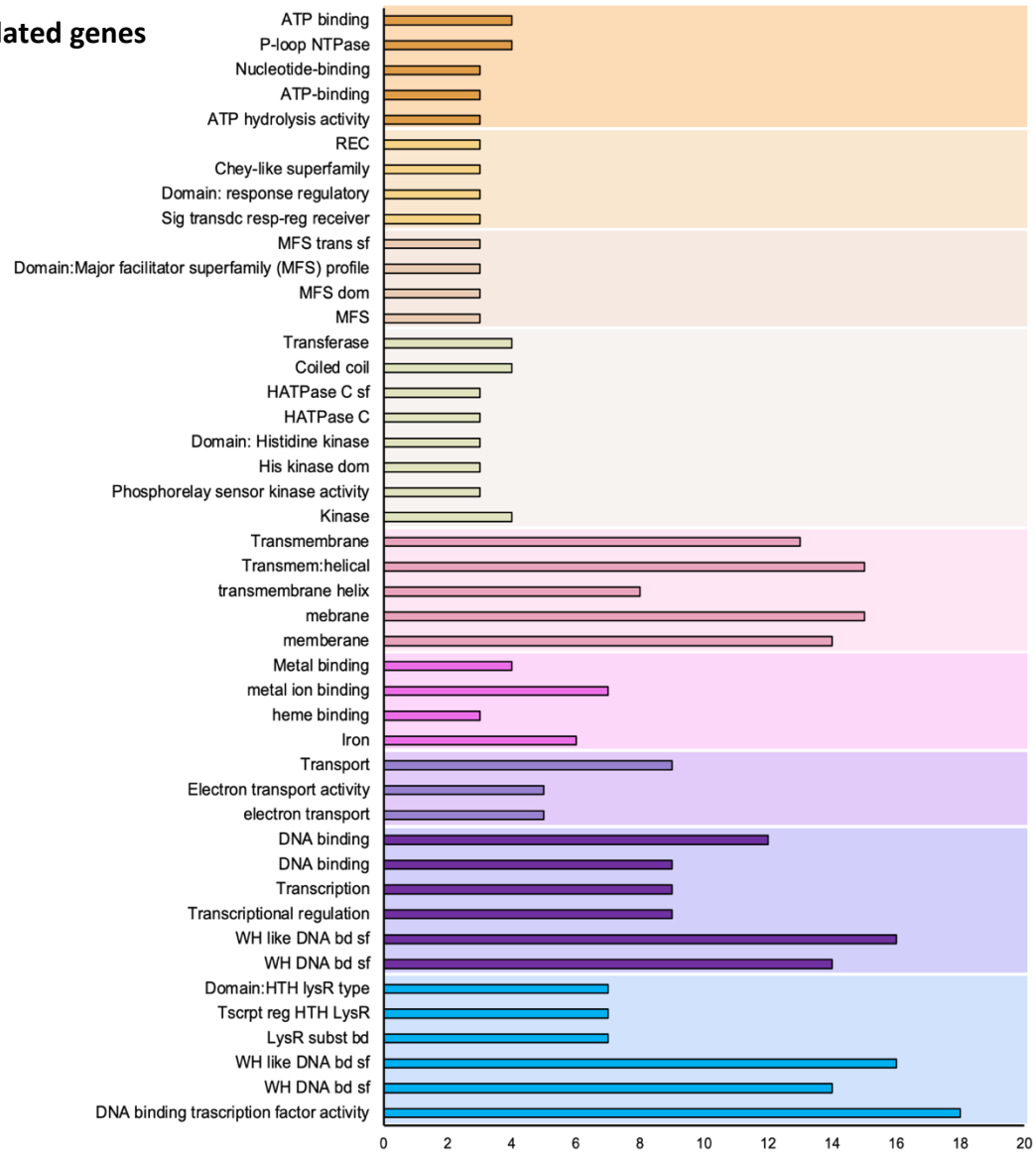
PCT/US10/23886 [7]. Caffeine and theophylline were seen at 0 h (Brown line) and caffeine and theophylline were completely converted to 7MX in 12 h (yellow line) by strain LJBJ221 (*P. putida* KT2440::*ΔglpR* pBTL-2-P_{tac}-*ndmABD*$_{(kan)}$) in 2X M9 minimal medium without externally added carbon source**. (B),** A total of 3.21 ± 0.08 mM of 7MX was produced at the end of 12 h.



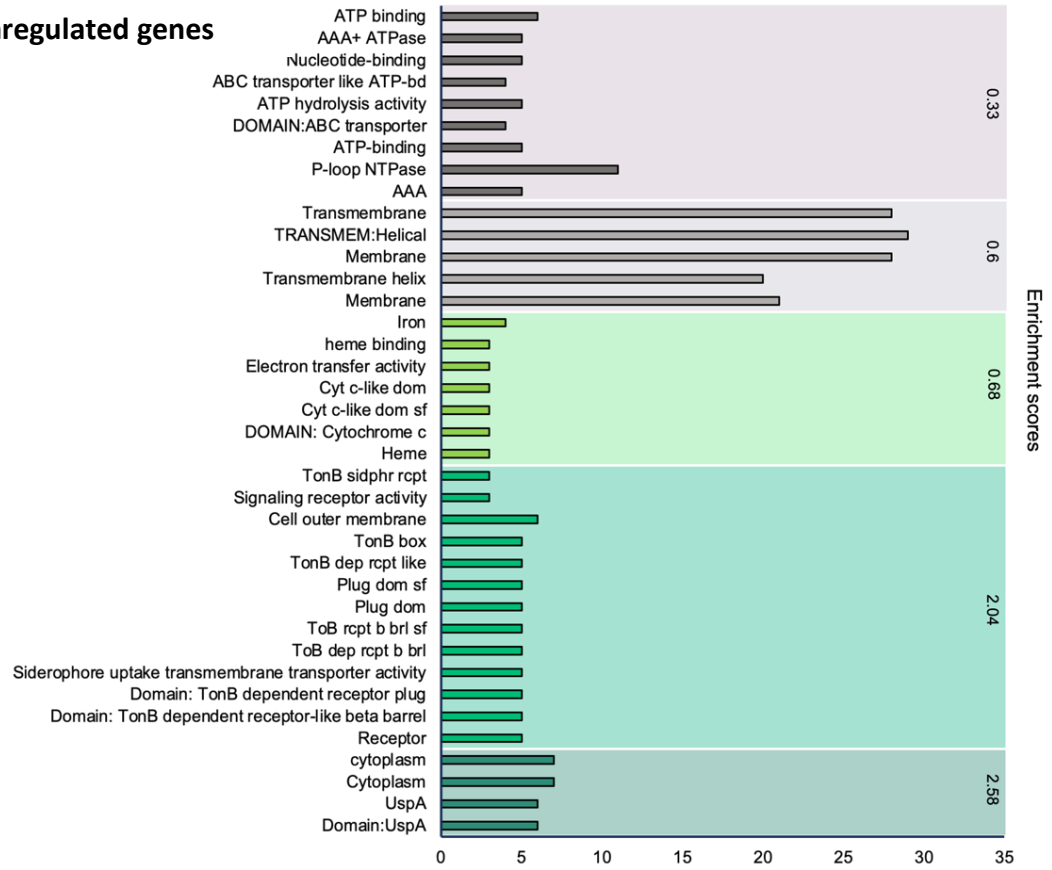
Supplementary Fig. S21: Caffeine utilization and 7-MX production of LJBJ600 in 50 mM tris HCL media (initial cell density of OD_{600}: 5).
The efficiency of the conversion was highest during the first 3 h and the efficiency was drastically reduced after 3 h. Hence the complete conversion of 10 mM of caffeine was not observed.

4.0 Functional annotation clustering of upregulated and down regulated genes in Glycerol-Caffeine condition, compared to Glycerol condition (>2 Log_2Fold)

4.1 Upregulated genes

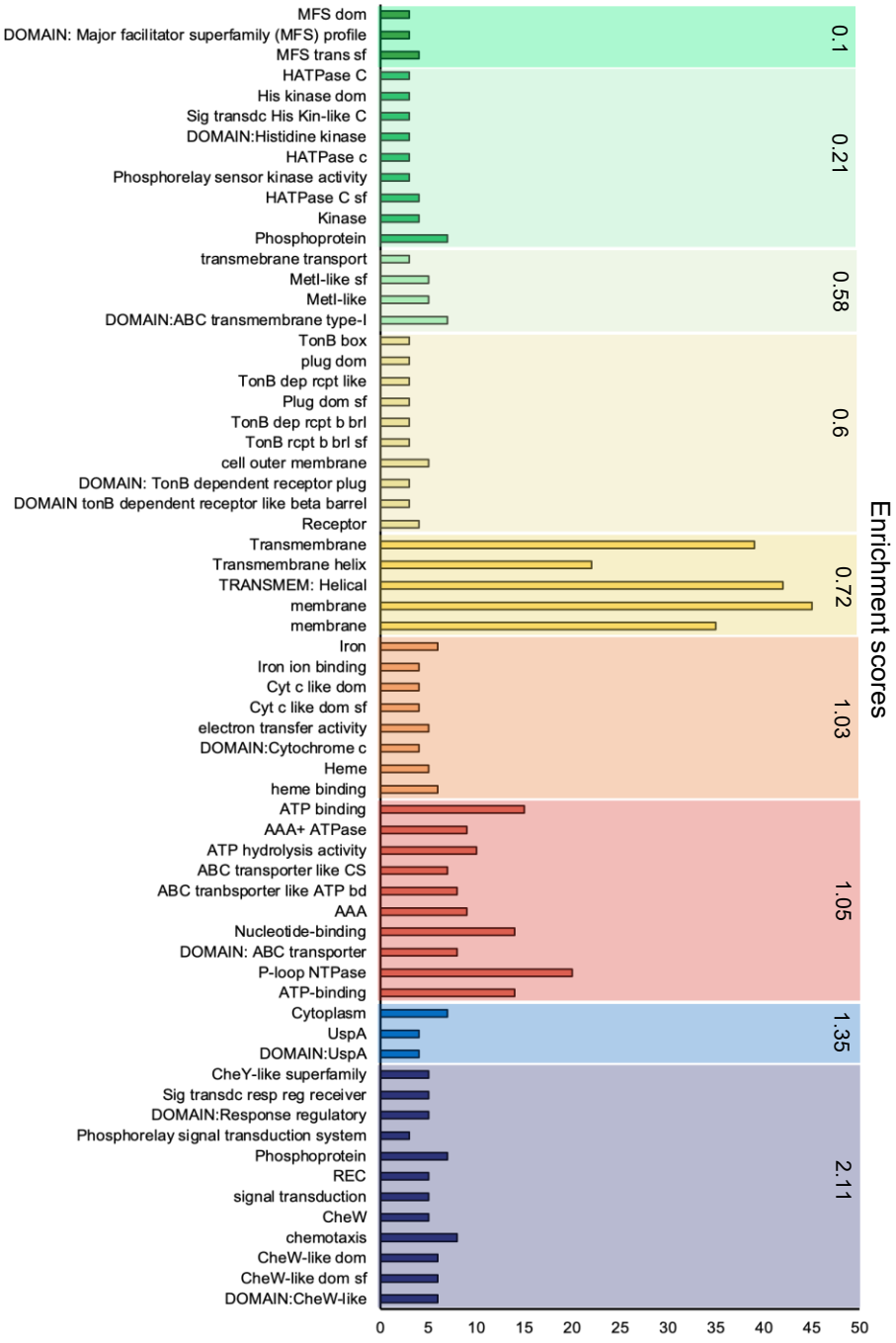


4.2 Downregulated genes

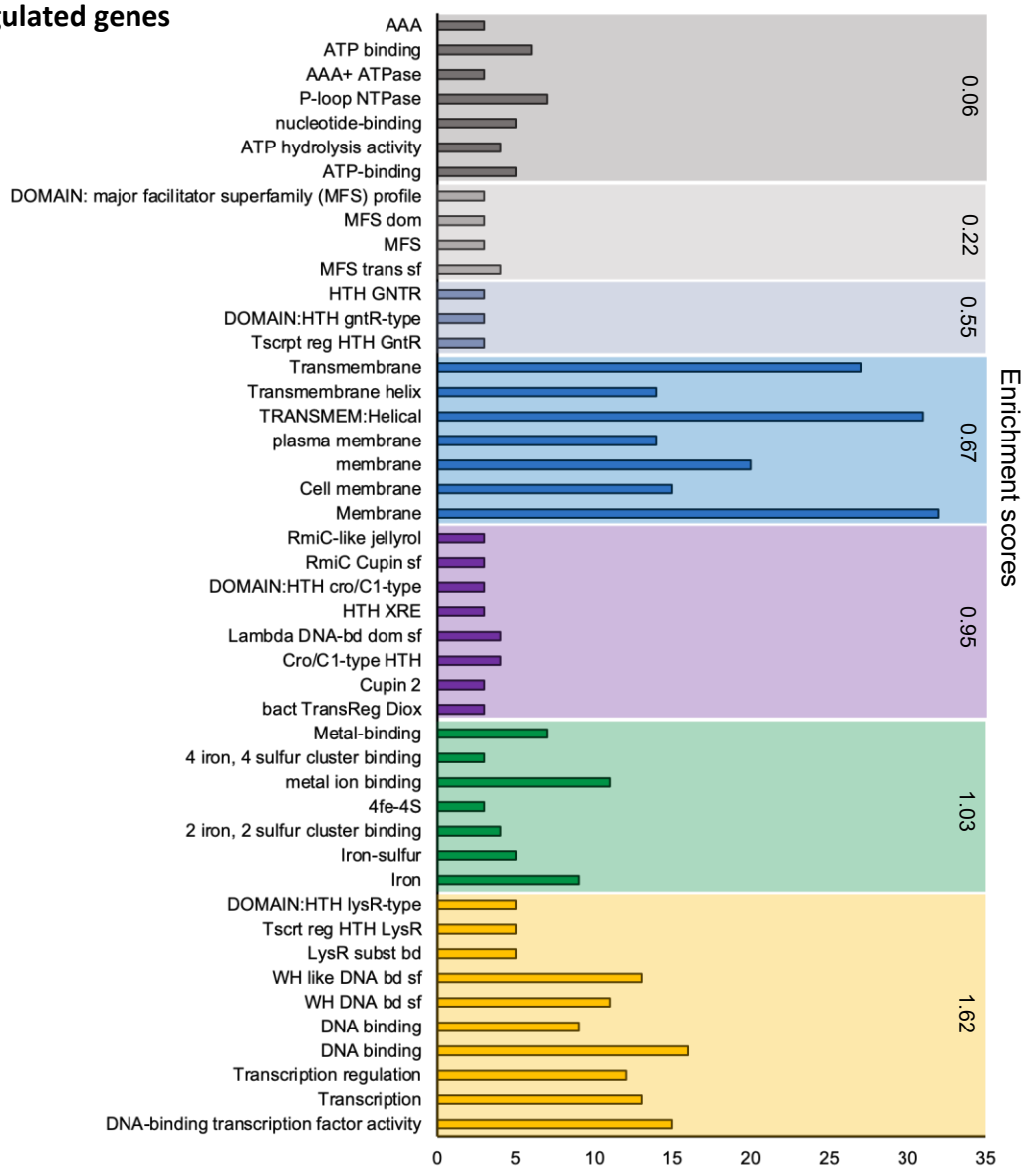


5.0 Functional annotation clustering of upregulated and down regulated genes in Glucose-Caffeine condition, compared to Glycerol-caffeine condition (>2 Log_2Fold)

5.1 Upregulated genes



5.2 Downregulated genes



6.0 The most highly upregulated and downregulated genes

6.1 Glycerol-caffeine vs glycerol

Gene	Description	log2FoldChange
gene-PP_RS13965	pyrroloquinoline quinone biosynthesis peptide chaperone PqqD	7.47
gene-PP_RS13930	pedF cytochrome c-550 PedF	7.44
gene-PP_RS13960	pqqA pyrroloquinoline quinone precursor peptide PqqA	7.29
gene-PP_RS15090	csiD carbon starvation induced protein CsiD	6.91
gene-PP_RS13870	hypothetical protein	6.79
gene-PP_RS01645	membrane dipeptidase	6.67
gene-PP_RS01650	hypothetical protein	6.64
gene-PP_RS13920	pentapeptide repeat-containing protein	6.53
gene-PP_RS13955	aldehyde dehydrogenase	6.45
gene-PP_RS13935	transporter substrate-binding domain-containing protein	6.42
gene-PP_RS17105	paaA 1,2-phenylacetyl-CoA epoxidase subunit A	6.41
gene-PP_RS13915	response regulator transcription factor	6.29
gene-PP_RS01655	dgcA dimethylglycine demethylation protein DgcA	6.28
gene-PP_RS13925	PQQ-dependent dehydrogenase, methanol/ethanol family	6.28
gene-PP_RS13940	quinoprotein dehydrogenase-associated SoxYZ-like carrier	6.15
gene-PP_RS17100	paaB 1,2-phenylacetyl-CoA epoxidase subunit B	6.04
gene-PP_RS13945	quinoprotein relay system zinc metallohydrolase 1	5.90
gene-PP_RS13905	ABC transporter substrate-binding protein	5.85
gene-PP_RS13885	PQQ-dependent catabolism-associated CXXCW motif protein	5.84
gene-PP_RS17095	paaC phenylacetate-CoA oxygenase subunit PaaC	5.83
gene-PP_RS13900	YVTN family beta-propeller repeat protein	5.78
gene-PP_RS13950	PQQ-dependent dehydrogenase, methanol/ethanol family	5.71
gene-PP_RS18750	DMT family transporter	5.61
gene-PP_RS13910	HAMP domain-containing sensor histidine kinase	5.59
gene-PP_RS13875	PAS domain-containing sensor histidine kinase	5.55
gene-PP_RS13895	ATP-binding cassette domain-containing protein	5.54
gene-PP_RS01660	dimethylglycine demethylation protein DgcB	5.47
gene-PP_RS24820	amino acid permease	5.42
gene-PP_RS01665	electron transfer flavoprotein subunit alpha/FixB family protein	5.22
gene-PP_RS23380	phenylalanine 4-monooxygenase	5.21
gene-PP_RS01725	sarcosine oxidase subunit delta	5.02
gene-PP_RS17090	phenylacetate-CoA oxygenase subunit PaaJ	4.96
gene-PP_RS01730	sarcosine oxidase subunit alpha	4.88
gene-PP_RS13865	porin	4.87
gene-PP_RS01720	sarcosine oxidase subunit beta family protein	4.86
gene-PP_RS21395	isocitrate lyase	4.69
gene-PP_RS13890	ABC transporter permease	4.66
gene-PP_RS01735	sarcosine oxidase subunit gamma family protein	4.65
gene-PP_RS01670	electron transfer flavoprotein subunit beta	4.65
gene-PP_RS17135	2,3-dehydroadipyl-CoA hydratase	4.64
gene-PP_RS27610	glutamine synthetase	4.46
gene-PP_RS01715	serine hydroxymethyltransferase	4.42
gene-PP_RS11325	formate dehydrogenase subunit gamma	4.35
gene-PP_RS17120	hydroxyphenylacetyl-CoA thioesterase PaaI	4.31
gene-PP_RS14985	catalase family peroxidase	4.17
gene-PP_RS18690	gamma-glutamyl-gamma-aminobutyrate hydrolase family protein	4.17
gene-PP_RS13880	response regulator transcription factor	4.16
gene-PP_RS17085	phenylacetate-CoA oxygenase/reductase subunit PaaK	4.15
gene-PP_RS26445	NirD/YgiW/YdeI family stress tolerance protein	4.15
gene-PP_RS28195	DeoR family transcriptional regulator	4.10

Gene	Description	log2FoldChange
gene-PP_RS17325	TonB-dependent siderophore receptor	-7.10
gene-PP_RS27915	GTP-binding protein	-7.01
gene-PP_RS17370	cytochrome c5 family protein	-6.89
gene-PP_RS17375	DUF2946 family protein	-6.85
gene-PP_RS17305	DUF4440 domain-containing protein	-6.83
gene-PP_RS27910	DUF1826 domain-containing protein	-6.75
gene-PP_RS16845	GNAT family N-acetyltransferase	-6.65
gene-PP_RS19505	glcF glycolate oxidase subunit GlcF	-6.63
gene-PP_RS25250	DUF2946 domain-containing protein	-6.33
gene-PP_RS19500	glcE glycolate oxidase subunit GlcE	-6.11
gene-PP_RS17385	LysR family transcriptional regulator	-6.04
gene-PP_RS19495	glcD glycolate oxidase subunit GlcD	-5.97
gene-PP_RS19510	hypothetical protein	-5.88
gene-PP_RS16860	Hsp20/alpha crystallin family protein	-5.76
gene-PP_RS17390	putative natural product biosynthesis protein	-5.63
gene-PP_RS22085	CcoQ/FixQ family Cbb3-type cytochrome c oxidase assembly chaperone	-5.61
gene-PP_RS14285	universal stress protein	-5.46
gene-PP_RS07465	TonB-dependent receptor	-5.44
gene-PP_RS25255	TonB-dependent copper receptor	-5.43
gene-PP_RS19685	chlorinating enzyme	-5.38
gene-PP_RS25245	copper chaperone PCu(A)C	-5.31
gene-PP_RS17330	DUF4198 domain-containing protein	-5.31
gene-PP_RS17300	hypothetical protein	-5.28
gene-PP_RS05235	arginine deiminase	-5.14
gene-PP_RS28075	DUF2933 domain-containing protein	-5.14
gene-PP_RS28070	isoprenylcysteine carboxylmethyltransferase family protein	-5.00
gene-PP_RS05230	ornithine carbamoyltransferase	-4.99
gene-PP_RS22080	cytochrome-c oxidase, cbb3-type subunit II	-4.92
gene-PP_RS13790	universal stress protein	-4.89
gene-PP_RS16840	erythromycin esterase family protein	-4.88
gene-PP_RS19680	acyl carrier protein	-4.86
gene-PP_RS28080	hypothetical protein	-4.78
gene-PP_RS04310	cytochrome o ubiquinol oxidase subunit III	-4.73
gene-PP_RS05985	DUF2025 family protein	-4.63
gene-PP_RS17320	GTP cyclohydrolase I FolE2	-4.55
gene-PP_RS15995	type VI secretion system tube protein Hcp	-4.51
gene-PP_RS25260	PepSY domain-containing protein	-4.47
gene-PP_RS17170	MBL fold metallo-hydrolase	-4.47
gene-PP_RS19675	radical SAM protein	-4.41
gene-PP_RS22090	cytochrome-c oxidase, cbb3-type subunit III	-4.41
gene-PP_RS16880	universal stress protein	-4.39
gene-PP_RS17165	universal stress protein	-4.37
gene-PP_RS12660	AraC family transcriptional regulator	-4.32
gene-PP_RS17175	hypothetical protein	-4.27
gene-PP_RS04315	cytochrome o ubiquinol oxidase subunit IV	-4.23
gene-PP_RS02690	ABC transporter ATP-binding protein	-4.23
gene-PP_RS02695	DUF2796 domain-containing protein	-4.20
gene-PP_RS12605	DUF1624 domain-containing protein	-4.19
gene-PP_RS17160	GNAT family N-acetyltransferase	-4.17
gene-PP_RS19975	alcohol dehydrogenase AdhP	-4.13

6.2 Glucose-caffeine vs glycerol-caffeine

Gene	Description	log2FoldChange
gene-PP_RS05325	porin	9.15
gene-PP_RS05320	sn-glycerol-3-phosphate ABC transporter ATP-binding protein UgpC	9.04
gene-PP_RS05310	sugar ABC transporter permease	9.00
gene-PP_RS05315	carbohydrate ABC transporter permease	8.81
gene-PP_RS17595	sugar kinase	8.57
gene-PP_RS17590	MFS transporter	8.46
gene-PP_RS05305	carbohydrate ABC transporter substrate-binding protein	8.35
gene-PP_RS17600	xylose isomerase	8.23
gene-PP_RS17305	DUF4440 domain-containing protein	7.21
gene-PP_RS17575	hypothetical protein	7.02
gene-PP_RS17585	D-glycerate dehydrogenase	6.73
gene-PP_RS27915	GTP-binding protein	6.55
gene-PP_RS03270	branched-chain amino acid ABC transporter permease	6.53
gene-PP_RS03275	branched-chain amino acid ABC transporter permease	6.31
gene-PP_RS27910	DUF1826 domain-containing protein	6.26
gene-PP_RS08960	cation acetate symporter	6.22
gene-PP_RS03260	ABC transporter ATP-binding protein	6.16
gene-PP_RS25250	DUF2946 domain-containing protein	6.09
gene-PP_RS00785	DUF1127 domain-containing protein	6.03
gene-PP_RS03265	ABC transporter ATP-binding protein	5.87
gene-PP_RS08955	DUF485 domain-containing protein	5.64
gene-PP_RS07465	TonB-dependent receptor	5.58
gene-PP_RS17325	TonB-dependent siderophore receptor	5.55
gene-PP_RS17375	DUF2946 family protein	5.54
gene-PP_RS05300	AGE family epimerase/isomerase	5.51
gene-PP_RS12260	propionyl-CoA synthetase	5.46
gene-PP_RS25255	TonB-dependent copper receptor	5.43
gene-PP_RS22085	CcoQ/FixQ family Cbb3-type cytochrome c oxidase assembly chaperone	5.36
gene-PP_RS17385	LysR family transcriptional regulator	5.30
gene-PP_RS17300	hypothetical protein	5.25
gene-PP_RS25245	copper chaperone PCu(A)C	5.21
gene-PP_RS01875	3'-5' exonuclease	5.19
gene-PP_RS05235	arcA arginine deiminase	5.18
gene-PP_RS17370	cytochrome c5 family protein	5.06
gene-PP_RS05230	ornithine carbamoyltransferase	5.01
gene-PP_RS28075	DUF2933 domain-containing protein	4.99
gene-PP_RS10580	aldolase	4.97
gene-PP_RS03255	Zn-dependent hydrolase	4.96
gene-PP_RS25260	PepSY domain-containing protein	4.92
gene-PP_RS01880	CBS domain-containing protein	4.86
gene-PP_RS05330	D-hexose-6-phosphate mutarotase	4.86
gene-PP_RS28070	isoprenylcysteine carboxylmethyltransferase family protein	4.85
gene-PP_RS06150	dicarboxylate/amino acid:cation symporter	4.85
gene-PP_RS01240	OprD family porin	4.84
gene-PP_RS12795	asparaginase	4.82
gene-PP_RS17390	putative natural product biosynthesis protein	4.81
gene-PP_RS22080	cytochrome-c oxidase, cbb3-type subunit II	4.81
gene-PP_RS17615	cytochrome c	4.80
gene-PP_RS07225	MFS transporter	4.74
gene-PP_RS17790	GntP family permease	4.73

Gene	Description	log2FoldChange
gene-PP_RS13925	PQQ-dependent dehydrogenase, methanol/ethanol family	-8.87
gene-PP_RS13930	cytochrome c-550 PedF	-8.35
gene-PP_RS13935	transporter substrate-binding domain-containing protein	-8.13
gene-PP_RS01650	hypothetical protein	-7.63
gene-PP_RS01645	membrane dipeptidase	-7.36
gene-PP_RS13940	quinoprotein dehydrogenase-associated SoxYZ-like carrier	-7.32
gene-PP_RS13920	pentapeptide repeat-containing protein	-7.26
gene-PP_RS13955	aldehyde dehydrogenase	-7.10
gene-PP_RS18750	DMT family transporter	-7.03
gene-PP_RS13945	quinoprotein relay system zinc metallohydrolase 1	-6.93
gene-PP_RS01655	dimethylglycine demethylation protein DgcA	-6.79
gene-PP_RS13865	porin	-6.61
gene-PP_RS01660	dimethylglycine demethylation protein DgcB	-5.89
gene-PP_RS13950	PQQ-dependent dehydrogenase, methanol/ethanol family	-5.85
gene-PP_RS01665	electron transfer flavoprotein subunit alpha/FixB family protein	-5.79
gene-PP_RS15090	carbon starvation induced protein CsiD	-5.68
gene-PP_RS13870	hypothetical protein	-5.65
gene-PP_RS13905	ABC transporter substrate-binding protein	-5.46
gene-PP_RS01670	electron transfer flavoprotein subunit beta	-5.28
gene-PP_RS18690	gamma-glutamyl-gamma-aminobutyrate hydrolase family protein	-5.16
gene-PP_RS14985	catalase family peroxidase	-4.90
gene-PP_RS14580	NAD(P)/FAD-dependent oxidoreductase	-4.70
gene-PP_RS26445	NirD/YgiW/YdeI family stress tolerance protein	-4.57
gene-PP_RS24820	amino acid permease	-4.53
gene-PP_RS13960	pyrroloquinoline quinone precursor peptide PqqA	-4.50
gene-PP_RS13890	ABC transporter permease	-4.49
gene-PP_RS01725	sarcosine oxidase subunit delta	-4.45
gene-PP_RS01730	sarcosine oxidase subunit alpha	-4.43
gene-PP_RS13910	HAMP domain-containing sensor histidine kinase	-4.41
gene-PP_RS14205	DUF3833 domain-containing protein	-4.41
gene-PP_RS23555	LysR family transcriptional regulator	-4.40
gene-PP_RS01735	sarcosine oxidase subunit gamma family protein	-4.40
gene-PP_RS03185	rpsT 30S ribosomal protein S20	-4.39
gene-PP_RS13895	ATP-binding cassette domain-containing protein	-4.35
gene-PP_RS01720	sarcosine oxidase subunit beta family protein	-4.31
gene-PP_RS14195	DUF1543 domain-containing protein	-4.30
gene-PP_RS14575	alpha/beta hydrolase	-4.29
gene-PP_RS02330		-4.29
gene-PP_RS12685	aminotransferase class V-fold PLP-dependent enzyme	-4.28
gene-PP_RS02335		-4.27
gene-PP_RS18745	LysR family transcriptional regulator	-4.26
gene-PP_RS13915	response regulator transcription factor	-4.25
gene-PP_RS13900	YVTN family beta-propeller repeat protein	-4.24
gene-PP_RS11325	formate dehydrogenase subunit gamma	-4.19
gene-PP_RS14200	periplasmic protein	-4.14
gene-PP_RS27035	hypothetical protein	-4.14
gene-PP_RS20840	translation initiation factor IF-1	-4.13
gene-PP_RS01715	serine hydroxymethyltransferase	-4.11
gene-PP_RS04455	Fe-S cluster assembly transcriptional regulator IscR	-4.08
gene-PP_RS13965	pyrroloquinoline quinone biosynthesis peptide chaperone PqqD	-4.06

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