Supplementary Materials

A hadal *Streptomyces*-derived echinocandin acylase discovered through prioritization of protein families

Xuejian Jiang [1], Hongjun Shu [1], Shuting Feng [1], Pinmei Wang [1,2], Zhizhen Zhang [1] and Nan Wang [1,2,*]

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Table S1. Marine derived proteins clustered with the probe FR9013179 acylase in SNN generated by EFI-EST

Origin	UniProt ID
Unknown proteins of intertidal origins	A0A2S1I7W9 A0A1S2PKA0 A0A2M9LMP4 A0A1Q5BWW0 A0A1Q5GMV2
Unknown sponge co-epiphytic microbial proteins	A0A3N6H9K9 A0A3N6DWY1 A0A3G4VTM0 A0A101URM3
Unknown microbial proteins derived from seafloor sediments	A0A4R1DSC3 H0BNF0 A0A3S9PDQ3 A0A1V0UK48 A0A6I6WZB9 A0A941FGI5 A0A1E7LNN9 F3NT93
Hadal origin protein studied	ECEA*

*not a UniProt accession ID

Table S2. Strains and plasmids used in this study.

Strain / Plasmid	Function	Source
E. coli DH5α	Subcloning	In-house collection
E. coli ET12567(PUZ8002)	Intergeneric Conjugation	In-house collection
Streptomyces lividans TK24	Host for ECEA production	In-house collection
Streptomyces sp. SY1965	Cloning of the *ecea* gene and biotransformation of ECE/FR9013179	[15]
pSET-*kasO*p**ecea*	Conjugal transformation plasmid	This study
Aspergillus nidulans LO8030-5.1	ECE production	[9]
Streptomyces lividans TKecea66	Biotransformation of ECE/FR9013179	This study

Table S3. Primers used in this study.

Primer	Nucleotide Sequence (5'-3')	Function
ECEA_F1	gccttaagatgacgttacgcaaccgtctgagactgc	*ecea* gene amplification
ECEA_R1	ctagtggtggtggtggtggtgccgccgctcgtgcac	*ecea* gene amplification
ECEA_F2	caccaccaccaccaccactagactccatctggatttgttc	Overlap integration
ECEA_R2	gctgaacgggggggttcgtgcacaca	Overlap integration
ECEAyz_F	ttgtaaaacgacggccagtgccaagct	Genotype PCR verification
ECEAyz_R	atgcttccggctcgtatgttgtgtgg	Genotype PCR verification
pSL_F5	cacgttttcccaggtcagaagc	Genotype PCR verification
pSL_R5	atgctcaactaaagtggggcg	Genotype PCR verification
pSL_F7	gaccatcgtgatcggcgtgt	Genotype PCR verification
pSL_R7	gcgtcgagccgcataatcagg	Genotype PCR verification

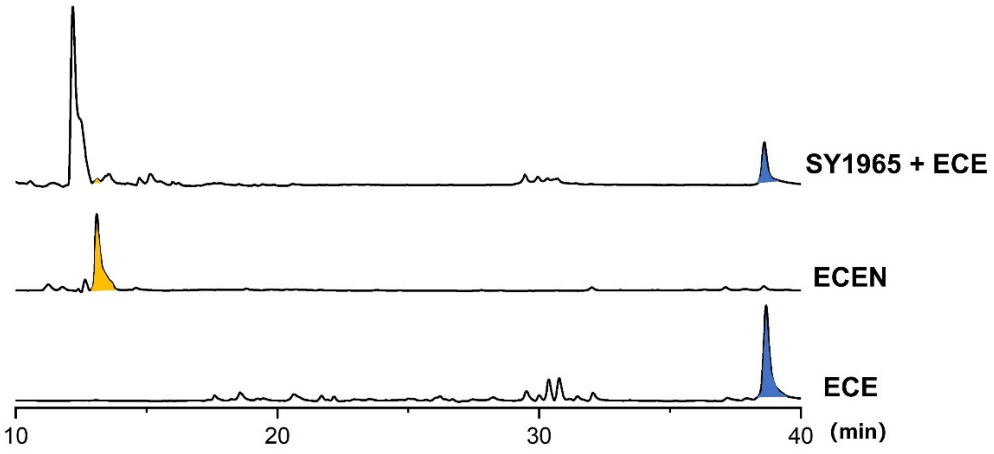
Table S4. DNA fragments for assembling the *ecea* expressing cassette

Fragment	DNA sequence (5′-3′)
The coding sequence of *ecea*	ttgacgttacgcaaccgtctgagactgctcggggtcgccggtctcgccctgttcaccgtgtcggcctcgctgccgcc ggccgccgcgtccggggccgggccacagcggcacccgtccggcggcgggctctccgccgtcatccggtacacg gagtacggcattccgcacatcgtggcgaaggactacgcgcagctcggcttcggcaccggctgggcgcaggcgg ccgaccaggtgtgcacgctggcggacggtttcctcacggtgcgcggggaacggtcgaggttcttcggcccggac gccgccccggacttctcgctctcctcggcggcgacgaacctctccagcgatctgtacttccggggtgtccgggaca gcggcacggtggagaagctgctgaaggtgcccgcgcccgccggtccgagccgggacgccaaggagacgatg cgcgggttcgccgccgggtacaacgcgtggatccagcagaaccggatcaccgatccggcctgccggggcgcgt cctgggtccgtccggtgacggcgctggacgtggcggcgcgcggctacgcgctggccgtgctcggcggtcaggg gcgcggcatcgacggcatcacggcggcgcggccgccgaccgccgctcccccggcggcgggggtcacgcccga ggaggcggcgacggcggccgagcggctgctgtcggcgcagaacgcggacatgggctccaacgcggtggcctt cgacggctccaccacggtgaacgggcgcgggctgctgctcggcaacccgcactacccgtggcagggcggacgc cgcttctggcagtcgcagcagaccattcccggtgagctgaacgtgtcgggtgcgtccctgctgggcgccacgacg atctcgatcgggcacaacgcggatgtggcgtggagccatacggtcgccacgggcgtcacgctgaatctgcatca gctgacgctggatccggccgacccgaccacgtatctggtggacgggaagcgggagcggatgacgaagcggac ggtgagcgtcccggtgaagggcgcggctcccgtgacccgcacccagtggtggacgcgctacggcccggtcgtc gactccatgggctcggcgctgccgttgccgtggacggcgagcacggcgtacgccctgaacgacccgaacgcga cgaatctgcggatggcggacaccggtctgggcttcagcagggcccgctccacggcggacgtggaacgctcact gcaccggaaccagggcatgccgtgggtgaacacgatcgcggcggaccgggcggggcactcgttcttcgcccag tcgcaggtcctgccgaggatcacggacgagctggcggagcgctgctcgaccgcgctgggccgggccacgtatc cggcgtccgggctggcggtgctggacggctcgcgcgaggactgcgcgctgggcagcgacccggacgcggtgc agtcgggcatcttcggcccgagccggatgcccacgctcaagaaccggccgtacgtggagaactccaacgacagc gcctggctgaccaacgcggagcagccgctgaccgggtacgagcgggtcttcggcacggtcgcgacgccccggt cgatgcggacgcgtggcgcgatcgaggatgtcgcggagatggcggacaagggccgcctccgggtcgcggatc tccagcggcagcagttcgccaaccgtgcgccggccggcgacctggccgcgtcggagacggcggggtggtgcg cggcgctgccgggcggcacggccgtgggcacgggcggtacgccggtcgatgtgtcggaggcctgccgggtgc tgcggcgctgggaccggagcgtggacagcgacagccggggcgcgctgctcttcgaccggttctggcggaggg cctcggcggtgcccgccgccgagctgtggcggaccccgttcgacccggccgacccggtgcgcaccccgcgcggt ctgaacaccgccgcgccgggtgtcggcacggcgctggcggacgcggtgacggagctgcgggcggcgggcat cacgctcgacgcaccgctgggcaagcaccagttcgtggtccggaacggcaagcggctcccgatcggcggcggg acggagtcgctgggcatctggaacaagaccgagcccgtgtggaacgcggcggcgggcggctatacggaggtg tcgacgggctccagctacatccaggcggtcggctgggacgacagccgctgcccggtggcccggacgctgctga cgtactcccagtcggagaacccgaagtcgccgcacttcagcgaccagacccggctgtacgcgggcgagcgctg

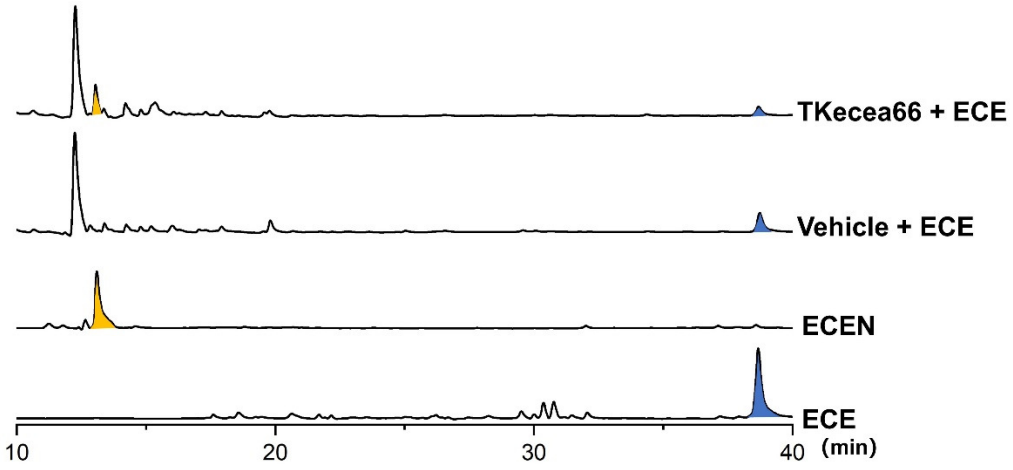
	ggtgacgtcccggttctgcgagaaggacatcgcccgctcgccggccctgcgggtggtgcgggtgcacgagcgg cggtag
*KasO*p*	tgttcacattcgaacggtctctgctttgacaacatgctgtgcggtgttgtaaagtcgtggccaggagaatacgacag cgtgcaggactgggggagtt
6×His	caccaccaccaccaccac
lambda t0 terminator	actccatctggatttgttcagaacgctcggttgccgccgggcgtttttat

Table S5. Amino acid sequence of ECEA including the signal peptide

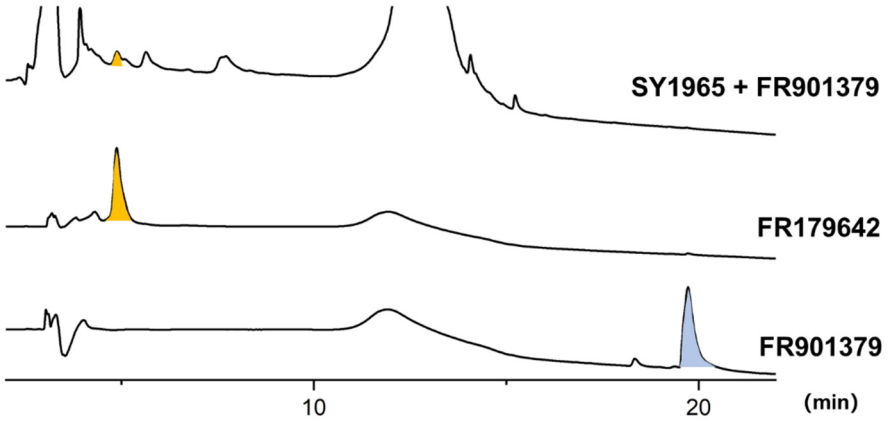
Amino acid sequence
LTLRNRLRLLGVAGLALFTVSASLPPAAASGAGPQRHPSGGGLSAVIRYTEYGIPHIVAKDY AQLGFGTGWAQAADQVCTLADGFLTVRGERSRFFGPDAAPDFSLSSAATNLSSDLYFRGV RDSGTVEKLLKVPAPAGPSRDAKETMRGFAAGYNAWIQQNRITDPACRGASWVRPVTAL DVAARGYALAVLGGQGRGIDGITAARPPTAAPPAAGVTPEEAATAAERLLSAQNADMGS NAVAFDGSTTVNGRGLLLGNPHYPWQGGRRFWQSQQTIPGELNVSGASLLGATTISIGHN ADVAWSHTVATGVTLNLHQLTLDPADPTTYLVDGKRERMTKRTVSVPVKGAAPVTRTQ WWTRYGPVVDSMGSALPLPWTASTAYALNDPNATNLRMADTGLGFSRARSTADVERSLH RNQGMPWVNTIAADRAGHSFFAQSQVLPRITDELAERCSTALGRATYPASGLAVLDGSRE DCALGSDPDAVQSGIFGPSRMPTLKNRPYVENSNDSAWLTNAEQPLTGYERVFGTVATPR SMRTRGAIEDVAEMADKGRLRVADLQRQQFANRAPAGDLAASETAGWCAALPGGTAVG TGGTPVDVSEACRVLRRWDRSVDSDSRGALLFDRFWRRASAVPAAELWRTPFDPADPVRT PRGLNTAAPGVGTALADAVTELRAAGITLDAPLGKHQFVVRNGKRLPIGGGTESLGIWNK TEPVWNAAAGGYTEVSTGSSYIQAVGWDDSRCPVARTLLTYSQSENPKSPHFSDQTRLYAG ERWVTSRFCEKDIARSPALRVVRVHERR*


SY1965 + ECE
ECEN
ECE
10
20
30
40
(min)

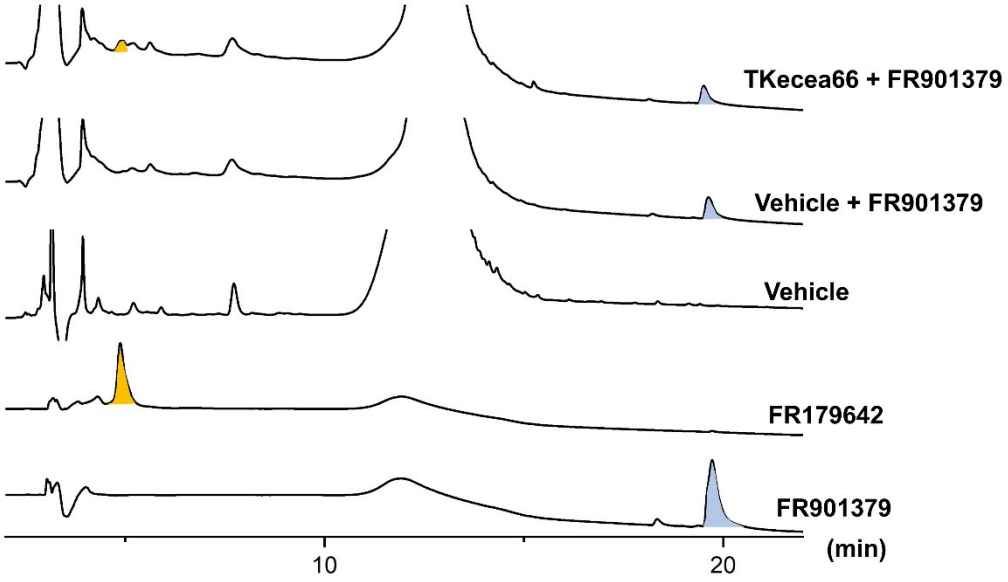
(a)


TKecea66 + ECE
Vehicle + ECE
ECEN
ECE
10
20
30
40
(min)

(b)


SY1965 + FR901379
FR179642
FR901379
10
20
(min)

(c)



(d)

Figure S1. HPLC profiles showing the deacylation activity of ECE and FR901379 by the hadal bacterium *Streptomyces* sp. SY1965 (a, c) and the recombinant echinocandin E acylase (ECEA) overproducing strain (TKecea66)(b, d).

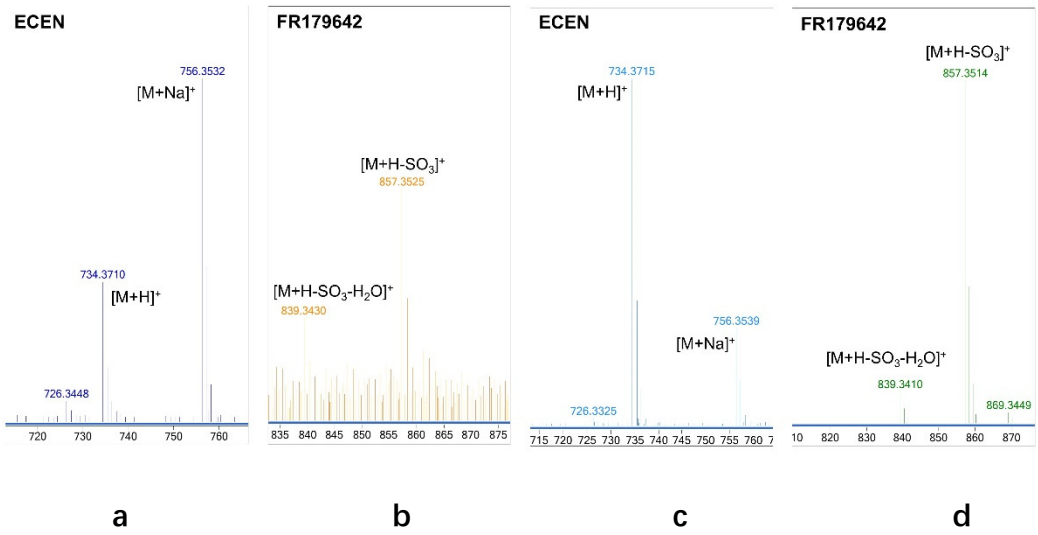
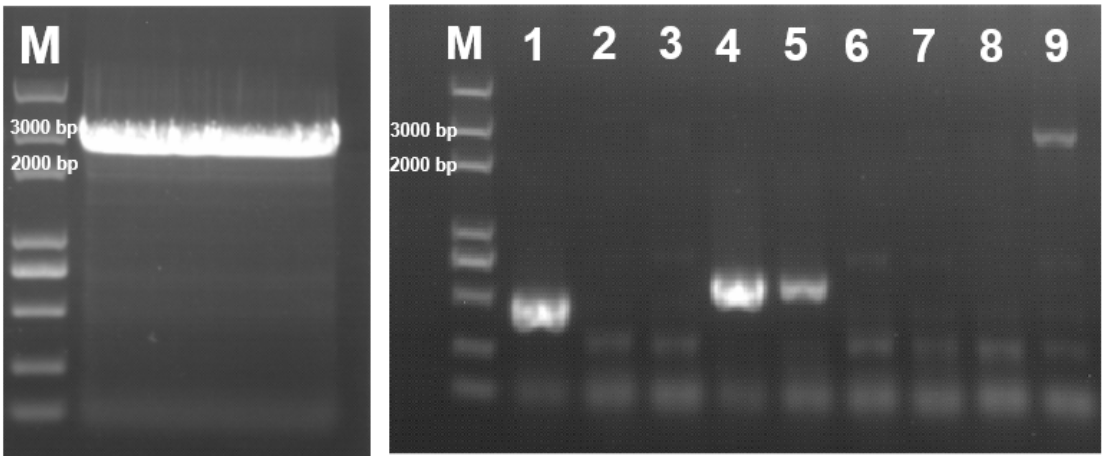


Figure S2. High-resolution mass spectra identification the deacylated products of ECEN and FR179642 from ECE and FR901379 by *Streptomyces* SY1965 (**a**, **b**) and recombinant strain *S. lividans* TKecea66 (**c**, **d**).



(a) **(b)**

Figure S3. Construction of the *ecea* gene overexpression plasmid for intergeneric conjugation. The DNA fragment (2415 bp) amplified from the genomic DNA of *Streptomyces* sp. SY1965 (a); PCR verification of recombinant plasmid pSET-*kasOp*ecea* (b). M: DNA Marker; left gel: lanes 1-9 are *E. coli* DH5α transformants; Lane 9 represents the correct construct.

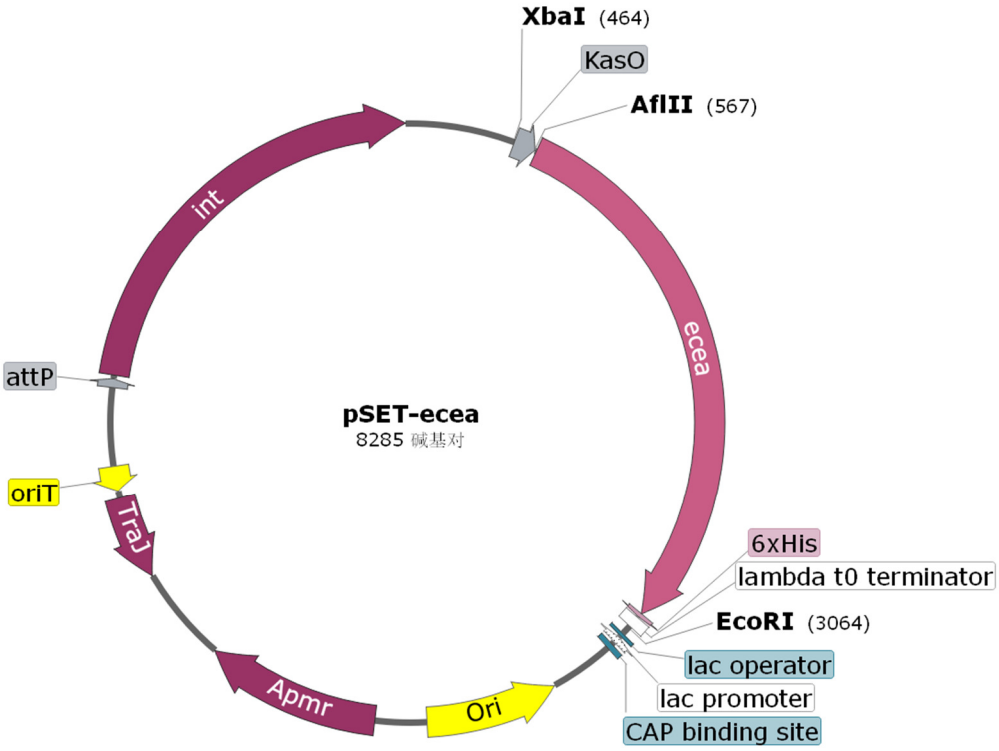


Figure S4. Plasmid map of pSET-*kasOp*ecea*. The *ecea* gene expression cassette consists of the *KasO*p* (with RBS) promoter, structure gene of *ecea*, 6×His tag and the Lambda T0 terminator.

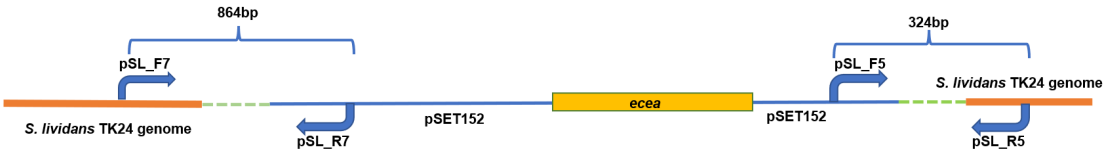
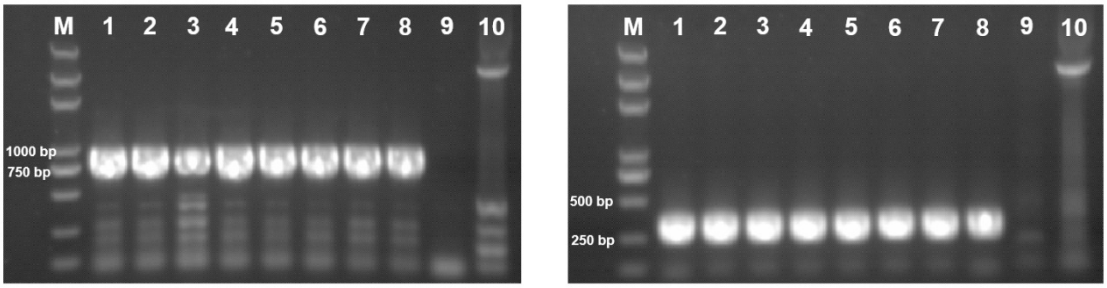


Figure S5. Schematic diagram showing the integration site of the *ecea* gene and PCR verification of the expression cassette on the chromosome of the recombinant strain, *Streptomyces lividans* TKecea66.



(a) **(b)**

Figure S6. PCR verification of the genotype of *S. lividans* TKecea66. Agarose (1%)/TAE gel electrophoresis showing the upstream (**a**) and downstream (**b**) junctions flanking the *ecea* gene. M: DNA Marker; lines 1-8: exconjugants; line 9: *S. lividans* TK24; line 10: exconjugant with empty plasmid.

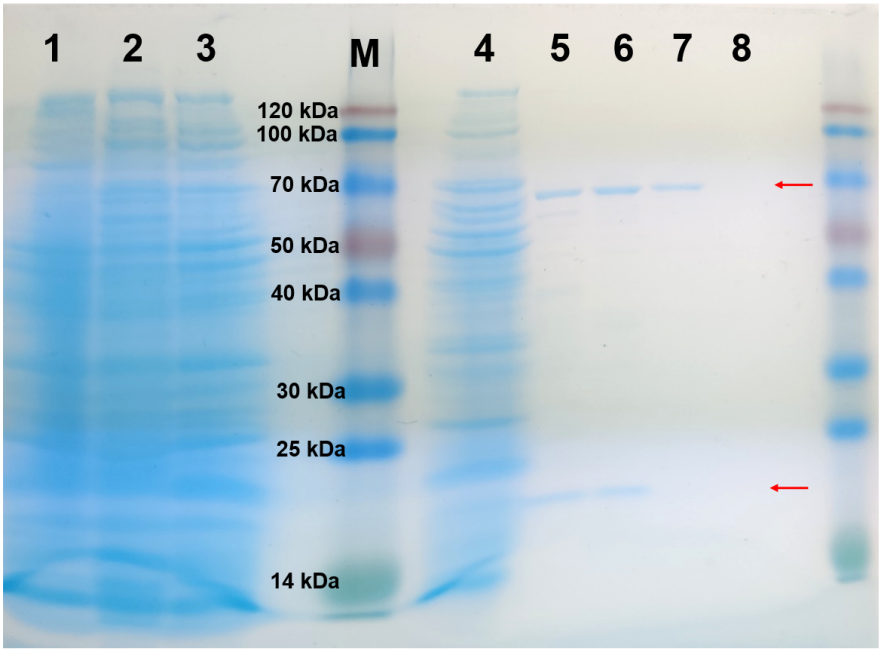


Figure S7. SDS-PAGE analysis of the ECEA produced by the recombinant strain *Streptomyces lividans* TKecea66. M: Marker; Lane 1: supernatant of *S. lividans* TK24; lane 2: supernatant of TKecea66; lane 3: *S. lividans* TK24 transferred with an empty pSET152 vector; lane 4: fractions eluted with buffer containing 20 mM imidazole; Lane 5: fractions eluted with buffer containing 50 mM imidazole; line 6: fractions eluted with buffer containing 100 mM imidazole; line 7: fractions eluted with buffer containing 300 mM imidazole; line 8: fractions eluted with buffer containing 500 mM imidazole.

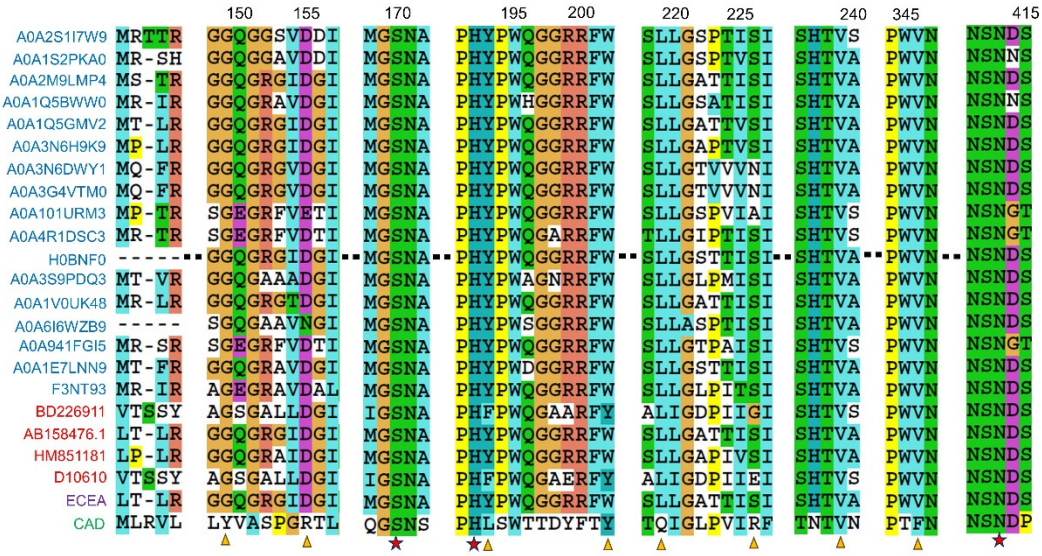
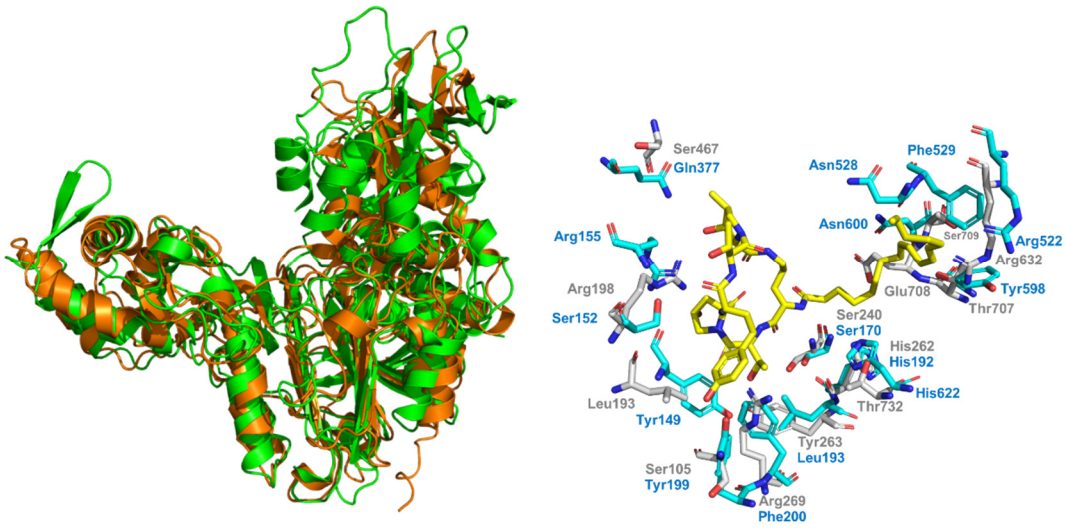
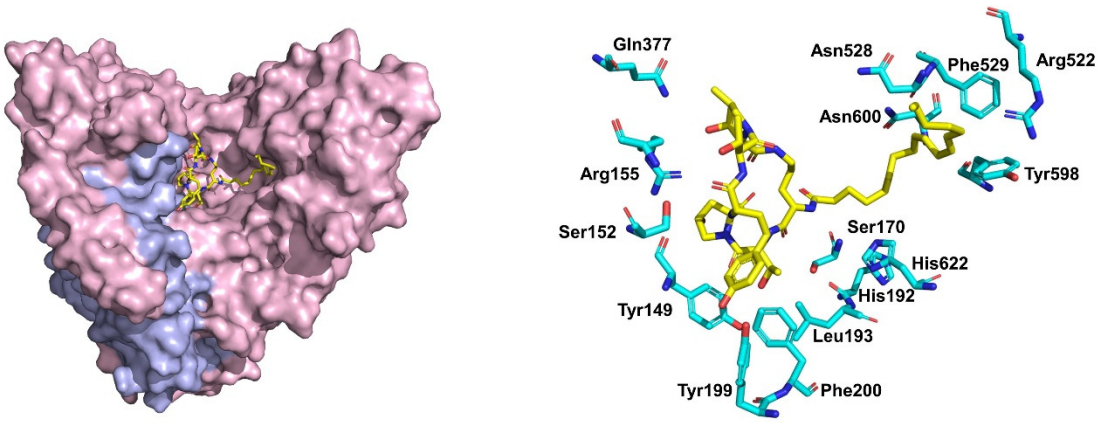


Figure S8. Multiple sequence alignment of ECEA, hypothetic ECE acylases (blue), known proteins (red) and a cephalosporin C acyltransferase (CAD, GenBank accession number: AF251710.1, green). Key catalytic sites marked with red stars and active pocket residues marked with triangles. echinocandin B acylase gene (GenBank accession numbers: BD226911, AB158476.1, HM851181, D10610).



(a) **(b)**

Figure S9. Comparison of the structures of ECEA (green) and CPC (orange, PDB code: 1JVZ) (**a**) RMSD= 2.469. The catalytically critical Ser170 in CPC (cyan) superimposed with the Ser240 of ECEA (gray) (**b**). The structure of ECEA was generated by Alphafold2.



(a) **(b)**

Figure S10. The structure (a) of ECE and CPC protein docking that α-subunit colored by light blue and β-subunit colored by light pink, and shown the key residues (cyan) surrounding substrate of ECE (yellow) within 6 Å (b).

References

10. Yu, X.; Jiang, Q.; Chen, X.; Shu, H.; Xu, Y.; Sheng, H.; Yu, Y.; Wang, W.; Keller, N.P.; Xu, J. and Wang, P. Unnatural tetradeoxy echinocandins produced by gene cluster design and heterologous expression. *Org. Biomol. Chem.* **2023**, *21*, 3552-3556.
16. Yi, W.; Qin, L.; Lian, X.-Y. and Zhang, Z. New Antifungal Metabolites from the Mariana Trench Sediment-Associated Actinomycete *Streptomyces* sp. SY1965. *Mar. Drugs* **2020**, *18*, 385.