The TetR family transcriptional regulator PccD negatively controls propionyl coenzyme A assimilation in *Saccharopolyspora erythraea*

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Table S1. The oligonucleotides used in this study

Oligonucleotides	Sequence (5' to 3')
Primers for real-time RT-PCR	
RT0026F	CCGCTGGTCACCTTCGTAGACA
RT0026R	ATCACCGAGTAGTAGGCGTTCTCC
RT3241F	ACCGACCTGAAGACGCCGTT
RT3241R	TAGGACCTGCCGACCATGTAGC
RT3398F	ACATGCTGCTCGACGAGGGTT
RT3398R	CGGTCTTCATCGCCAGGTCCAT
RT3400F	GTCGTATCTGGACATCGGCAAGG
RT3400R	AGCGAACGCCACCACCTCAT
RT3856F	GGTGATCTCGCTGGTGGCTTCA
RT3856R	AACTTGCCCTCGGCGGTGAA
RT4237F	AGGCAGCGTTCCAGCACCAA
RT237R	ACCTTCTGGCGGTAGCTGTTCA
RT6509F	GTCGTATCTGGACATCGGCAAGG
RT6509R	AGCGAACGCCACCACCTCAT
RT7038F	GCTGACCGTCACCTACCAGGAA
RT7038R	GTGTGCTCCATCTTCATCGCCTC
RT3396F	ACTCCTACATCACCTTCGCCTACG
RT3396R	GGCATCGGTGCGGAACATCA
RT3398F	ACATGCTGCTCGACGAGGGTT
RT3398R	CGGTCTTCATCGCCAGGTCCAT
RT3400F	GTCGTATCTGGACATCGGCAAGG
RT3400R	AGCGAACGCCACCACCTCAT
Construction of the pET-*pccD* plasmids for PccD overexpression	
pccR-F	GGAATTCCATATGatggccgcagcaggcagcaagggg
pccR-F	ATTGGATCCCTACCCATAAGTGAATGCCCGATAGGCATCGG
Primers for PCR amplification of EMSAs probe with biotin labeling	
EMSA3398F	AGCCAGTGGCGATAAGCAAGTGCGTGGGGGCGCGGCTTTAG

EMSA3398R	AGCCAGTGGCGATAAGCGTGGTTGCGCCGGTAGAGGTCGG
EMSA3400F	AGCCAGTGGCGATAAGAGCAATTCCCCGCGCGAGCTAGGAGC
EMSA3400R	AGCCAGTGGCGATAAGATGTCCAGATACGACTCCGCCGCCGTC
Synthesized short biotin-labeled ssDNA containing PccD-binding site	
M-*pccA*-F	AGCCAGTGGCGATAAGATGACGGTGTTGTCTTATCGCCACTGGCT
M-*pccA*-R	AGCCAGTGGCGATAAGACAACACCGTCATCTTATCGCCACTGGCT
M-*pccBC*-F	AGCCAGTGGCGATAAGTTGACGCTGCTGACTTATCGCCACTGGCT
M-*pccBC*-R	AGCCAGTGGCGATAAGTCAGCAGCGTCAACTTATCGCCACTGGCT
Construction of the *pccD* in-frame deletion mutant	
pUC3396upF	CCCAAGCTTATGACCGCGAACTCCGAGACGCTCGAC
pUC3396upR	GCTCTAGAGTGCCGGTAGAGCGCGGAGGGC
pUC3396dwF	CGGGGTACCGGTTCATCGACATCCTGGTCACCGAGGTC
pUC3396dwR	CCGGAATTCGGCGGATGATGCCGTTCCACTCCTGC
Construction of the *pccD* overexpression strain	
3396overF	GGAATTCCATATGatggccgcagcaggcagcaagggg
3396overR	ATAAGAATGCGGCCGCCTACCCATAAGTGAATGCCCGATAGGCATCGGT
Construction of T-vector pMD-18T plasmid with the promoter region of SACE_3398	
pMD18T-3398F	CAAGTGCGTGGGGGCGCGGCTTTAG
pMD18T-3398R	GCGGTGGTGTGGATGTCCGGTGCCT
Primers for mutant confirmation by DNA sequencing	
Qc3396JLF	TGTGCCGGAAAGGGTGGGAATG
Qc3396JLR	TCTCCTGGCTCGTTGCGGATGA
UPF	GTACGCGGTTGAGGTGACCAGGAACTGCGG
Ut1	CAGAACATACCGGTCCGCCTCATCGACTCCTCG
Dt1	CGGAGAGAACGACGGGAAGGGAGAAGACGTAACC
DWR	CACGCCAGGTTGATGTCGGCACCGAGG
Primers for Identification of co-transcription of of *S. erythraea* acyl-CoA carboxylases.	
sqRT0026/0027F	TCGTGGACGGCGTGGTCG
sqRT0026/0027R	CGAAGGGTGGCGAGGCTGTC
sqRT0027/0028F	GTCGAGGAGGTGTGACCGTGTT
sqRT0027/0028R	GCGGCTCCGATGATGTTGGG
sqRT3241/3242F	RT3241/3242F
sqRT3241/3242R	RT3241/3242R
sqRT3398/3399F	RT3398/3399F
sqRT3398/3399R	RT3398/3399R
sqRT3399/3400F	CGCACCTCCGCCCTTCCC
sqRT3399/3400R	GCACGAACACCGCATCCAC
sqRT6509/6510F	RT6509/6510F
sqRT6509/6510R	RT6509/6510R
sqRT7038/7039F	RT7038/7039F
sqRT7038/7039R	RT7038/7039R

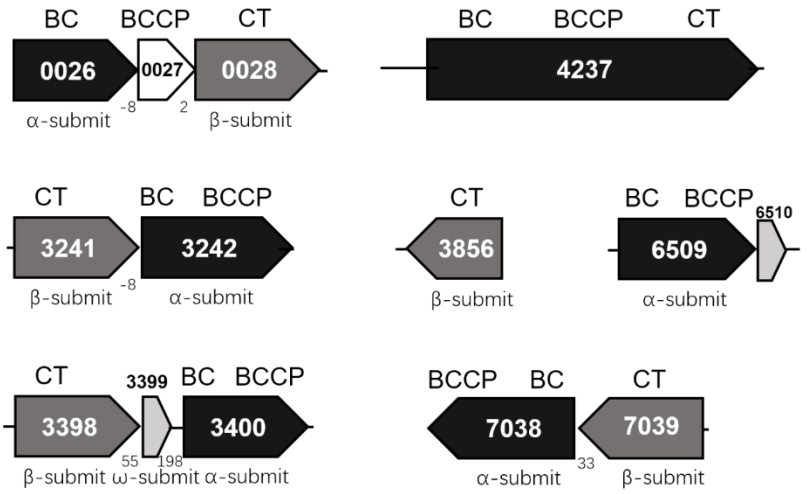

Figure S1. Genetic organization of monocistronic or operon of *S. erythraea* acyl-CoA carboxylases. The letters BC, BCCP, CT refer to the biotin carboxylase, biotin carboxy carrier protein, and carboxytransferase activities respectively.

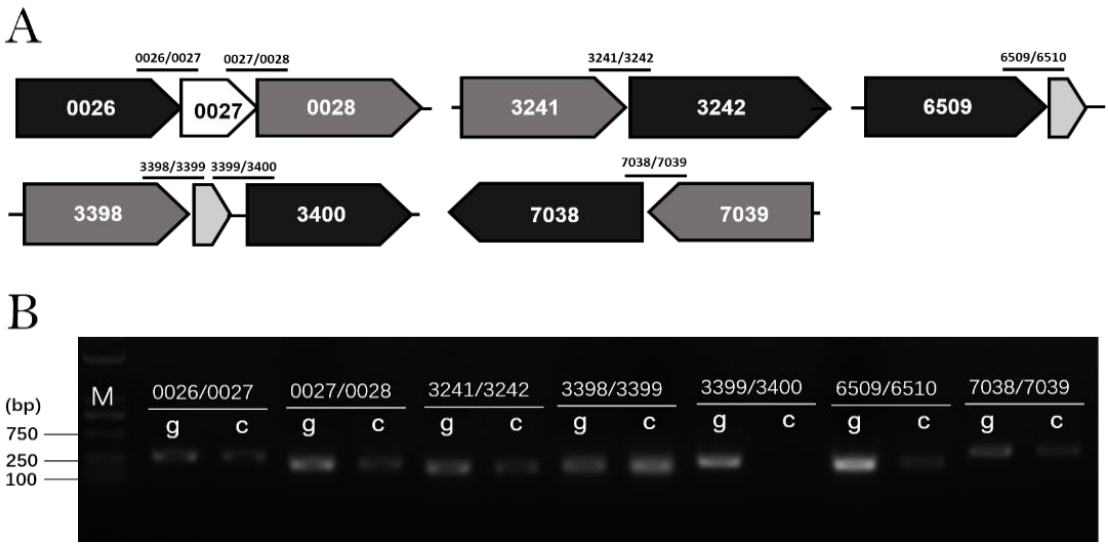

Figure S2. Identification of co-transcription of of *S. erythraea* acyl-CoA carboxylases.

A. Alignment of the selected acyl-CoA carboxylases locus. Primers were design cross subunit genomic DNA as indicated above the gene structure schematic.

B. Identification of the co-transcription of regions indicated in (A). Genomic DNA (g) was used as a positive control, and cDNA (c) was reverse transcribed from RNA extracted from WT cultured in TSB and subjected to semi-quantitative RT-PCR, PCR products were evaluated on 1% agarose gels.

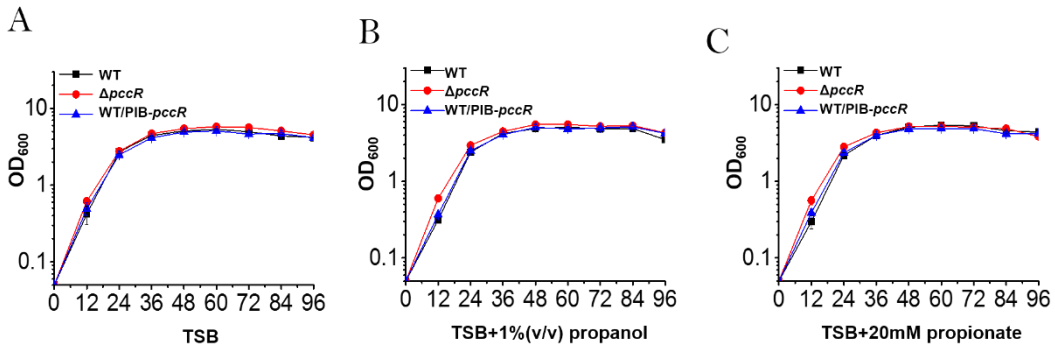

Figure S3. Growth curves of *S. erythraea* strains WT, Δ*pccD* and WT/PIB-*pccD* grown on TSB liquid media (A), TSB supplemented with 1% (v/v) propanol (B) and TSB supplemented with 20 mM propionate (C).

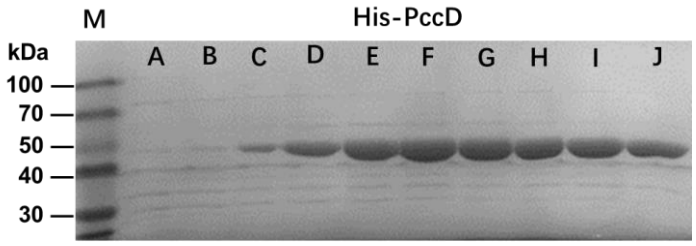

Figure S4. Purification of Histidine tagged PccD. His-PccD was purified by Ni-NTA Superflow column (Qiagen), and eluted with 5 mL buffer containing 250 mM imidazole. A-J refer to every 0.5 mL eluate collected constantly. The protein in fraction G, H and I were used for the gel shift assay.

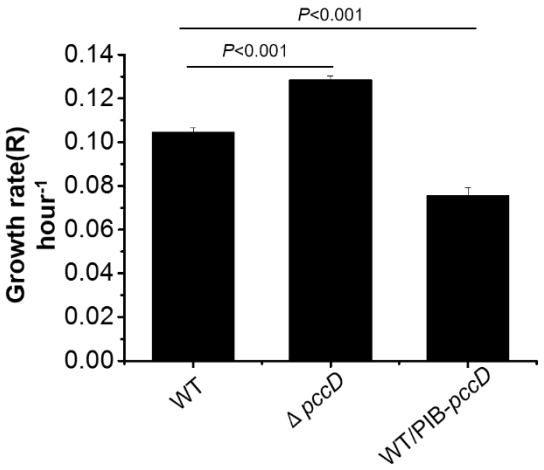

Figure S5. The growth rates of *S. erythraea* strains WT, Δ*pccD,* and WT/PIB-*pccD* grown on the Evans medium supplemented with 20 mM sodium propionate as the sole carbon source. Error bars indicate standard deviation from three biological replicates.

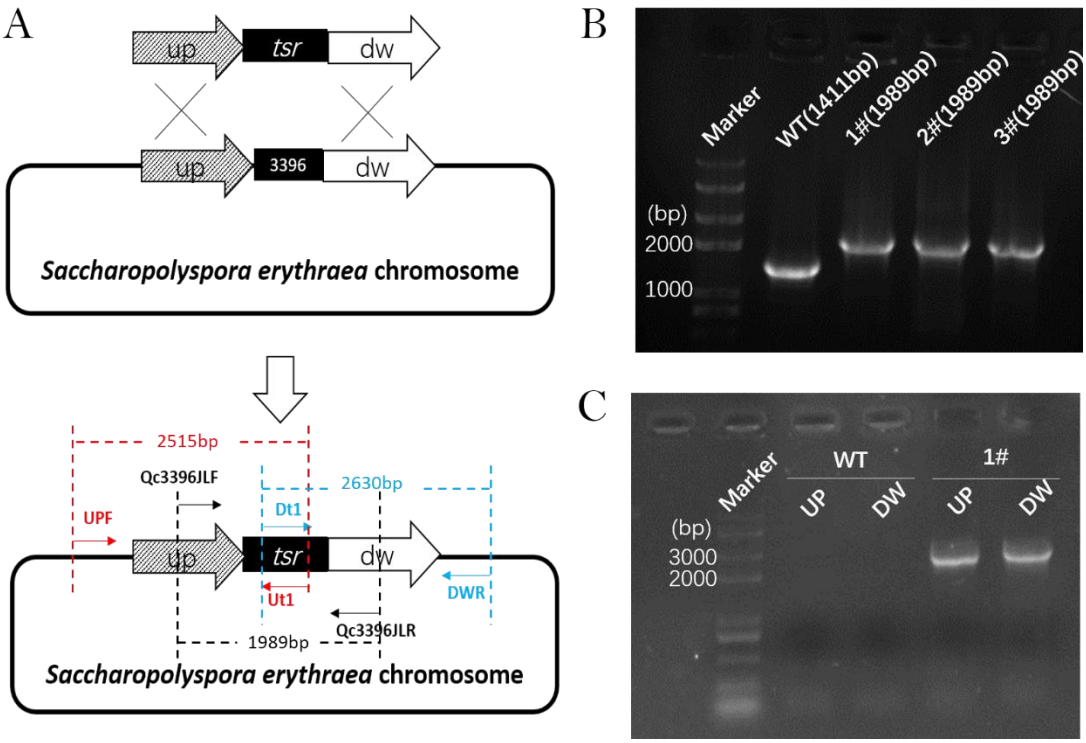

Figure S6. Identification of *pccD*-deleted mutant.

A. Stratergy of double crossover gene knock-out method for *pccD* (SACE_3396).

B. PCR screening of intergrant clonies using primer Qc3396JLF/R.

C. PCR screening of double-exchange clonies using primer UPF/Ut1 and primer Dt1/DWR.