Supporting Information

Genome mining, heterologous expression, antibacterial and antioxidant activities of lipoamides and amicoumacins from compost-associated *Bacillus subtilis* fmb60

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Table S1. Strains and plasmids in this study

Name	Description	
E. coli strains		
GB2005	F-mcrA Δ (mrr-hsdRMS-mcrBC) φ 80lacZ Δ M15 Δ lacX74 recA1 endA1 araD139 Δ (ara, leu) 7697 galU galK λ rpsL nupG fhuA::IS2 recET red α , phage T1-resistant	
GB05-dir	GB2005, araC-BAD-ΕΤγΑ	
	(GB2005, mtaA-genta) a pPant transferase gene from myxobacterium Stigmatella aurantiaca	
GB05-MtaA	DW4/3-1 was randomly transposed into the chromosome for expression of secondary metabolite	
GB05-MtaA-ami	Plasmid in <i>E. coli</i> GB05-MtaA, for expressiong of NRPS/PKS gene cluster, Cm ^R	
GB05-MtaA-ami-ace	GB05-MtaA- <i>ami-ace</i> Plasmid in <i>E. coli</i> GB05-MtaA, for expressiong of NRPS/PKS and <i>ace</i> gene cluster, Cm ^R	
Plasmids		
p15A-cm-tet ^R -ccdB-hyg	PCR template to generate a linear vector for direct cloning	(23)
p15A-cm-ami	recombinant plasmid with entire ami gene cluster	This study
pBR322-apra-OriT	PCR template to generate a linear vector for <i>ace</i> gene cluster cloning	Lab stored
pBR322- <i>apra-ace</i> recombinant plasmid with entire <i>ace</i> gene cluster		This study

Table S2. List of primers used in this study

Primer	Sequence (5'-3')	Description	
	<u>GCTGTAATCTCATGTAAAGCAGGAGAAACGCCGCCCA</u>		
YM-ami-5	GCTGGGTAAAAGCAACGATCGGCACAGATCCGAAAA	Clone ami gene cluster	
	CCCCAAGTTACG		
	CACGACTTCCGGAATGATATCGCCCGCTTTTTTGATGA		
YM-ami-3	CGACCTTATCCAAAATCCGAATGTTTCGGCCTTGAATT	Clone ami gene cluster	
	GATCATATGC		
ace-pBR322-HAF	<u>CAAATTGTCAACAGACAAGAACTAAAGAGAGTGATG</u>	Clone ace gene cluster	
	CAGGTACATGAACGCTCAGTGGAACGAGGT		
ace-pBR322-HAR	CTGATTTTGCGAAAGATATCGAAACAACTGAAGGAAG	Clone <i>ace</i> gene cluster	
	CCCTGAATAACGCGTTGCTGGCGTTTTTC		
ace-F	TTATTCAGGGCTTCCTTCAGT	Clone ace gene cluster	
ace-R	TCATGTACCTGCATCACTCT	Clone ace gene cluster	

Clusters	Synthetase type	Most similar known cluster	Location	MIBiG BGC-ID
Clusters	Synthetase type		Location	
Cluster 1	Microcin		5271883567	
Cluster 2	NRPS	Surfactin biosynthetic gene cluster (78% genes show similarity)	255220320611	BGC 0000433_c1
Cluster 3	Microcin		521848541996	
Cluster 4	NRPS/Type I PKS	xenocoumacin biosynthetic gene cluster (28% genes show similarity)	592175673526	BGC0001054_c1
Cluster 5	Type I PKS	Bacillaene biosynthetic gene cluster (59% genes show similarity)	854869954351	BGC0001089_c1
Cluster 6	Terpene			
Cluster 7	NRPS	Fengycin biosynthetic gene cluster (100% of genes show similarity)	18446561928055	BGC 0000407_c1

Table S3. Secondary Metabolite Biosynthetic Gene Clusters Identified by AntiSMASH

Cluster 8	Terpene		19956302017528	
Cluster 9	Type III PKS		20641932105290	
Cluster 10	Microcin		29142532934401	
Cluster 11	NRPS	Bacillibactin biosynthetic gene cluster (92% genes show similarity)	30500573099798	BGC 0000309_c1
Cluster 12	Thiopeptide		31172663146494	
Cluster 13	Sactipeptide	Subtilosin A biosynthetic gene cluster (87% of genes show similarity)	36515703673181	BGC0000602_c1
Cluster 14	Other	Bacilysin biosynthetic gene cluster (100% of genes show similarity)	36881603729578	BGC0001184_c1
Cluster 15	Microcin		40564154076563	
Cluster 16	Microcin		40769574097105	
Cluster 17	Microcin		41374094150267	

Protein	Size (aa)	Proposed function	Accession No	Identity (%)
Orf 1	397	Putative MFS family major facilitator transporter (WP_003240131.1)	OEI73252.1	98
AmiA	1498	Amino acid adenylation domain-containing protein (WP_003240128.1)	OEI73253.1	96
AmiB	501	β-lactamase (WP_003240126.1)	OEI73254.1	93
AmiC	327	Hypothetical protein (WP_003240124.1)	OEI73255.1	95
AmiD	233	Thioesterase (WP_003240123.1)	OEI73256.1	97
AmiE	284	3-hydroxybutyryl-coA dehydrogenase (WP_003240121.1)	OEI73257.1	98
AmiF	353	Methoxymalonyl-ACP biosynthesis protein (WP_003240119.1)	OEI73258.1	98
AmiG	89	Acyl carrier protein (WP_003240117.1)	OEI73259.1	100
AmiH	380	Acyl-CoA dehydrogenase (NADP(+)) (WP_003240115.1)	OEI73260.1	98

Table S4. Proteins encoded by *ami* cluster and open reading frames adjacent to *ami* cluster as well as their proposed function and size

AmiI	3032	Nonribosomal peptide synthetase-polyketide synthase hybrid (WP_003240114.1)	OEI73261.1	95
AmiJ	889	Nonribosomal peptide synthetase subunit (WP_003240112.1)	OEI73262.1	94
AmiK	1507	Putative polyketide synthase PksJ (PKS) (WP_003240111.1)	OEI73263.1	95
AmiL	2517	Polyketide synthase subunit (WP_003240108.1)	AFI27291.1	96
AmiM	2142	Polyketide synthase subunit (WP_003240106.1)	OEI73264.1	96
AmiN	333	Putative kinase (WP_003240104.1)	OEI73265.1	98
AmiO	459	Alkaline phosphatase (WP_003240102.1)	OEI73266.1	96
Orf 2	230	Membrane component (WP_003240098.1)	OEI73267.1	99





Figure S4. COSY spectrum of 1 in methanol-*d*₄.





Figure S6. HMBC spectrum of 1 in methanol-*d*₄.



Figure S7. ¹H-NMR spectrum of **4** in methanol- d_4 .



Figure S8. ¹³C-NMR spectrum of **4** in methanol- d_4 .







Figure S10. HSQC spectrum of 4 in methanol-*d*₄.







Figure S12. NOESY spectrum of 4 in methanol-*d*₄.



Figure 14. ¹³C-NMR spectrum of **5** in methanol- d_4 .







Figure S16. HSQC spectrum of 5 in methanol-*d*₄.



Figure S17. HMBC spectrum of **5** in methanol-*d*₄.



Figure S18. NOESY spectrum of 5 in methanol-*d*₄.



Figure S19. (A) Digestion map of NRPS/PKS gene cluster. M: DL15000 DNA Marker, 1,2: plasmid p15A-*cm-ami* digested by restriction enzyme *AflIII*. (B)
Digestion map of *ace* gene cluster. M: DL5000 DNA Marker, 1,2,3: plasmid p15A-*apra-ace* digested by restriction enzyme *Ava* I and *Eco* RV.