

Supplemental Information
For
VapA of Rhodococcus equi Binds Phosphatidic Acid

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Table S1. Plasmids used in this study.

Plasmids	Description	Source
pHis-Parallel1	N-terminal His tag expression vector; Carb ^R	(Sheffield et al., 1999)
pGO35	yeast N-terminal GFP fusion expression plasmid	(Burd and Emr, 1998; Odorizzi et al., 1998)
pVapALMW	<i>vapA</i> (94-567) in pHis-Parallel1; Carb ^R	This Study
pVapGLMW	<i>vapG</i> in pHis-Parallel1; Carb ^R	This Study
pVapBLMW	<i>vapB</i> in pHis-Parallel1; Carb ^R	This Study
pVapK2LMW	<i>vapK2</i> in pHis-Parallel1; Carb ^R	This Study
pGFP-VapA	<i>vapA</i> in pGO35	This Study
pGFP-VapA ³²⁻¹⁸⁹	<i>vapA</i> (94-567) in pGO35	This Study
pGFP-VapG ²⁷⁻¹⁷²	<i>vapG</i> (79-519) in pGO35	This Study
pGFP-VapB ³⁵⁻¹⁹⁷	<i>vapB</i> (103-594) in pGO35	This Study
pGFP-VapK2 ³²⁻²⁰²	<i>vapK2</i> (94-609) in pGO35	This Study
pGFPmut2	constitutive GFP expression plasmid; Hyg ^R	(Burton et al., 2015)

Table S2. Primers used in this study.

Name	Sequence ¹	Plasmid Generated
VapA F (BamHI)	5'-GTAGGATCCGACCGTTCTTGATTCCGGTAG-3'	pVapALMW
VapA R (Sall)	5'-CTAGTCGACCCGGAAGTACGTGCAG-3'	pVapALMW
VapG F (EcoRI)	5'-GAATTCGCGGAACTTCAATGGTATCCAC-3'	pVapGLMW
VapG R (SpeI)	5'-CTTACTAGTGTGAGTGCGCCGTCC-3'	pVapGLMW
VapB F (EcoRI)	5'-GTTGAATTCGCGGCTGTGCTGGATTCC-3'	pVapBLMW
VapB R (Sall)	5'-CATGTCGACCTATGCGTTATGCAACCTC-3'	pVapBLMW
VapK2 F (EcoRI)	5'-GTTGAATTCGAACCGCTGGACGTTG -3'	pVapK2LMW
VapK2 R (SpeI)	5'-CCTACTAGTCGAACGTTATGTTCCGCC-3'	pVapK2LMW
GFP-VapA NSS F (BglII)	5'-ATGGATGAACTATAACAAGTCCGGACTC AGATCTATGACCGTTCTTGATTCCGGTAGC-3'	pGFP-VapA ³²⁻¹⁸⁹
GFP-VapA SS F (BglII)	5'-ATGGATGAACTATAACAAGTCCGGACT CAGATCTATGGTGAAGACTCTTCACAAGAC-3'	pGFP-VapA
GFP-VapA R (BglII)	5'-GTCGACTGCAGAATTCGAAGCTTGAG CTCGAGATCTCTAGGCGTTGTGCCAGCTAC-3'	pGFP-VapA ³²⁻¹⁸⁹ , pGFP-VapA
GFP-VapG NSS F (BglII)	5'-ATGGATGAACTATAACAAGTCCGGACTC AGATCTATGGGAACTTCAATGGTATCCAC-3'	pGFP-VapG ²⁷⁻¹⁷²
GFP-VapG R (BglII)	5'-GTCGACTGCAGAATTCGAAGCTTGA GCTCGAGATCTCTATTGCCACCCTCCGGTTC-3'	pGFP-VapG ²⁷⁻¹⁷²
GFP-VapB NSS F (BglII)	5'-ATGGATGAACTATAACAAGTCCGGACT CAGATCTATGGCTGTGCTGGATTCCGGAGGC-3'	pGFP-VapB ³⁵⁻¹⁹⁷
GFP-VapB R (BglII)	5'-GTCGACTGCAGAATTCGAAGCTTGAGC TCGAGATCTCTATTATGCAACCTCCCAGTTG-3'	pGFP-VapB ³⁵⁻¹⁹⁷

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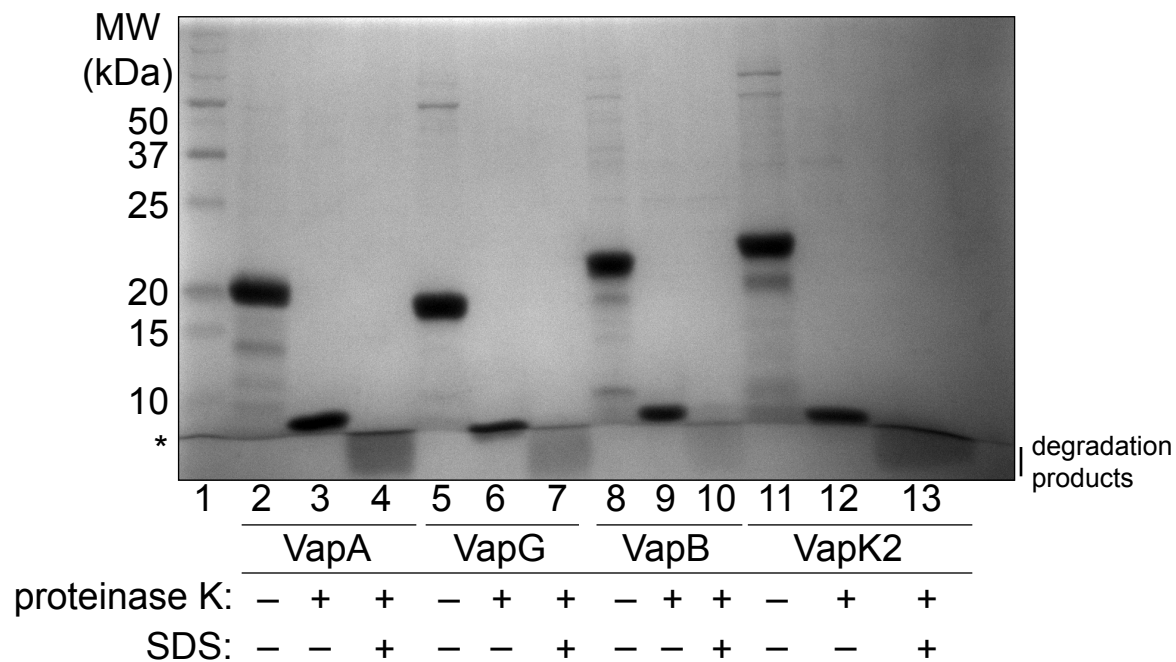


Figure S1. Proteinase K digestion of each recombinant Vap shows a stable protein core. 3 μ g of indicated recombinant Vap protein was incubated with or without Proteinase K (500:1 protein:Proteinase K molar ratio) and, where indicated, 1% SDS for 75 min at 37 $^{\circ}$ C. Reactions were separated via SDS-PAGE and stained with Coomassie Brilliant Blue. The asterisk (*) indicates the dye front of the gel.