0							
Primer name	Sequence	Reference					
Oligonucleotides $(5' \rightarrow 3')$ used to amplify <i>etnE</i> sequences							
CoM-F1L	AACTACCCSAAYCCSCGCTGGTACGAC	(1, 2)					
CoM-R2E	GTCGGCAGTTTCGGTGATCGTGCTCTTGAC	(1, 2)					
JS623RTAkMOF	TCACTTCTGGCACCAACATC	This study					
JS623RTAkMOR	GACGACCCACTCTTCCCATA	This study					
JS623WEtnEF	TAGCCTGGCAGCTGGACTAT	This study					
JS623WEtnER	CAGTCGCGACATCACAGATT	This study					
JS623PMDF	GGCGTTCAAAGAGGACTTCA	This study					
JS623PMDR	TTCGGTGATCGTTGATTTGA	This study					
		-					
Oligonucleotides $(5' \rightarrow 3')$ used for primer walking on fosmid clones							
JS623EtnAF	TTTGGGAACCAATCGAAGAT	This study					
JS623EtnBR	AACTGGCATGACTGGAGAGC	This study					
JS623EtnDF	CGGATTTAGCGGTGGATGTA	This study					
JS6231F	CGTGGACGAAGGAACTGATA	This study					
JS6232F	CGTGAACTGGAAAACGAACA	This study					
JS6233R	TCGCTGAGTTCGTTCATGTC	This study					
JS6234F	ACAGTCTGGCGACACTCAAC	This study					
pCC1FOS5F	GGATGTGCTGCAAGGCGATTAAGTTGG	Epicentre					
pCC1FOS5R	CTCGTATGTTGTGTGGAATTGTGAGC	Epicentre					

Table S1. Oligonucleotides $(5' \rightarrow 3')$ used in this study

			Zn	243	257
		200		V	▼
EtnE-JS614	:	WEIEVLNAAVEGVS	SQIWVHTCWGNYSGTP	GY FPDDEDT EFGAWVLD <mark>H</mark> RI	PTDAPAPOAAVP
EtnE-B276	:	WEIEILNKAVEGVD	C <mark>Q</mark> FWVHTCWGNYSGTP	AYLP <mark>DDEE</mark> REFGAWVLD <mark>H</mark> RI	PTDAPAPHGAEA
EtnE-623a	:	WEV <mark>D</mark> AINYAMEGIS	CDFWVHTCWGNYSGTP	GYLPEENEKEFGA <mark>G</mark> VLDKR	SHSAGTERAAA
EtnE-623c	:	WEV <mark>D</mark> AINYAMEGIS(C <mark></mark> DFWVHTCWGNYSGTP	GYLPEENEKEFGA <mark>r</mark> vldkr:	ISHSAGTERAAA
EtnE-623b	:	WEV <mark>D</mark> AINYAMEGIS(C <mark></mark> DFWV <mark>HTCWGNY</mark> SGTP	GYLPEENEKEFGAWVLDKR	ISHSAGTE <mark>L</mark> AAA
EtnE-623e	:	WEV <mark>D</mark> AINYAMEGIS(C <mark></mark> DFWVHTCWGNYSGTP	GYLPEEN <mark>E</mark> KEFGA <mark>G</mark> VLDKR'	ISHSAGTE <mark>l</mark> aaa
EtnE-623d	:	WEV <mark>D</mark> AINYAMEGIS(C <mark></mark> DFWVHTCWGNYSGTP	GYLPEENEKEFGAW <mark>G</mark> LDKR'	ISHSAGTERAAA
EtnE-623wt	:	WEV <mark>D</mark> AINYAMEGIS(C <mark></mark> DFWV <mark>HTCWGNY</mark> SGTP	GYLPEENEKEFGAWVLDKR	ISHSAGTERAAA
EtnE-624	:	WEVEAINFAMEGIS(C <mark></mark> DFWV <mark>HTCW</mark> GN <mark>Y</mark> SGTP	GYLPEEN <mark>E</mark> KEFGAWVLDKR:	ISHSAGTERAAA
EtnE-625	:	WEVEAINFAMEGIS(C <mark></mark> DFWV <mark>HTCWGNY</mark> SGTP	GYLPEEN <mark>E</mark> KEFGAWVLDKR:	ISHSAGTERAAA
EtnE-619	:	WEVEAINFAMEGIS(C <mark></mark> DFWV <mark>HTCWGNY</mark> SGTP	GYLPEEN <mark>E</mark> KEFGAW <mark>A</mark> LDKR'	ISHSAGTERAAA
EtnE-617	:	WEVEAINFAMEGIS(C <mark></mark> dfwv <mark>hacw</mark> gn <mark>y</mark> sgtp	GYLPEENEKEFGAWVLDKR:	ISHSAGTERAAA
EtnE-616	:	WEVEAINFAMEGIS(C <mark></mark> dfwv <mark>hacw</mark> gn <mark>y</mark> sgtp	GYLPEEN <mark>E</mark> KEFGAWVLDKR:	ISHSAGTERAAA
EtnE-622	:	weveainy <mark>a</mark> mdgis(C <mark></mark> DFWV <mark>HTCW</mark> GN <mark>Y</mark> SGTP	GYLPEEN <mark>E</mark> KEFGAWVLDKR:	ISHSAGTERAAA
EtnE-61	:	WEVEAINYAMEGIS(C <mark></mark> DFWV <mark>HTCW</mark> GN <mark>Y</mark> SGTP	GYLPEEN <mark>E</mark> KEFGAWVLDKR:	ISHSAGTE <mark>C</mark> AAA
EtnE-AJ	:	WEVEAINYAM <mark>D</mark> GIS(C <mark></mark> DFWVHTCWGNY <mark>A</mark> GTP	GYLPEEN <mark>D</mark> KEFGAWVLDKR:	IS <mark>E</mark> SA <mark>QP</mark> ERAAA
EtnE-621	:	WEVEAINY <mark>A</mark> MDGIS(C <mark></mark> DFWVHTCWGNYSGTP	GYLPEEN <mark>D</mark> KEFG <mark>V</mark> WVLDKR:	IS <mark>E</mark> SA <mark>QP</mark> ERAAA
EtnE-60	:	WEVEAINYAM <mark>d</mark> gis(C <mark></mark> DFWV <mark>HTCW</mark> GN <mark>Y</mark> SGTP	GYLPEEN <mark>D</mark> KEFGAWVLDKR'	IS <mark>E</mark> SA <mark>QP</mark> ERA <mark>P</mark> A
EtnE-TD	:	weveainy <mark>a</mark> mdgis(C <mark></mark> DFWV <mark>HTCW</mark> GN <mark>Y</mark> SGTP	GYLPEEN <mark>D</mark> KEFGAWVLDKR:	IS <mark>E</mark> SA <mark>QP</mark> ERAAA
XecA-Py2	:	WAIEAFNAAVDGVKI	N-AKIIA <mark>HV</mark> CWGNWGGT P	AYYPDETAASGEIFDLTKR	KAEATKATATGS
MetE-Mlepr	:	MANKAFRLATSGVA	DSTQIHT <mark>H</mark> L <mark>CY</mark> SEFGEVI	GATADLDAD	
MetE-Ecoli	:	ØGVEAFRINAAVAKI	ddtqiht <mark>h</mark> m <mark>cy</mark> cefndim	DSIAALDRD	
MetE-Therm	:	MAINAFNL <mark>A</mark> AN-AR	PETQIHA <mark>H</mark> MCYSDFNEII	EYI <mark>HQLEFD</mark>	
		297		Zn	
EtnE-JS614		GTTDVKSTVTETADI	VADRTRAVLEEVPAERL	GISTOCGLINIPRMISAGKI	RALADGAATVR
EtnE-B276	÷	GVVDVKSTVTETADI	VADRTRAVLEYVPADRL	GLSTDCGLTNLPRMTAASKI	RALADGAATVR
EtnE-623a	÷	GVIDVKSTITETAD	VADRIRSVLEFVPAEOL	ALSTDCGLINLNRMISASKI	HALADGAAIVR
EtnE-623c	÷	GVIDVKSTITETADI	VADRIRSVLEFVPAEOL	ALSTDCGLINLNRMISASKI	HALADGAATVR
EtnE-623b		GVIDVKSTITETAD	VADRIRSVLEFVPAEOL/	ALSTDCGLINLNRMISASKI	HALADGAAIVR
EtnE-623e	÷	GVIDVKSTITETAD	VADRIRSVLEFVPAEOL	ALSTDCGLINLNRMISASKI	HALADGAAIVR
EtnE-623d	÷	GVIDVKSTITETAD	SVADRIRSVLEFVPAEOL	ALSTDCGLINLNRMISASKI	HALADGAAIVR
EtnE-623wt	:	GVIDVKSTITETADE	VADRIRSVLEFVPAEOL	ALSTDCGLINLNRMISASKI	HALADGAAIVR
EtnE-624	:	GVID			
EtnE-625	:	GVID			
EtnE-619	:	GVID			
EtnE-617	:	GVIDVKSTITETAD-			
EtnE-616	:	GVIDVKSTITETAD-			
EtnE-622	:	GVID			
EtnE-61	:	GVIDVKSTITETAD-			
EtnE-AJ	:	GVIDVKSTITETAD-			
EtnE-621	:	GVIDVKSTITETAD-			
F+ nF_60		GVIDVKSTITETAD-			
6CH6-00	:				
EtnE-TD	:	GVIDVKSTITE <u>TAD</u> -			
EtnE-TD XecA-Pv2	:	GVIDVKSTITETAD- GVIDVKS <u>TITETAD</u> I	VANRIRRLLEIVPADRL	GVTTDCGLILLQRYIAODKI	HALVEGTKIVR
EtnE-TD XecA-Py2 MetE-Mlepr	::	GVIDVKSTITETAD GVIDVKSTITETAD GVYDIHSPRVPSTDI	VANRIRRLLEIVPADRL SIAKSLRAALKAIPMORL	GVTTDCGLILLQRYIAQDKI WVNPDCGLKTRSVDEVSASI	JALVEGTKIVR JONMVAAAROVR
EtnE-TD XecA-Py2 MetE-Mlepr MetE-Ecoli	: : : : : : : : : : : : : : : : : : : :	GVIDVKSTITETAD GVIDVKSTITETAD GVYDIHSPRVPSTDI GVYDIHSPNVPSVEI	VANRIRRLLEIVPADRL SIAKSLRAALKAIPMQRL VIEALLKKAAKRIPAERLU	GVTTDCGLILLQRYIAQDKI WVNPDCGLKTRSVDEVSASI WVNPDCGLKTRGWPETRAAI	HALVEGTKIVR JONMVAAAROVR JANMVQAAQNLR

Figure S1. Regions from an alignment of zinc-containing transferases EtnE, XecA, and MetE, numbered according to the JS623 EtnE enzyme. The His-Cys-Cys motif involved in zinc binding corresponds to H219-C221-C337 in JS623. Also indicated are the W243 and R257 residues predicted to be important for VC adaptation in JS623. EtnE, XecA, and MetE sequences are identified according to their source strain (*Nocardioides* JS614, *Gordonia* B-276,

Mycobacterium JS623, JS624, JS625, JS619, JS617, JS616, JS622, JS61, JS621, and JS60, *Pseudomonas* AJ, *Ochrobactrum* TD, *Xanthobacter* Py2, *Mycobacterium leprae* (Mlepr), *E.coli*, and *Thermotoga*). Other abbreviations: wt: wild-type, a-e identify JS623 EtnE variants.



Figure S2: Ethene (•) biodegradation and epoxyethane (\blacktriangle) accumulation in recombinant JS623 strains A) JS623(pMV*etnE*), B) JS623(pMV*etnE1*), and C) JS623(pMV*etnE2*). The data points are the averages from analysis of three replicate bottles and the error bars are the standard deviation. In some cases, the error bars are smaller than the symbols. The behavior of JS623(pMV*etnE1*) JS623(pMV*etnE2*) cultures was confirmed in at least two independent experiments.

References

- 1. **Coleman, N. V., and J. C. Spain.** 2003. Distribution of the coenzyme M pathway of epoxide metabolism among ethene- and vinyl chloride-degrading *Mycobacterium* strains. Appl. Environ. Microbiol. **69:**6041-6046.
- 2. **Mattes, T. E., N. V. Coleman, J. C. Spain, and J. M. Gossett.** 2005. Physiological and molecular genetic analyses of vinyl chloride and ethene biodegradation in *Nocardioides* sp. strain JS614. Arch. Microbiol. **183:**95-106.