

CHEMBIOCHEM

Supporting Information

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for

A Type I/Type III Polyketide Synthase Hybrid Biosynthetic Pathway for the Structurally Unique *ansa* Compound Kendomycin

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Ken_TE	ADGATGPADSGIADLYWSANDAGHYEAAATGLLRAVAALRPAFDEDTADRHAPRPLRLARG	60
Aves_TE	-----RIEESMALLSAASFRRPAFTDPSDIP-EPTFVRLAQGEARAQGEA-----LARG	48
DEBS_TE	SGTPAREASSALRDGYRQAGVSGFRVRSYLDLLAGLSDFREHFDGSDGFSLLDLVDMADGPG	60
Tyl_TE	TGAAPADAGSGLPALYREAVRTGRAAEEMAEELAAASRFRPAFGTADRQFVALVPLADGAE	60
Ken_TE	DAR-PALVCLPSPSPASGPHEYARFAAALR---GDREVVWALPEPGLDGGQALPADVDALV	116
Aves_TE	ETR-PALICLPTVAAVSSVYQYSRFAAGLN---GHRDVWYVPAPGFELEGEPLPSGIGAVT	104
DEBS_TE	EV---TVICCAGTAALISGPHEFTRLAGALR---GIAPVRAVDPQGYEEGEPLPSMAAVA	114
Tyl_TE	DTGLPLLVGCGATAVASGPVEFTAFAGALADLPAAAPMAALPQPGFLPGERVPEATPEALF	120
Ken_TE	AAHAHALATDGPGTAPVLVGRSAAGWIAHALAARLEAEGRPAAALVLLDTYSP-DALARR	175
Aves_TE	RMFADAIVRFTDGAIFALAGHSAGGWVYAVTSHLERLGVRPEAVVTMDAYLPDDGIAP-	163
DEBS_TE	AVQADAVIRTQGDKPFVAVAGHSAGALMAYALATELLDRGHPPRGVVLDIVYPPGHQDAMN	174
Tyl_TE	EAQAEALLRYAAGRPFVLLGHSAGANMAHALTRHLEANGGGPAGLVLMDIYTPADPGAMG	180
Ken_TE	DWVRTAMTRATSGRESALVLRNETRLAATGGYDRIFTGWAPGPLRTPILLVRAADPFSTE	235
Aves_TE	--VASALTSEIFDRVTQFVDVDYTRLVAMGGYFRIFSGWSPPDITTPALFLRGRD-----	216
DEBS_TE	-AWLEELTATLFDR--ETVRMDDTRLTALGAYDELGTQWRPRETGLPTLLVSAGEPMG--	229
Tyl_TE	-VWRNDMFQVWVRR--SDIPDDHRELTAMGAYHRLLLDWSPTPVRAPVHLHRAAEPMG--	235
Ken_TE	LLGLAEFGDWTAAWEPSHDAVTVPGTHFTILEERSADTAGAVE	279
Aves_TE	----GEQM---PPPWGVPHTVLDIQGNHFTMLEQFADSTARHVD	253
DEBS_TE	--PWP--DDSWKPTWPFHDTVAVPGDHFTMVQEHADAIARHID	269
Tyl_TE	--DWPPGDTGWQSHWDGAHTTAGIPGNHFTMMTEHASAAARLVH	277

Figure S1: Sequence alignment of the kendomycin TE and macrolactone-forming TEs from other PKS systems (Ken, kendomycin synthase; Aves, avermectine synthase; DEBS, 6-deoxyerythronolide B synthase; Tyl, tylactone synthase). Many of the conserved residues identified by Stroud et al.^[57] (highlighted in grey) are presumably important for maintaining the TE fold, forming the active site and lining the channel as well as interacting with the ACP domain.

Table S1: Plasmids generated in this study.

Plasmid	Fragment	Recipient vector
pKen14	17.5 kb XhoI/EcoRI fragment from cosmid F10	XhoI/EcoRI pBluescript II SK +
pKen15	5.3 kb EcoRI/XhoI fragment from cosmid F3	EcoRI/XhoI pBluescript II SK +
pKen22	4.3 kb MluI fragment from cosmid D11	BssHII pBC SK +
pKen24	2.1 kb MluI/XhoI fragment from cosmid D11	MluI/XhoI pCR-XL-TOPO
pKen26	4.1 kb BglII fragment from cosmid A7	BamHI pBC SK +
pKen18	14.5 kb EcoRI fragment from cosmid F3	EcoRI pSET152
pKen19	1.25 kb PCR product generated with the oligonucleotide pair Ken14/Ken15 and cosmid H4 as template	pCR2.1-TOPO
pKen41	1.3 kb EcoRI fragment from pKen19	EcoRI pKC1132