Supplementary table 1. Description of previously selected antibiotic-catabolizing soil isolates

Isolate name[a]	Prior designation	Organism	Initial selection	β-lactam catabolism	
				Penicillin	Carbenicillin
ABC02	CA-S3F-1	*Paraburkholderia caledonica* CA-S3F-1	Carbenicillin	+	+
ABC07	PE-S1G-1	*Pseudomonas sp*. PE-S1G-1	Penicillin	+	-
ABC08	PE-S2R-1	*Pandoraea sp*. PE-S2R-1	Penicillin	+	-
ABC10	PE-S2T-3	*Pandoraea sp*. PE-S2T-3	Penicillin	+	-

[a]Data, isolate selection and growth described in Dantas *et al* (2008)[15]

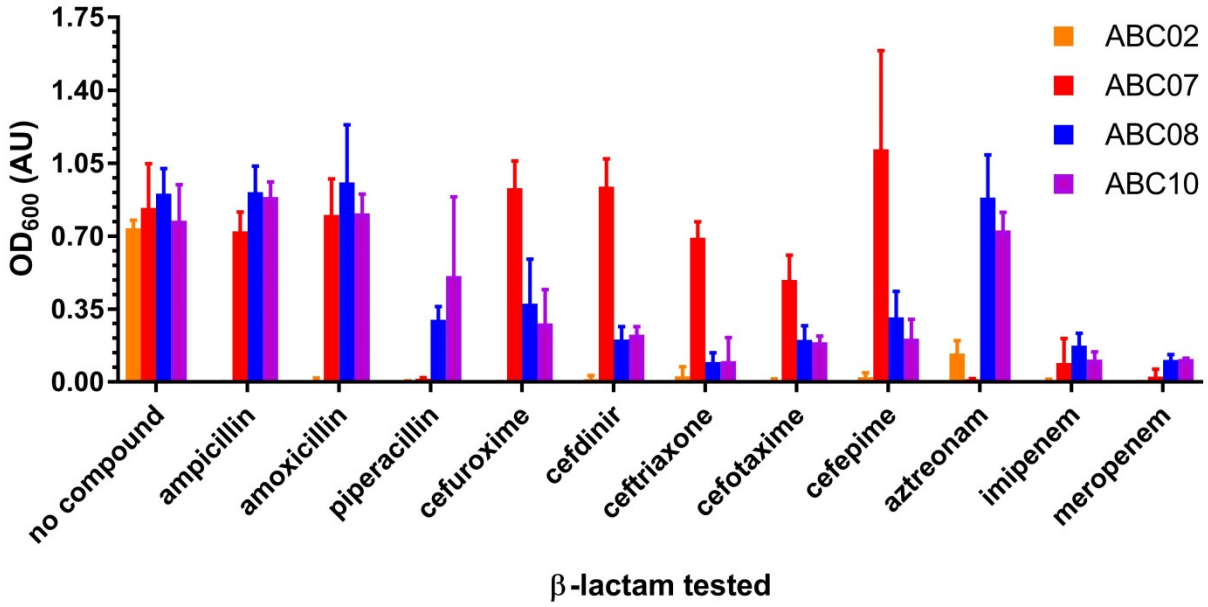
Supplementary table 2. Total β-lactamase and β-lactamase-like genes identified in ABC strain genomes

Ambler class	ABC02	ABC07	ABC08	ABC10	Total
Class A	1	0	0	0	1
Class B	0	0	0	0	0
Class C	5	2	4	4	15
Class D	0	0	1	1	2
Incomplete annotation*	3	8	3	3	17
Total	9	10	8	8	35

*Includes β-lactamases without Ambler class identification, and putative β-lactamases annotated as serine proteases or, predominantly, MBL fold metallo-hydrolases

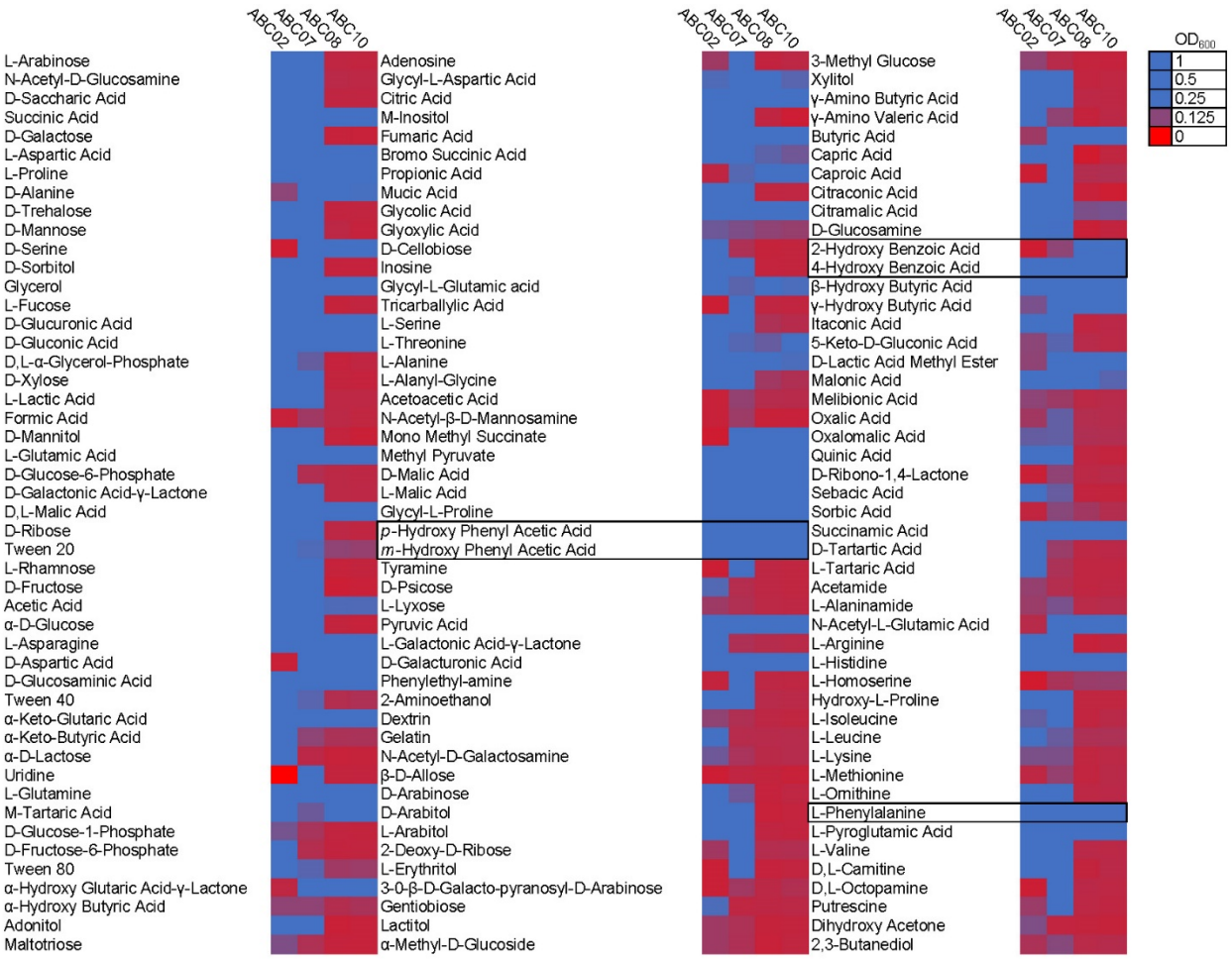
Supplementary table 3. β-lactamases up-regulated by growth on penicillin in ABC strain genomes

Ambler class	ABC02	ABC07	ABC08	ABC10	Total
Class A	1	0	0	0	1
Class B	0	0	0	0	0
Class C	1	2	1	1	5
Class D	0	0	1	1	2
Total	2	2	2	2	8



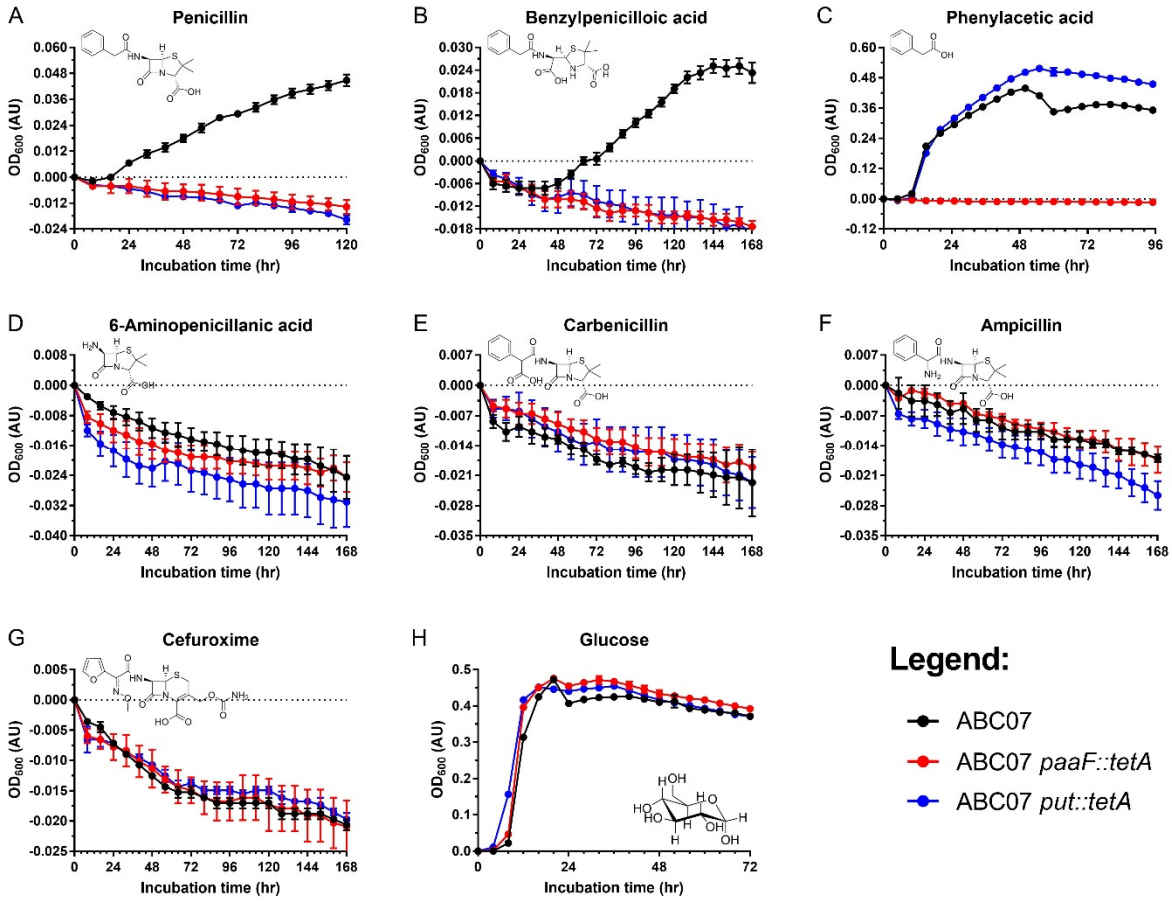
Supplementary figure 1. ABC strains exhibit multi-class, cross-generational resistance to β-lactam antibiotics

ABC strains were grown in rich media with 1 g/l β-lactam antibiotics including 3rd and 4th generation penicillins (ampicillin/amoxicillin and piperacillin respectively), 2nd, 3rd, and 4th generation cephalosporins (cefuroxime, cefdinir/ceftriaxone/cefotaxime, and cefepime respectively), a monobactam (aztreonam) and two carbapenems (imipenem and meropenem). Average culture density measured by OD_{600} of three cultures with standard deviation is shown.



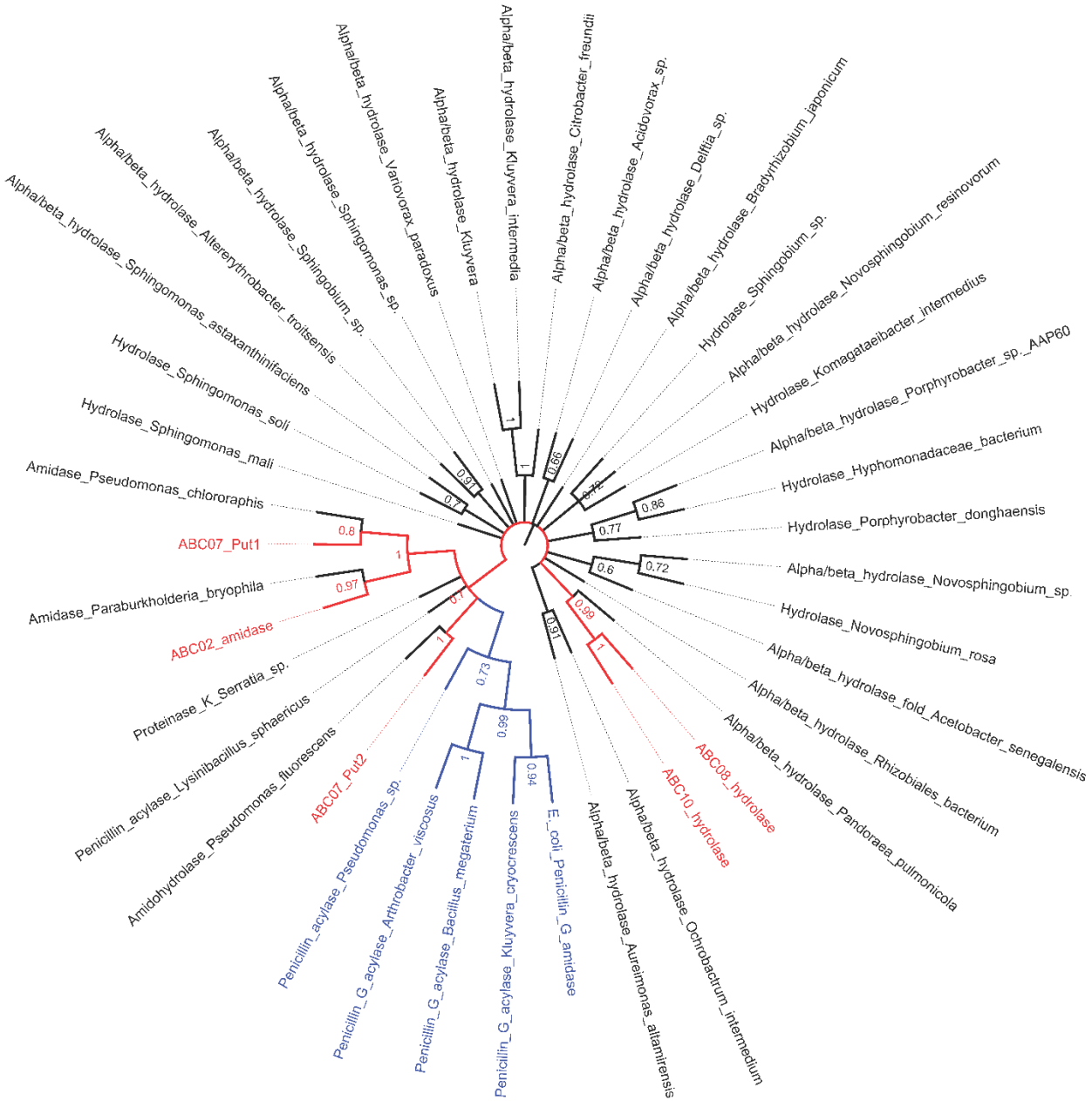
Supplementary figure 2. ABC strains catabolize diverse substrates including aromatic compounds

Growth of four ABC strains were assayed on 190 different carbon sources. Only carbon sources for which growth exceeded OD_{600} 0.05 for at least one strain are displayed. Aromatic carbon sources are boxed for emphasis.

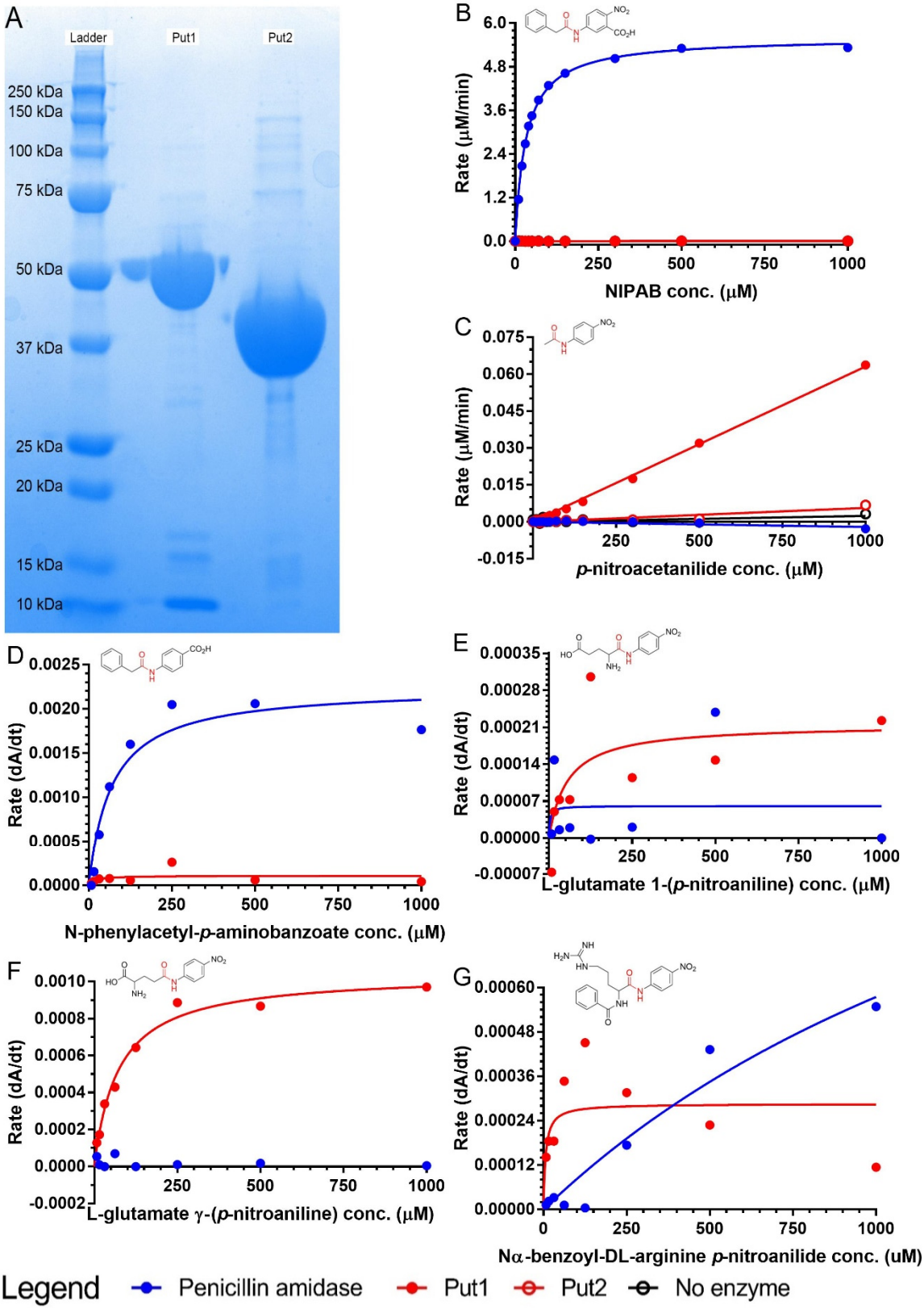


Supplementary figure 3. Growth of ABC07, *paaF*, and *put* knock-outs on penicillin degradation products and other β-lactam antibiotics

Growth curves of wild-type ABC07 (black), and *paaF::tetA* (red) and *put::tetA* (blue) strains in minimal media containing (a) penicillin, (b) benzylpenicilloic acid, (c) phenylacetic acid, (d) 6-aminopenicillanic acid, (e) carbenicillin, (f) ampicillin, (g) cefuroxime, or (h) glucose as sole carbon source. Measurements are the average of triplicate cultures with standard error of the mean displayed.



Supplementary figure 4. Phylogenetic analysis of penicillin-responsive amidases, amidohydrolases, and hydrolases from ABC strains compared to penicillin amidases
Maximum Likelihood tree of penicillin amidase amino acid sequences (shown in blue), including *E. coli* penicillin amidase and penicillin-upregulated amidase, amidohydrolase, and hydrolase amino acid sequences from ABC strains (shown in red). Node support is given by bootstrap fraction.



Supplementary figure 5. *In vitro* characterization of ABC07 Put1 and Put2 from *E. coli* overexpression

(a) SDS-PAGE gel of his-tagged Put1 and Put2 following over expression in *E. coli* and purification by IMAC resin. Expected protein masses are approximately 54.4 kDa and 44.0 kDa for Put1 and Put2 respectively. (b through g) Enzyme kinetic analysis of Put1, Put2 (panels b and c only), commercial *E. coli* penicillin amidase, or no enzyme with (b) 6-nitro-3-(phenylacetamido)benzoic acid (NIPAB) (penicillin amidase K_M 70.12±16.13 μM), (c) *p*-nitroacetanilide, (d) N-phenylacetyl-*p*-aminobenzoate (penicillin amidase K_M 68.97±24.45 μM), (e) L-glutamate 1-(*p*-nitroaniline), (f) L-glutamate γ-(*p*-nitroaniline) (Put1 K_M 73.33±10.53 μM), or (g) Nα-benzoyl-DL-arginine *p*-nitroaniline (penicillin amidase K_M 1.99±1.93 mM) as substrates.

A

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                                    *                    #
ABC07 Put1          132 QTYNSVFGTTGNAY-------...------VMSLCESALKDFAAL 292
E. coli             280 ANGLAGYPTTSNMWVIGKSKA...NPQSGYIANWNNSPQKDYPAS 540
K. cryocrescens     280 ANGLAGYPTTSNMWVIGKNKA...NPQSGYIANWNNSPQKDYPAS 539
B. megaterium       256 EELGLPLKIGSNAAIVGSEKS...NPKNGYVVNWNNKPSKEWVN- 519
A. viscosus         256 EELGLPLKIGSNAAIVGSEKS...NPKNGYVVNWNNKPSKEWVN- 519
Pseudomonas sp.     230 GDASDAAGGGSNNWAVAPGRT...DPPGGLIVTANNRVVADDHP- 490
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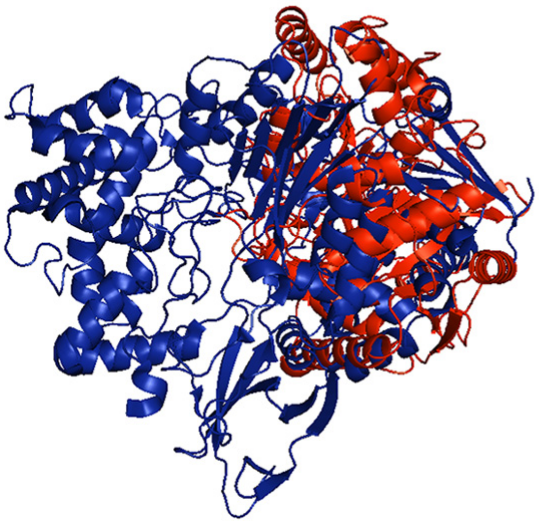
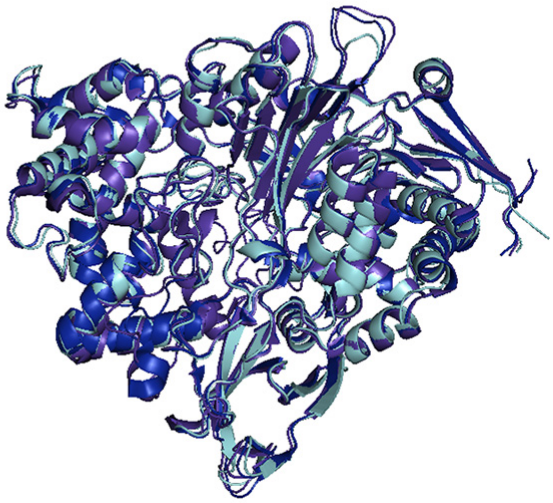
B

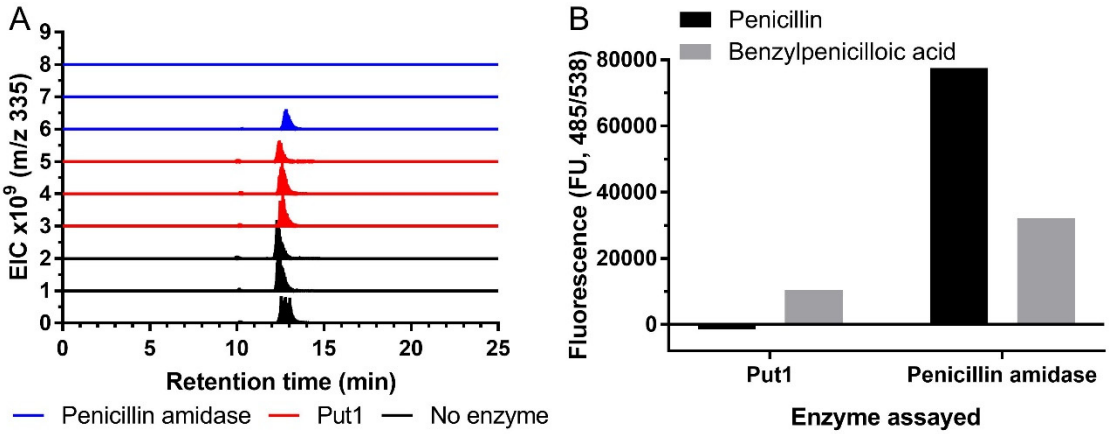


C

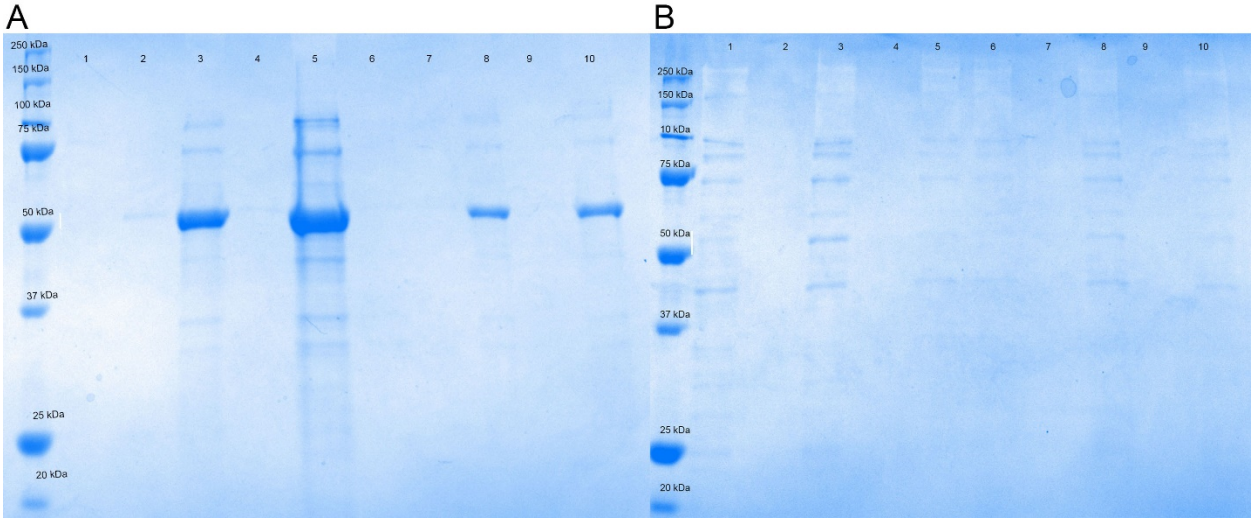


Supplementary figure 6. Sequence and structural comparison of Put1 with penicillin amidase enzyme family

(a) Multiple sequence alignment of Put1 enzyme alongside penicillin G amidase homologs. Amino acid residues conserved among penicillin amidases and N-terminal nucleophile hydrolases, the catalytic serine (*) and the oxyanion hole (#), are highlighted. Overlays and fit (RMSD/aligned residues) of *E. coli* penicillin amidase with (b) the Phyre predicted Put1 three dimensional structure (6.79 Å/88 residues) and (b) penicillin G amidases homologs from *P. rettgeri* (1.71 Å/744 residues), and *K. citrophilia* (1.39 Å/552 residues).



Supplementary figure 7. Put1 hydrolyzes the amide bond of benzylpenicilloic acid but shows little degradative activity with penicillin
(a) LCMS analysis of penicillin degradation by *E. coli* penicillin amidase or Put1 by extracted ion count monitoring at 335 m/z corresponding to singly protonated penicillin. Within each condition traces represent (bottom to top) incubation for 30 min, 300 min, and 22.5 hr. (b) Put1 and *E. coli* penicillin amidase were incubated with penicillin or benzylpenicilloic acid as substrates overnight. Hydrolysis of an amide bond (the phenylacetamide bond in penicillin and benzylpenicilloic acid or the β-lactam ring in penicillin) was detected by derivatization of newly revealed amino groups by the reagent NBD-Cl followed by fluorescent quantification.



Supplementary figure 8. Test conditions for the expression and purification of Put1 from *E. coli*
SDS-PAGE gels of (a) insoluble and (b) soluble protein fractions from cell-free extracts of *E. coli* BL21(DE3) cells expressing *put1* on pET28B vector under the following conditions: TB brand MB induced with (1) no IPTG, (2) 100 µM IPTG at inoculation, (3) 100 µM IPTG at density, (4) 500 µM IPTG at inoculation, (5) 500 µM IPTG at density, and TB brand FB induced with (6) no IPTG, (7) 100 µM IPTG at inoculation, (8) 100 µM IPTG at density, (9) 500 µM IPTG at inoculation, and (10) 500 µM IPTG at density. For all conditions the majority of Put1 protein (MW 54.4 kDa) is found in the insoluble fraction.