Increased Hydrolysis of Oximino-β-Lactams by CMY-107, a Tyr199Cys Mutant of CMY-2 Produced by Escherichia coli

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Supplemental Material

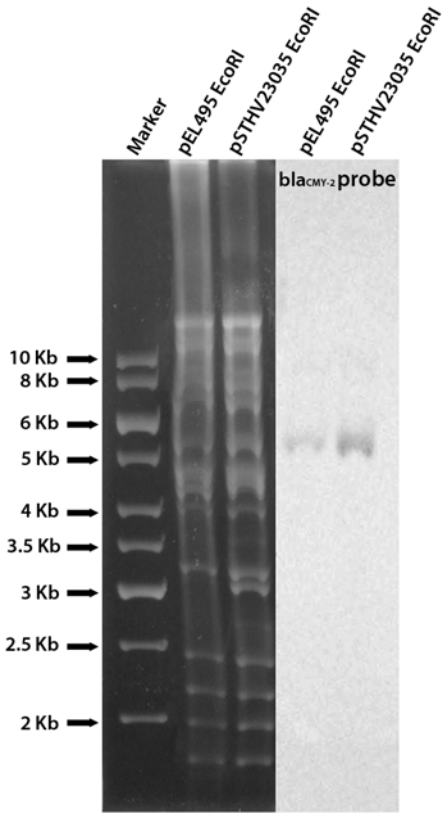

Figure S1: Comparison between the RFLP profiles of pEL495 ($bla_{CMY-107}$; *E. coli*) and pSTHV23035 (bla_{CMY-2}; *S. enterica* sv. Typhimurium) IncI plasmids. The two plasmids share a highly similar EcoRI restriction electrophoretic profile (left part). In addition a bla_{CMY} specific probe hybridized with a common fragment (~5500 bp) of both plasmids (right part).

Table S1: Primers used in sequencing reactions of pEL495

Primer	Sequence (5'-3')	Target*	Ref
E9	GAGAAAGAGGCTGTCAG	nt2384-2400; blaCMY reverse	1
CITM-F	TGGCCAGAACTGACAGGCAAA	nt2687-2707; blaCMY forward	2
CITM-R	TTTCTCCTGAACGTGGCTGGC	nt3128-3148; blaCMY reverse	2
E4/F	TGGGTTCAGGCCAACATGGATGC	nt3107-3129; blaCMY forward	3
SugE-R	GCCTGATATGTCCTGGATCGT	nt4424-4444; sugE forward	3
SugE-Rin	CATAAGCGGTTCCTACAGG	nt4260-4278; sugE reverse	this study
TrbA/Fw	CGACAAATGCTTCCGGGGT	nt6214-6232; trbA reverse	4

* Numbering according to sequence GQ398239 (GenBank)

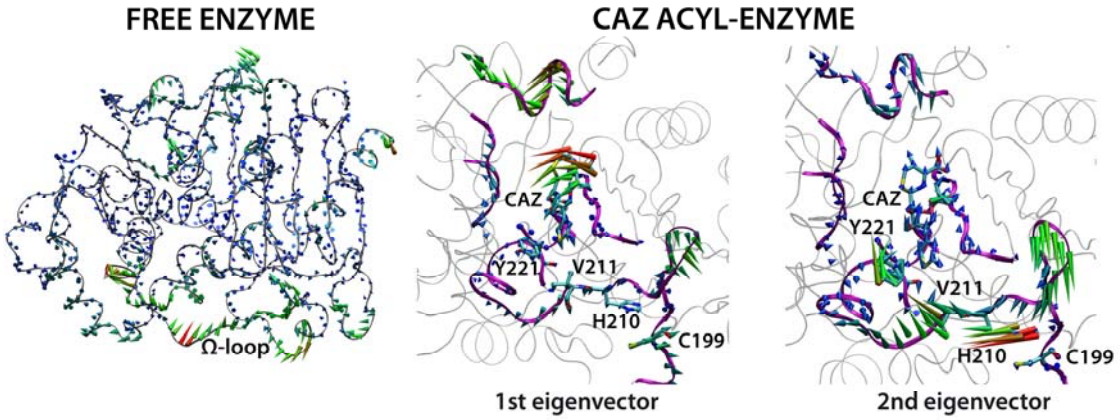

Figure S2: Porcupine plots of principle movements observed in the free enzyme of CMY-107 and in its CAZ acyl-enzyme (the projections of the first two eigenvectors are shown). Secondary structures forming the edges of the active site as well as the bound substrate in CMY-107 had similar dynamic properties with those of ES 211 variants of CMY-2, CMY-30 and CMY-42, that differed from the respective ones of the parental enzyme in respect of both intensity and direction (published elsewhere; 5).

References

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