

The σ^{54} System Directly Regulates Bacterial Natural Product Genes

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Supplementary Table S1. Bacterial strains and plasmids used in the current study

Bacterial Strains	Relevant characteristics	Source or reference
Strains		
<i>M. xanthus</i>		
DK1622	Wild type	Lab collection
MM201	$\Delta nla28$ derivative of DK1622	This study
<i>E. coli</i>		
Top 10	Cloning host;	Invitrogen
BL21(DE3)	Nla28-DBD protein expression host;	New England Biolabs
Plasmids		
pCR 2.1 TOPO	Cloning vector; Km ^r	Invitrogen
pREG1727	Vector containing promoterless <i>lacZ</i> ; Km ^r	Lab collection
pSWV22	Vector used for <i>nla28</i> inactivation; Tet ^r	Lab collection
pMAL-c5x	Maltose-binding protein (MBP) fusion vector for Nla28-DBD protein expression; Amp ^r	New England Biolabs
pMM101	WT MXAN1286 promoter in pREG1727; Km ^r	This study
pMM101a	-12 region mutant MXAN1286 promoter in pREG1727; Km ^r	This study
pMM101b	-24 region mutant MXAN1286 promoter in pREG1727; Km ^r	This study
pMM101c	-12/24 spacer mutant MXAN1286 promoter in pREG1727;	This study
pMM101d	Half Nla28 EBP binding site mutant MXAN1286 promoter in pREG1727; Km ^r	This study
pMM102	WT MXAN1579 promoter in pREG1727; Km ^r	This study
pMM102a	-12 region mutated MXAN1579 promoter in pREG1727; Km ^r	This study
pMM102b	-24 region mutated MXAN1579 promoter in pREG1727; Km ^r	This study
pMM102c	-12/24 spacer mutated MXAN1579 promoter in pREG1727;	This study
pMM102d	Half Nla28 EBP binding site mutated MXAN1579 promoter in pREG1727; Km ^r	This study
pMM103	WT MXAN1603 promoter in pREG1727; Km ^r	This study
pMM103a	-12 region mutated MXAN1603 promoter in pREG1727; Km ^r	This study
pMM103b	-24 region mutated MXAN1603 promoter in pREG1727; Km ^r	This study
pMM103c	-12/24 spacer mutated MXAN1603 promoter in pREG1727;	This study
pMM104	WT MXAN3778 promoter in pREG1727; Km ^r	This study
pMM104a	-12 region mutated MXAN3778 promoter in pREG1727; Km ^r	This study
pMM104b	-24 region mutated MXAN3778 promoter in pREG1727; Km ^r	This study
pMM104c	-12/24 spacer mutated MXAN3778 promoter in pREG1727;	This study
pMM104d	Half Nla28 EBP binding site mutated MXAN3778 promoter in pREG1727; Km ^r	This study
pMM301	600-bp partial fragment of <i>nla28</i> gene in pSWV22 used for <i>nla28</i> inactivation; Tet ^r	This study
pMM302	Fragment for Nla28-DBD expression in pMAL-c5x; Amp ^r	This study

Supplementary Table S2. Oligonucleotides used in the current study

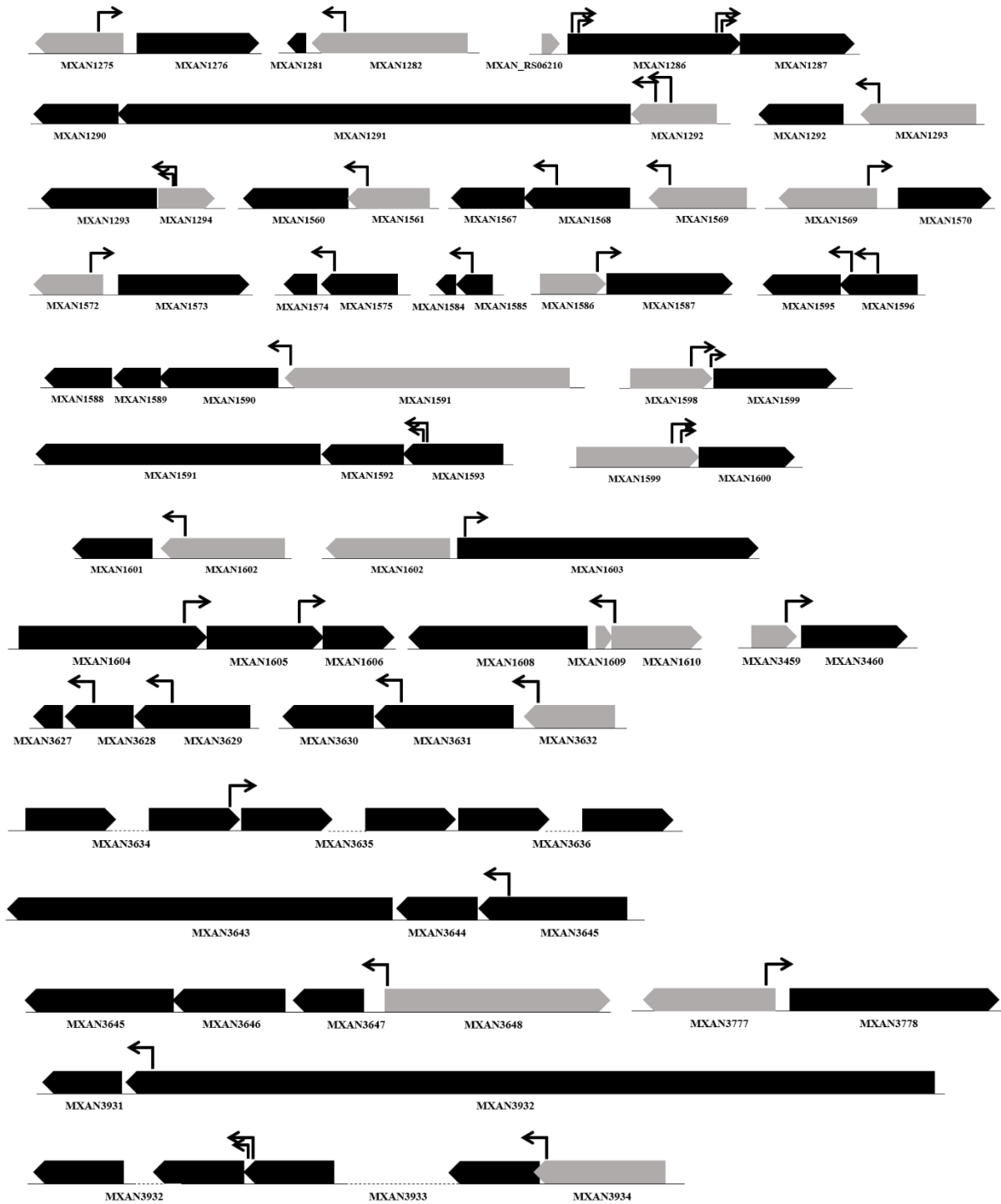
Application of oligonucleotides	Name	Sequence
Amplification of WT putative σ^{54} promoter fragments		
MXAN1286	MM101F	5' gaaagcttaacgccgctcgcaagg 3'
	MM101R	5' atcctagggaggtcggcgatgtg 3'
MXAN1579	MM102F	5' gcaagcttggggttgcgtagaagat 3'
	MM102R	5' atcctagggaggaacaacccccg 3'
MXAN1603	MM103F	5' ataagcttgacgggctcgtgggg 3'
	MM103R	5' cacctagggcctcccgcatcaa 3'
MXAN3778	MM104F	5' aagctttcaccaccgggatgccgg 3'
	MM104R	5' cctagaagggcagcttcacgacgagc 3'
2-bp mutation in -12 region of WT σ^{54} promoter fragments		
MXAN1286	MM101aF	5' cgaggtagagctgaacctccgcgccaac 3'
	MM101aR	5' gttggcgcggaaggttcagctctacctgcg 3'
MXAN1579	MM102aF	5' ccaccgcgcaaaaggattgcgccccatccc 3'
	MM102aR	5' gggatggggcgcaatcctttgcgcgggtgg 3'
MXAN1603	MM103aF	5' ccgcgcgccgagaaaccaccaggcc 3'
	MM103aR	5' ggcctgggtggtttctcggcgcgcg 3'
MXAN3778	MM104aF	5' gacgacccggctcactcaaaatccgtctcaacatc 3'
	MM104aR	5' gatgttgagacggagattttgagtgagccgggtcgtc 3'
2-bp mutation in -24 region of WT σ^{54} promoter fragments		
MXAN1286	MM101bF	5' gccttcgcgaaaacgcgcgattccggtgga 3'
	MM101bR	5' tccaccggaatcgcggttttcgcggaaggc 3'
MXAN1579	MM102bF	5' cgcaaggattgcgaaccatccccgccg 3'
	MM102bR	5' cgggcggggatggttcgaatccttcg 3'
MXAN1603	MM103bF	5' accaccaggaaacttcgggcgacaccaga 3'
	MM103bR	5' tctgggtgtcgcccgaagtttctgggtggt 3'
MXAN3778	MM104bF	5' cgcaatctccgtcaaaacatccccctctgtcg 3'
	MM104bR	5' cgacaggagggggatgtttgacggagattgcg 3'
1-bp deletion in -12/-24 region spacer of WT σ^{54} promoter fragments		
MXAN1286	MM101bF	5' gcgttggcgcggaagcttaacgccg 3'
	MM101bR	5' cggcgtaagctttccgcgccaacgc 3'
MXAN1579	MM102bF	5' gggatggggcgcaataagcttgggggttg 3'
	MM102bR	5' caacccaagcttattgcgccccatccc 3'
MXAN1603	MM103bF	5' gaagtggcctgggaagcttgacgggc 3'
	MM103bR	5' gcccgtcaagcttccaggccacttc 3'
MXAN3778	MM104bF	5' gggatgttgagacgggattgcgagtgagcc 3'
	MM104bR	5' ggctcactcgcaatccccgtctcaacatccc 3'

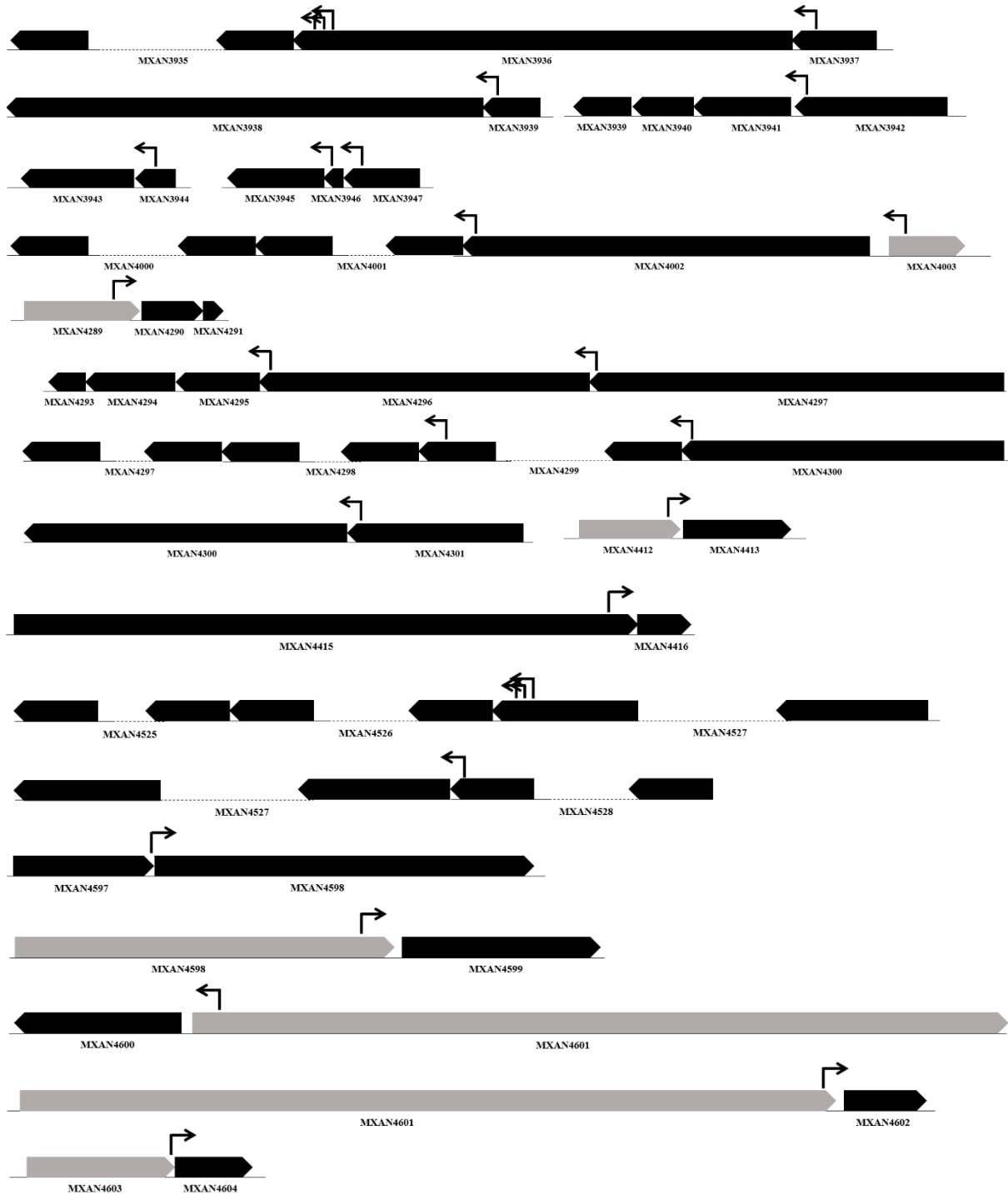
**Amplification of 5'-labelled Cy5 WT σ^{54}
promoter fragment for EMSAs**

Cy5-P ₁₂₈₆	MM109F	5' Cy5-gctcgtgtcgcgtccctacatcca 3'
	MM109R	5' acagggacagcgtccacacgacc 3'
Cy5-P ₁₅₇₉	MM110F	5' Cy5-gcgtcttcttgccgtaccgcgga 3'
	MM110R	5' atacacccgaaaggaaggaacgccg 3'
Cy5-P ₃₇₇₈	MM110F	5' Cy5-aagtgggtctcgaagcgcacgtcgc 3'
	MM110R	5' cagcaggcgctcgaggtcctcaa 3'
Fragment for Nla28-DBD expression	MM302F	5' ctggcgctcaacgtgacgggcgt 3'
	MM302R	5' acctgcaggctacgactcggcctccg 3'

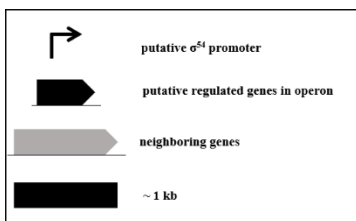
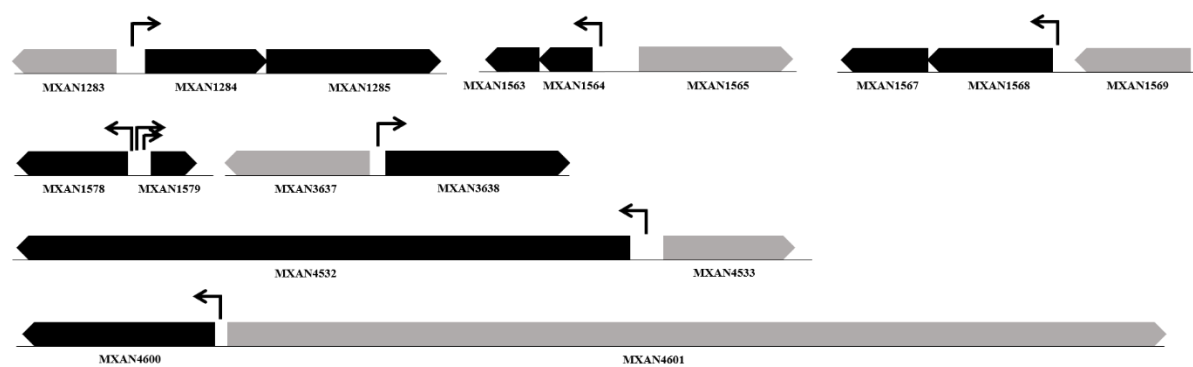
Supplementary Figure S1

A. Locations of putative intragenic σ^{54} promoters in PK/NRP gene clusters in *M. xanthus*





B. Locations of putative intergenic σ^{54} promoters in PK/NRP gene clusters in *M. xanthus*



Supplementary Table S3. Putative intragenic and intergenic σ^{54} promoters in *M. xanthus* PK/NRP gene clusters

Intragenic			
Regulated genes/operons	Functions	Putative promoter -24/-12 sequence ^a	Position ^b
MXAN1276	Glutamate-cysteine ligase family 2 protein	GT <u>GG</u> CGCGGCCCTT <u>GAA</u>	-476
MXAN1281	hypothetical protein	CT <u>GGA</u> ACGCGTGCAG <u>GCA</u>	-712
MXAN1286-1287	ABC transporter/ATP-binding protein;	TT <u>GGA</u> CCCGGCTCGT <u>GCG</u>	+15
	hypothetical protein	TT <u>GGA</u> CGCGGAAGGC <u>GCA</u>	+111
MXAN1287	hypothetical protein	ATT <u>G</u> CCCCGCGCCCT <u>GCT</u>	-308
		GAG <u>G</u> CCCCAACTGTT <u>GCG</u>	-233
MXAN1290-1291	Phosphotransferase; non-ribosomal peptide synthetase	CT <u>GGA</u> CACGTGACGT <u>GTT</u>	-285
		GT <u>GGA</u> TGCGGCGGAT <u>GCA</u>	-488
MXAN1292	hypothetical protein	CT <u>GGA</u> CGCGCGAGGA <u>ACT</u>	-521
MXAN1293	protoporphyrinogen oxidase	CC <u>GGA</u> TACACACGTT <u>GGA</u>	-285
		GAG <u>G</u> ACCCGGCGTT <u>GTT</u>	-324
MXAN1560	class I aminotransferase	GT <u>GGA</u> CACGTGCTG <u>GTTG</u>	-284
MXAN1567	urea amidolyase-like protein	CT <u>GGA</u> CGCGGTTGCT <u>GCG</u>	-407
MXAN1567-1568	hydrolase family protein;	CT <u>GGA</u> CACGCGCCTG <u>GCG</u>	-443
MXAN1570	class V aminotransferase	AT <u>GGA</u> CTCGCAGCTT <u>GCG</u>	-293
MXAN1573	AMP-binding domain protein	CT <u>GGA</u> CCTCCACGGT <u>GGA</u>	-409
MXAN1574	TfoX domain-containing protein	CT <u>GGA</u> CGCGCGCCGG <u>GGA</u>	-212
MXAN1584	hypothetical protein	GT <u>GGA</u> CGCGGCCGCT <u>GCT</u>	-182
MXAN1587	hypothetical protein	GT <u>GGA</u> GACATCCGGT <u>GCG</u>	-146
MXAN1588-1590	Aminotransferase; putative para-aminobenzoate	GAG <u>GGA</u> CACGCCGCAC <u>GCT</u>	-122
MXAN1591-1592	hypothetical proteins	CT <u>GCT</u> TCTGCACGGAG <u>GCT</u>	-217
		CT <u>GGA</u> AGTGGAACCT <u>GCT</u>	-229
MXAN1595	hypothetical protein	AT <u>GGA</u> CACGGGGGAC <u>GAA</u>	-115
		GAG <u>GGA</u> TGACCGGCT <u>GCT</u>	-413
MXAN1599	putative methyltransferase	GT <u>GGA</u> CCAGCCGGGC <u>GCG</u>	-281
		GT <u>GGA</u> TGCCGCTGCT <u>GCT</u>	-44
MXAN1600	class I aminotransferase	GAG <u>GGA</u> CCCGCTCCGAG <u>GCT</u>	-402
		CT <u>GGA</u> CTCGGGCGTT <u>GCG</u>	-270
MXAN1601	fatty acid desaturase family protein	GT <u>GGA</u> CCCGGGCACT <u>GCG</u>	-384
MXAN1603	putative non-ribosomal peptide synthetase	GT <u>GGA</u> CCTGGGTGGT <u>GCC</u>	+84
MXAN1605	putative permease	CT <u>GGA</u> AAGGTCGCT <u>GCT</u>	-265
MXAN1606	hypothetical protein	GT <u>GGA</u> CCGCCTTCAT <u>GCT</u>	-260
MXAN1608	hypothetical protein	TAG <u>GGA</u> ACGCACGAC <u>CCT</u>	-286
MXAN3460	Gfo/Idh/MocA family oxidoreductase	CT <u>GGA</u> TCCACATCAT <u>GCT</u>	-182
MXAN3627	hypothetical protein	AT <u>GGA</u> CGGAAGAGCT <u>GCT</u>	-305
MXAN3627-3628	hypothetical protein; non-ribosomal peptide biosynthesis	CT <u>GGA</u> CACGCCAGCAG <u>GCT</u>	-383
MXAN3630	polyketide synthase type I	CT <u>GGA</u> CGGACATCTG <u>GCA</u>	-315
MXAN3630-3631	polyketide synthase type I	AAG <u>GGA</u> ACAGCTGTT <u>GCG</u>	-290
MXAN3635-3636	non-ribosomal peptide synthase/polyketide synthase	AAG <u>GGA</u> CGTGCGCATT <u>GCT</u>	-193
MXAN3643-3644	non-ribosomal peptide synthase; isochorismatase	GT <u>GGA</u> AAAACCATCT <u>GCT</u>	-297

MXAN3645-3647	2,3-dihydroxybenzoate-AMP;	GT <u>GG</u> CACGCGAAAT <u>CCC</u>	-254
MXAN3778	DnaK family protein	TT <u>GAG</u> ACGGAGATT <u>GCG</u>	-284
MXAN3931	hypothetical protein	GT <u>GG</u> GCCGGTTCCG <u>GCT</u>	-289
MXAN3932	polyketide synthase	CAG <u>GA</u> CACGCCCATC <u>GCT</u>	-58
		TT <u>GAAAA</u> ACCGTT <u>GCT</u>	-75
MXAN3933	mixed type I polyketide synthase-peptide synthetase	GT <u>GG</u> TACTTCGCTT <u>ACT</u>	-222
MXAN3935	non-ribosomal peptide synthase/polyketide synthase Ta1	CT <u>GG</u> CACGAACCCT <u>GGG</u>	-205
		TT <u>GG</u> CGCTGAGCGT <u>GCC</u>	-301
		TT <u>GG</u> CTCGCGTCTG <u>GCT</u>	-413
MXAN3936	polyketide synthase	TT <u>GG</u> TCCGCCCGGGC <u>GGT</u>	-318
MXAN3938	polyketide synthase	CT <u>GT</u> CGCTCGTGAC <u>GCT</u>	-140
MXAN3939-3941	enoyl-CoA hydratase; polyketide beta-ketoacyl:acyl	CT <u>GG</u> CTCTGCAACT <u>GCG</u>	-229
MXAN3943	cytochrome P450 family protein	GT <u>GG</u> CGCATGCCTT <u>TCT</u>	-436
MXAN3945	polyketide TA biosynthesis protein TaF	CT <u>GG</u> ACAGCCTGCG <u>GCT</u>	-82
MXAN3945-3946	polyketide TA biosynthesis protein TaF; putative acyl	CT <u>GT</u> CGCGCGTGGT <u>GCT</u>	-205
MXAN4000-4001	non-ribosomal peptide synthase/polyketide synthase	CT <u>CG</u> CCCCGAGGTT <u>GCA</u>	-161
MXAN4002	nonribosomal peptide synthetase	AG <u>GG</u> CACGGCCCCGT <u>GAT</u>	-451
MXAN4290-4291	putative thioesterase; hypothetical protein	CT <u>GG</u> AGCGCGTGCT <u>GCT</u>	-354
MXAN4293-4295	hypothetical protein; transporting ATPase;	AT <u>GT</u> CGCCGTGCTT <u>GCA</u>	-118
MXAN4296	non-ribosomal peptide synthetase	CT <u>GGG</u> AGTGACCT <u>GCT</u>	-134
MXAN4297-4298	polyketide synthase type I	AT <u>GG</u> GTCGGCAGTT <u>GCT</u>	-364
MXAN4299	non-ribosomal peptide synthase/polyketide synthase	AT <u>GG</u> CGCTCGAGTT <u>GCG</u>	-164
MXAN4300	polyketide synthase type I	CT <u>GG</u> CGAAGCGGCT <u>GCT</u>	-167
MXAN4413	hypothetical protein	CT <u>GG</u> ACGACCGGTT <u>GGT</u>	-186
MXAN4416	cephalosporin hydroxylase family protein	CT <u>GGG</u> CCACGGATT <u>GCT</u>	-332
MXAN4525-4526	non-ribosomal peptide synthase; polyketide synthase type I	AT <u>GG</u> CGCTGGAGCT <u>GCG</u>	-243
		AT <u>GG</u> CGCTGCTGTT <u>GGA</u>	-359
		GT <u>GG</u> CAGGGCAGGT <u>GCG</u>	-452
MXAN4527	polyketide synthase	CT <u>GG</u> CTGAACAACCT <u>GCA</u>	-156
MXAN4598	non-ribosomal peptide synthase	AT <u>GGA</u> ACGGCGCAT <u>CCT</u>	-41
MXAN4599	M28 family peptidase	CT <u>GGG</u> GCTACGGGGT <u>GCT</u>	-534
MXAN4600	radical SAM domain-containing protein	GT <u>GG</u> CCTGGGCGTC <u>GGT</u>	-493
MXAN4602	hypothetical protein	CT <u>GG</u> CCCCGTCTGGAG <u>GCT</u>	-241
MXAN4604	hypothetical protein	AT <u>GT</u> CGCTCGACTT <u>GCT</u>	-62

Intergenic

Regulated genes/operons	Functions	Putative promoter ^a -24/-12 sequence	Position ^b
MXAN1284-1285	2-isopropylmalate synthase/homocitrate synthase family protein; tryptophan halogenase	AG <u>GG</u> CAAGGCATTT <u>CCA</u>	-121
MXAN1563-1564	alkyl hydroperoxide reductase C	CT <u>GG</u> CACGGTGACT <u>GCT</u>	-69
MXAN1567-1568	hydrolase family protein; LamB/YcsF	CT <u>GG</u> CACGCCAGCGT <u>TCT</u>	-44
MXAN1578	metallo-beta-lactamase family protein	AC <u>GG</u> CGCAGCGCTT <u>GCT</u>	-45
MXAN1579	hypothetical protein	GG <u>GG</u> CGCAATCCTT <u>GCG</u>	-104
		GG <u>GG</u> CGCAGGTCTT <u>GCG</u>	-75

MXAN3638	M19 family peptidase	CT <u>GG</u> TACTTCGAGT <u>GCA</u>	-32
MXAN4532	non-ribosomal peptide synthase	GT <u>GG</u> CACAAGCTGC <u>GCT</u>	-173
MXAN4600	radical SAM domain-containing protein	GC <u>GG</u> TAAAGTCTTT <u>GCT</u>	-41

^aPutative -12 and -24 regions were identified in a previous study³⁷.

^b*Distance between the -12 region of the promoter relative to the initiation codon.*