

Supplementary Information

Identification of the first Riboflavin Catabolic Gene Cluster isolated from *Microbacterium maritopicum* G10

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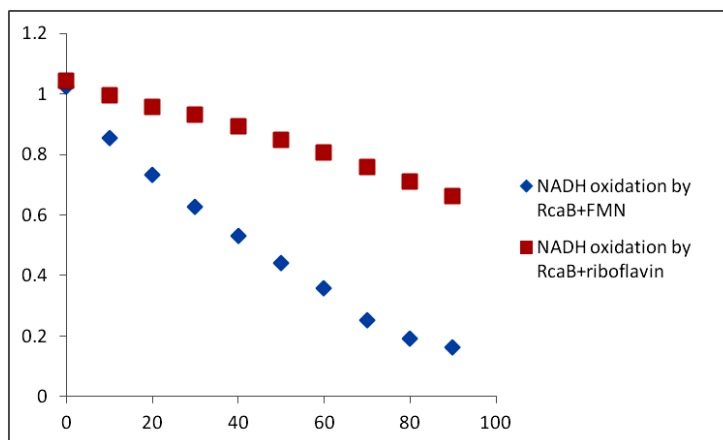


Figure S1: Comparative NADH oxidation rates by FMN (blue boxes) vs riboflavin (red boxes) in the presence of RcaB. The rate of reduction of FMN (shown by the decrease in absorbance of NADH at 340 nm) is ~ 10 times faster than the rate of reduction of riboflavin.

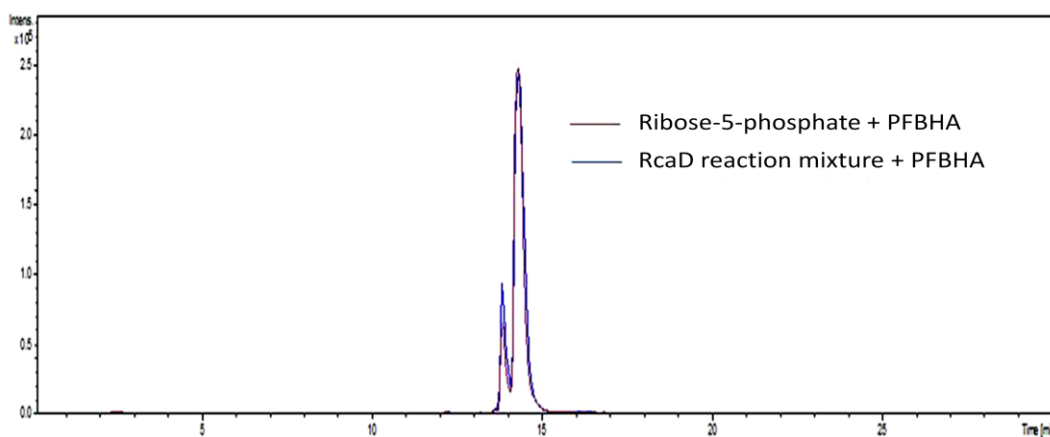


Figure S2: LC-MS trace showing the co-elution of the PFBHA oxime of the RcaD product with synthesized ribose-5-phosphate PFBHA oxime.

Table S1: Strains and plasmids used

Strain/plasmid	Relevant characteristics	Reference or source
<i>Actinomyces</i>		
<i>Microbacterium maritypicum</i> G10	Wild-type strain catabolizing riboflavin	This work
<i>Streptomyces lividans</i> 1326	Heterologous host for screening riboflavin catabolic gene cluster	1
<i>E. coli</i>		
Epi100™	Host cell for construction of genomic library	Epicentre
S17-1	TpR SmR <i>recA</i> , <i>thi</i> , <i>pro</i> , <i>hsdR</i> -M+RP4: 2-Tc:Mu: Km Tn7 λpir	3
DH5α	F- <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG</i> Φ80dlacZΔM15 Δ(<i>lacZYA-argF</i>)U169, <i>hsdR17</i> (r _K ⁻ m _K ⁺), λ-	Invitrogen
BL21(DE3)	F- <i>ompT gal dcm lon hsdS_B</i> (r _B ⁻ m _B ⁻) λ(DE3 [<i>lacI lacUV5-T7 gene 1 ind1 sam7 nin5</i>])	Novagen
plasmid		
pOJ446	<i>aac(3)IV</i> , SCP2, <i>rep</i> ^{PMB1*} , attΦC31, <i>oriT</i>	1
pSET152	<i>aac(3)IV</i> , <i>lacZ</i> , <i>rep</i> ^{PMB1*} , attΦC31, <i>oriT</i>	1

Table S2: Comparison of gene annotations between the two gene clusters

Genes from 5H7	Genes from 1A4	Protein homolog	Identity (%)
RcaA		Flavokinase <i>Arsenicococcus bolidensis</i>	
RcaB	RcaB2	Flavin reductase <i>Marine actinobacterium PHSC20C1</i>	132/160 83%
RcaC	RcaC2	PadR family transcriptional regulator <i>Salinibacterium sp. PAMC 21357</i>	185/194 95%
RcaD	RcaD2	Ribokinase <i>Llyobacter polytropus DSM 2926</i>	220/284 77%
RcaE	RcaE2	N5,N10-methylene tetrahydromethanopterin reductase <i>Bacillus sp. 10403023</i>	434/459 95%
RcaF	RcaF2	Transporter <i>Marine actinobacterium PHSC20C1</i>	269/282 95%
	RcaJ2	Hypothetical protein	
RcaG	RcaG2	ABC transporter ATP-binding protein <i>Marine actinobacterium PHSC20C1</i>	251/265 95%
RcaH		Phenol hydroxylase <i>Natronorubrum tibetense</i>	