Table S1. Primers used in this study					
SOE					
5'F1	5'-TATACTTGGTGCGGTTGCTGCT-3'				
5'R1-SOE	5'-TCTTGCCAAGCACGCAGCGCATATTCTTCCATTGC-3'				
3'R1	5'-ATTCGCAAGCATCGTCACTTGGTC-3'				
3'F1-SOE	5'-ATATGCGCTGCGTGCTTGGCAAGAAATAAATGCGAAAGAC-3'				
QRT-PCR					
RpoA F	5'-TGCTTCTTCTGGGCTGATACTTCC-3'				
RpoA R	5'-TGCAAGGTCCTGGTGTAGTAACTG'3'				
LpIA1 F	5'-TGTCATCGTTGTGCGCAGACTTTC-3'				
LpIA1 R	5'-ACGCCTAAACGTTTCAGAGCTTCC-3'				
LplA2 F	5'-ATCGACGTTCTCCGCCGTTTATCT-3'				
LplA2 R	5'-AGCATTTACACCAAGCTTGCGGAG-3'				
Complementation					
EcLplA F	5'-GCCCATGGCATCCACATTACGCCTGCTCA-3'				
EcLplA R	5'-CCCGTCGACTCAGTGGTGGTGGTGGTGGTGGTGCCTTACAGCCCCCGC-3'				
LmLplA1 F	5'-GCCCATGGCATATTTTATAGATAACAATAATGAGAAAGATCCA-3'				
LmLplA1 R	5'-				
	CCCGTCGACTCAGTGGTGGTGGTGGTGGTGATAAAGTAAATCTAAAAATT				
	CATCTTTAGTAAT-3'				
LmLplA2 F	5'-GCTCATGACAATTTATTTAGATAACGAAGATGTACTTGAT-3'				
LmLplA2 R	5'-				
	CCCCTCGAGTCAGTGGTGGTGGTGGTGGTGTTCAAATAACATATCTAAAA				
	TTGCTTCTTT-3'				

Figure S1. Amino Acid Alignment of Lipoate Ligases. The predicted protein sequences of LpIA1 and LpIA2 align with 47% identity and 72% similarity using the Needleman-Wunsch global alignment (EMBOSS). For comparison, LpIA from E. coli NP_418803) Streptococcus pneumoniae TIGR4 (Accession and (accession Np_345629.1) are aligned as well. Stars indicate identity, while two dots indicate greater similarity than one dot. Microbial and human lipoyl transferases share three signature sequence motifs, identified by grey outline boxes: RRXXGGGXV(F/Y)HD (residues 68-79), KhXGXA (residues 131-136), and HXX(L/M)LXXX(B/N)LXXLXXhL (residues 147-163) (Kim et al., 2005). Highly conserved residues that line the surface of the lipoyl-AMP binding pocket in Thermoplasma acidophilum LplA (SWISS-PROT accession code: Q9HKT1) are identified by dark boxes behind each amino acid (Kim et al., 2005).

Figure S2. Analysis of Synthetic DK^LA and Digestion Products. (A) HPLC chromatogram of synthetic lipoyl tripeptide DK^LA eluting from a C18 column on a HP1090 instrument (Konishi et al., 1996). The only peak eluted at 10.89 min and contained 100% of the area, indicating there were no byproducts from synthesis that were not pure DK^LA . (B) LC-MS mass spectrum of Anaspec synthesized DK^LA injected onto a C18 column. DK^LA exhibited a peak at 521.7 Da (C) LC-MS mass spectrum of aminopeptidase M digested DK^LA injected onto a C18 column and analyzed in positive ion mode on a nanoAcuity/Qtof premier instrument. DK^LA exhibited a peak at 521.2381 Da, DK^L exhibited a peak at 450.1994 Da, and K^L exhibited a peak at 335.1571 Da.

Figure S3. Heterologous Expression of *L. monocytogenes* **Lipoate Ligases in** *E. coli*. *E. coli* TM131 transformed with an empty IPTG inducible vector or vector containing a C-terminal histidine-tagged *E. coli* LplA, *L. monocytogenes* LplA1 or LplA2. Strains were grown in LB with 1mM IPTG. Bacterial pellets were lysed with SDS-PAGE buffer, boiled and analyzed by SDS-PAGE, followed by immunoblot with **A**) an anti-his antibody and **B**) an anti-lipoic acid antibody. The bands appearing at 37kDa on the anti-histidine blot correspond to the predicted size of *E. coli* LplA and *L. monocytogenes* LplA1, and the 75kDa band in the anti-lipoic acid blot corresponds with lipoylated E2-PDH from *E. coli*.

Figure S1

Lm	LplA1	MYFI	DNNNEKDPRINLAVEEFILTELN	LDEPVLLFYINKPSIII <mark>G</mark> RNQN	49	
Lm	LplA2	MIYLI	DNEDVLDQAYNFAMEEYALRSLD	ENETYFMFYRMKPTIIV <mark>G</mark> KNQN	49	
Sp	LplA	XGSDKIHHHHHHXKY	IINHSNDTAFNIALEEYAFKHLL	DEDQIFLLWINKPSIIV <mark>G</mark> RHQN	60	
Ec	LplA	MSTLRL	LISDSYDPWFNLAVEECIFRQMP.	ATQRVLFLWRNADTVVIGRAQN	51	
			* *:*:** : :	: :::: :::*: **		
Lm	LplA1	TVEEIDTEYVEKNDV	IVVR <mark>RLS</mark> GGG <mark>AVYH</mark> DEG <mark>N</mark> LNFSF	ITEDDGESFHNFAKFTQPIVEA	109	
Lm	LplA2	TLEEINHPFVKDHHII	dvlrrlsgggavyndegnisfsm	ITKDDGNSFQNFAKFTEPVIRA	109	
Sp	LplA	TIEEINRDYVRENGI	EVVRRISGGGAVYHDLNNLNYTI	ISKEDENKAFDFKSFSTPVINT	120	
Еc	LplA	PWKECNTRRMEEDNVI	RLAR <mark>RSSGGGAVFHD</mark> LG <mark>N</mark> TCFTF	MAGKPEYDKTISTSIVLNA	108	
		. :* : : :	: ** *****::* .* :::	:: . : : : : . :		
Ьm	LplA1	LKRLGVNAELKGRND	LLIDGF <mark>K</mark> VSGN <mark>A</mark> QFATKGK	MFSHGTLMYDLNLDNVAASIKP	165	
Lm	LplA2	LRKLGVNAELSGRND	IEVNGK <mark>K</mark> ISGN <mark>A</mark> QFATKGR	LYS <mark>H</mark> GTLLFDVDLSM <mark>LEKA</mark> LQV	165	
Sp	LplA	LAQLGVKAEFTGRND	LEIDGK <mark>K</mark> FCGN <mark>A</mark> QAYINGR	IXHHGCLLFDVDLSVLANALKV	176	
Еc	LplA	LNALGVSAEASGRND	LVVKTVEGDR <mark>KVSGS</mark> AYRETKDR	GFH <mark>HGTLLLNADLSRLANYL</mark> NP	168	
		* ***.** .****	: :. **.* :.:	** *: : :*. : *:		
Lm	LplA1	RKDKIESKGIKSVRSI	R <mark>V</mark> ANISDFMDQEMTTEEFRDLLL	LYIFGVEKVEDVKEYKLTAADW	225	
Lm	LplA2	DPEKYLSKGVKSVRSI	R <mark>V</mark> TTIREHLAEDIDILTFKQILL	ESIFETKDIPRYTFTEADK	222	
Sp	LplA	SKDKFESKGVKSVRA	R <mark>V</mark> TNIINELPKKITVEKFRDLLL	EYXKKEYPEXTEYVFSEEEL	234	
Еc	LplA	DKKKLAAKGITSVRSI	R <mark>V</mark> TNLTELLPGITHEQVCEAITE	AFFAHYGERVEAEIISPNKTPD	228	
		.* :**:.***:	**:.:::: .::	: : .		
Lm	LplA1	EKIHEISAKRYGNWD	WNYGKSPKFDLTRTKRFPVGAVD	VRLNVQKGVITDIKIFGDFFGV	285	
Lm	LplA2	QGIEKLRTERYRNWD	WTYGKSPKATIKRKKRFPAGTIE	FQVSLEKGQVKEATIYGDFFGT	282	
Sp	LplA	AEINRIKDTKFGTWD	WNYGKSPEFNVRRGIKFTSGKVE	VFANVTESKIQDIKIYGDFFGI	294	
Еc	LplA	LPNFAETFARQSSWE	WNFGQAPAFSHLLDERFTWGGVE	LHFDVEKGHITRAQVFTDSLNP	288	
		: .*:	*.:*::* :*. *::	: :. : :: * :.		
Lm	LplA1	KNVADIEEKLVNTTY	KREVLAEALVDIDVKEYFGNITK	DEFLDLLY 331		
Lm	LplA2	EDVAELAEKIIGCRFERKSIQNAWQEINAKDYFGGIEKEAILDMLFE 329				
Sp	LplA	EDVAAVEDVLRGVKYEREDVLKALKTIDITRYFAGISREEIAEAVVG 341				
Еc	LplA	APLEALAGRLQGCLYRADMLQQECEALLVDFPEQEKELRELSAWMAGAVR 338				
: : : . : : : : : : :						
Lm	LplA1	vs Lm LplA2	Lm LplA1 vs Ec LplA	Lm LplA2 vs Ec LplA		
Ide	entity	: 46.8%	Identity: 27.7%	Identity: 22.5%		
Sir	nilarit	cy: 70.9%	Similarity: 49.5%	Similarity: 40.7%		
-						
Sn						
	LplA v	vs EC LpIA	Lm LplAl vs Sp LplA	Lm LplA2 vs Sp LplA		
Ide	LplA v entity:	7 5 EC LpIA : 22.4%	Lm LplAl vs Sp LplA Identity: 45.6%	Lm LplA2 vs Sp LplA Identity: 43.8%		

Figure S2





640

560

Mass (m/z)

720

Figure S3

