Identification of the Gene Cluster Involved in Muraymycins Biosynthesis from *Streptomyces* sp. NRRL 30471

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Table S1. Strains and plasmids used in this study

Strain/phage/ plasmid	Relevant characteristics	Reference or source
***Streptomyces* sp. NRRL 30471**		
30471	Wild-type strain producing muraymycins	NRRL
CL-1	The *ca.* 0.5-kb fragment of *mur16* gene in 30471 was replaced by 1.5-kb of *neo* cassette of SuperCos1	This study (Figure. S1)
CL-2	The *ca.* 0.9-kb of *mur17* gene of 30471 was replaced by 1.5-kb of *neo* cassette of SuperCos1	This study (Figure. S1)
E. coli		
DH10B	F$^-$ *mcr*A Δ (*mrr-hsd*RMS-*mcr*BC) ϕ80d *lacZ*Δ M15 Δ *lac*X74 *deo*R *rec*A1*end*A1*ara*Δ139 D(*ara*, *leu*)1697 *gal*U *gal*Kλ $^-$ *rps*L *nup*G	GIBCO BRL
EPI300-T1R	Host cell for construction of genomic cosmid library	EPICENTER
BW25113 (pIJ790)	BW25113/pIJ790 K-12 derivative: Δ*araBAD* Δ*rhaBAD*/λ-Red(*gam bet exo*) *cat araC rep101*(Ts)	[1]
ET12567 (pUZ8002)	*dam dcm hsdS* pUZ8002	[2]
Bacillus subtilis	Indicator strain sensitive to muraymycins	(Lab collection)
Plasmids		
pOJ446	*aa (3)I V,* SCP2, *rep*^{pMB1*}, attΦC31, *ori*T	[3]
pSET152	*aa (3)I V*, *lacZ*, *rep*^{pMB1*} attΦC31, *ori*T	[3]
pJTU2463	A derivative of pOJ446 with SCP2 replicon replaced by *int* and *attp* from pSET152	(Yao *et al.* unpublished)

pJTU2463b	A derivative of pJTU2463 with XbaI and SpeI sites blocked	(Chen *et al*. unpublished)
SuperCos1	*neo, bla, cos,ori*^{pUC}	Stratagene
pJTU5622	A *ca*. 8 kb ScaI fragment (Fig. 2) from cosmid 18F3 was cloned into the EcoRV site of pOJ446	This study
pJTU5627	A *ca*. 0.5-kb fragment of *mur16* gene was replaced with 1.5-kb of *neo* cassette gene by PCR-targeting	This study
pJTU5628	A *ca*. 0.9-kb fragment of *mur17* gene was replaced with 1.5-kb of *neo* cassette by PCR-targeting	This study

*ori*T, origin of transfer of plasmid RK2; *aac(3)IV,* apramycin resistance gene; *cat*, chloramphenicol resistance gene; *neo*, Neomycin resistance gene; *rep*^{pMB1*}, mutated *rep*^{pMB1}.

Table S2. PCR primers used in this study

Primers	**Sequence**
cpz14degenerate1	5'-- CGACTTGTACAACCGSTAYTTYTTC --3'
cpz14degenerate2	5'-- GGAAGGTCTTGTGSGTSGAKCCKCC --3'
16F	5'-- GAAGTGAACGACGTCGTACGTTACCGATACGGCGCCGCT TCTAGAGCTATTCCAGAAGT --3'
16R	5'-- GACGTCATTGCGGAAGATCAATGCCTCGCCCGCTCCCAG ACTAGTCTGGATGCCGACG --3'
16A	5'-- TACCATATGGTGCTCCCTGGACCGGGTAG--3'
16B	5'-- ATCAAGCTTCTAGATCAGGGGCTCTCCAGGTAGAG--3’
17F	5'-- CTGTTGGCGCTCCTCGGCGAGATCGAGAAGGAACAGCGC TCTAGAGCTATTCCAGAAG--3'
17R	5'-- CCCGAGGCGGAGCGCCGGTTCCGGGATGTCGGGGAGCTC ACTAGTCTGGATGCCGACG--3'
17A	5'-- TACCATATGACCTCTTCGGACGACTGC--3’
17B	5'-- ATTGAATTCAGCCATGGAAGAGTCCGG--3'

S, C/G; Y, C/T; K, T/G.

Figure Legends

Figure S1: The corresponding conserved amino acids positions of cpz14degenerate1 and cpz14degenerate2 in Cpz14 and its homologs. Cpz14, BAI23322, AF484556_32 and YP_632761 are proteins from *Streptomyces* sp. MK730-62F2, *S. griseus*, *S. atroolivaceus* and *M. xanthus* DK 1622 respectively.

Figure S2: PCR Identification of the CL-1 (Δ*mur16*) and CL-2 (Δ*mur17*) Mutants of *Streptomyces* sp. NRRL 30471. **A,** PCR identification of *mur16* mutation in NRRL 30471, as *ca*. 0.5-kb of *mur16* gene was replaced by *ca.* 1.5-kb *neo* cassette, the resultant *mur16* mutant produces 1.8-kb PCR product (Lane 1), and the intact *mur16* gives a PCR product of 0.8-kb (Lane 2). **B,** PCR confirmation of *mur17* mutation in NRRL 30471, as *ca.* 0.9-kb of *mur17* gene was replaced by *ca.* 1.5-kb *neo* cassette, the resultant *mur17* mutant produces 1.9-kb PCR product, while the intact *mur17* gives a PCR product of *ca.* 1.3-kb.

Figure S3: MS and MS/MS Analysis of the muraymycin D1 and C1 components from wild-type. **A,** MS and MS/MS analysis of the muraymycin D1 component. The component generated a $[M+H]^+$ ion at m/z 930.4, which was fragmented into main ions of 462.2, 668.2, 813.3 and 912.4, *etc*. **B,** MS and MS/MS analysis of the muraymycin C1 component. The component generated a $[M+H]^+$ ion at m/z 946.4, which was fragmented into main ions of 442.2, 684.2, 829.3 and 928.4, *etc*. **C,** MS/MS Fragmentation pattern of the muraymycin D1. **D,** MS/MS Fragmentation pattern of the muraymycin C1.

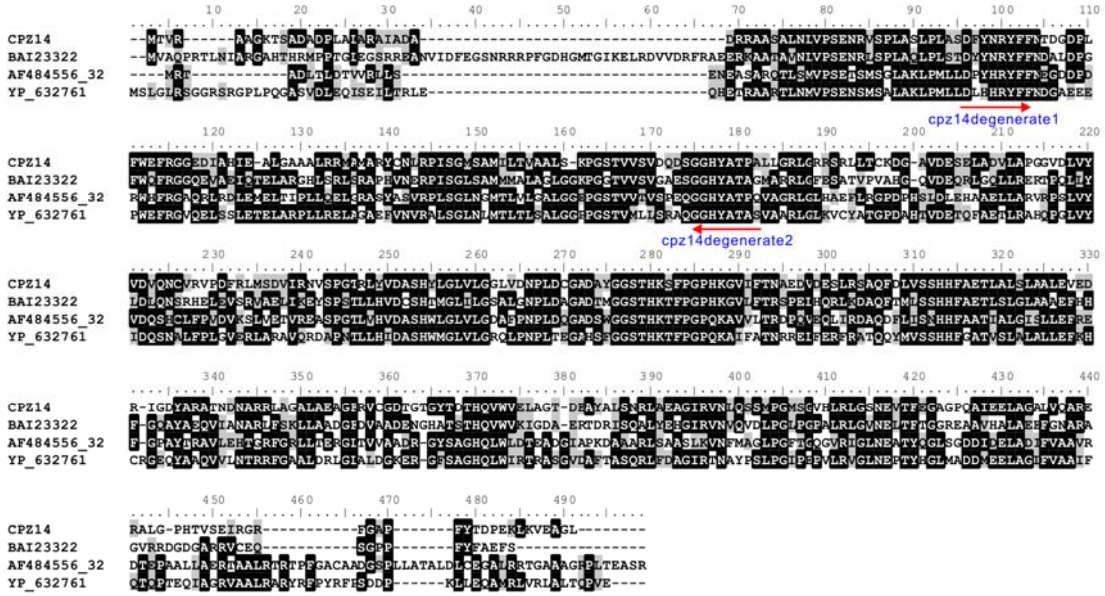

Figure S1

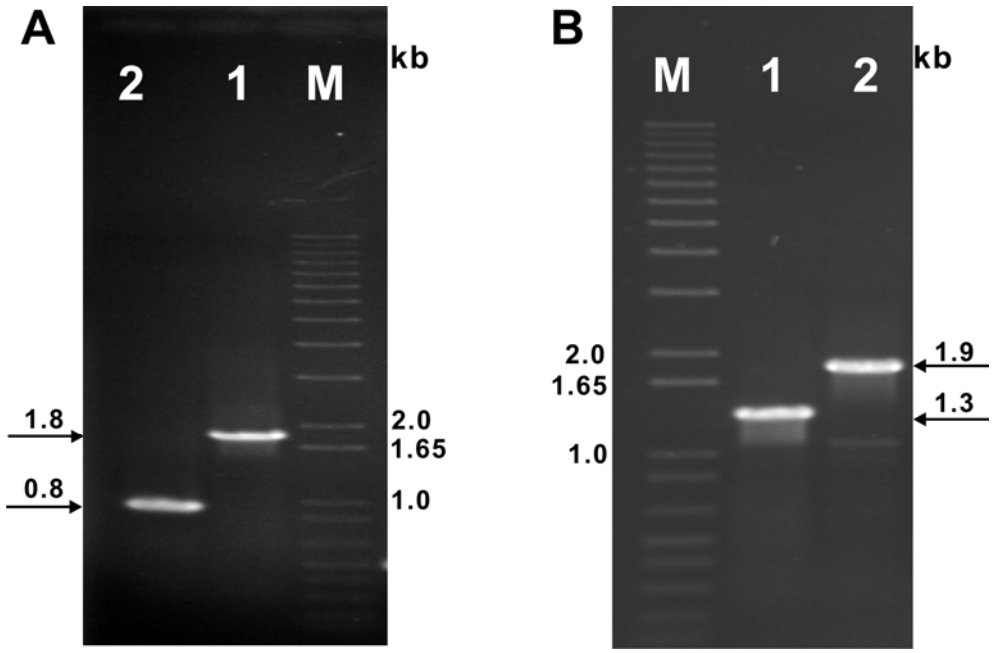

Figure S2

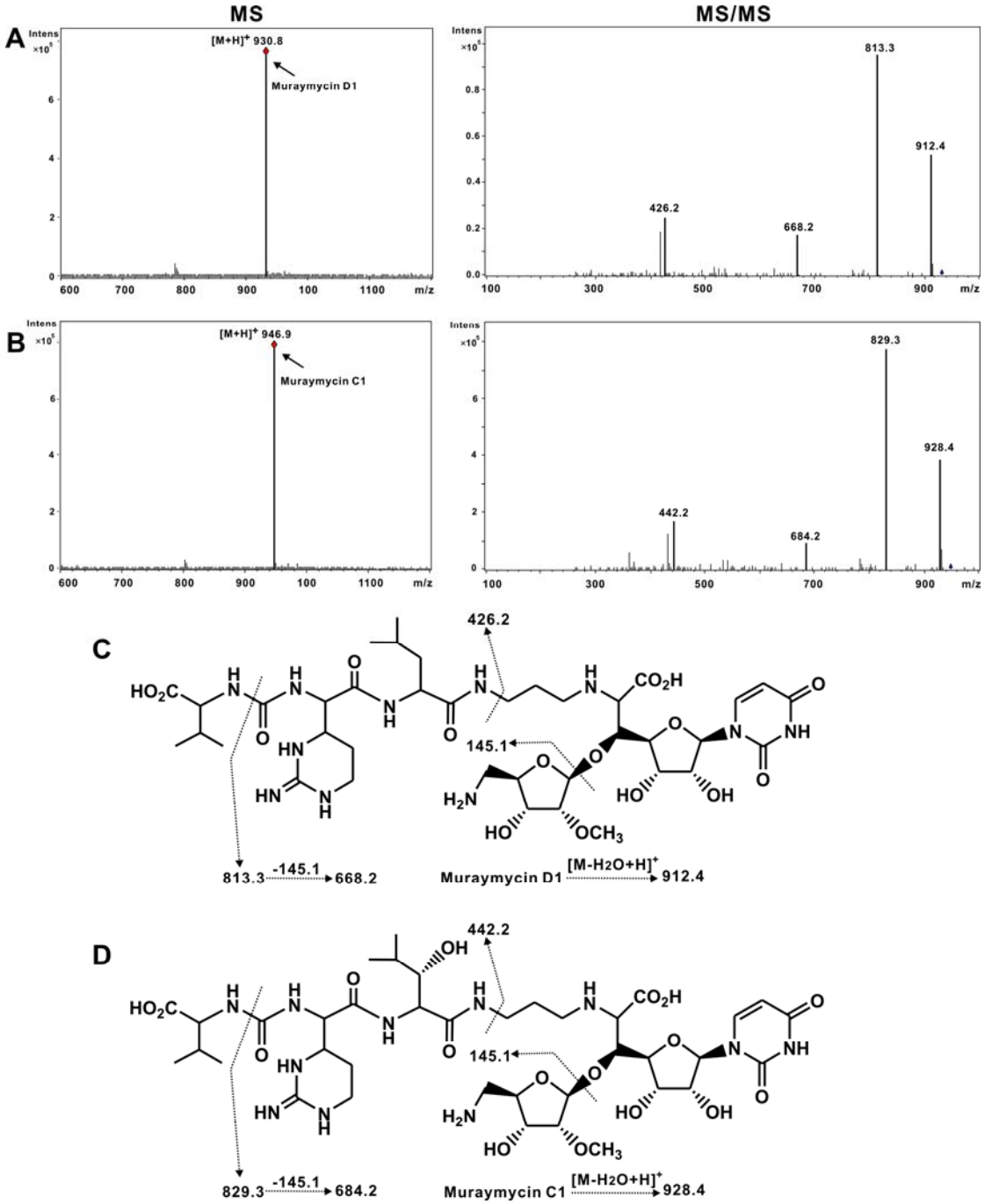

Figure S3

References

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2. M. S. Paget, L. Chamberlin, A. Atrih, S. J. Foster and M. J. Buttner, *Journal of bacteriology*, 1999, **181**, 204-211.
3. M. Bierman, R. Logan, K. O'Brien, E. T. Seno, R. N. Rao and B. E. Schoner, *Gene*, 1992, **116**, 43-49.